Human genetics = génétique humaine : proceedings of the fourth International Congress of Human Genetics/comptes rendus du quatrième Congrès international de génétique humaine, Paris, 6-11 September 1971 / editors/rédacteurs, J. de Grouchy, F.J.G. Ebling and I. W. Henderson.

Human Genetics

excerpta medica

The Principles of Human Biochemical Genetics

Frontiers of Biology, Vol. 19

by *Harry Harris*

1970, clothbound xiv + 328 pages 39 tables 90 illus.
1st reprint 1971, paperback

In recent years there have been remarkable advances in our understanding of the nature of inherited biochemical diversity among humans. The interrelationship between seemingly different phenomena of biochemical genetics can now be formed into a consistent framework and thus provide a more systematic and analytical approach to the subject. The purpose of this book is to present a general account of the subject, and, in particular, explain the principal concepts which underlie modern research in this field.

"For the student and those who wish to gain insight into fundamental advances in human biology, this book is essential reading."

The Medical Journal of Australia

"...provides an excellent introduction to biochemical genetics for medical students and workers in practically every branch of medicine."

British Journal of Haematology

"The book is recommended to all workers in the field of human genetics and related subjects."

Human Heredity

A North-Holland publication

Human Genetics

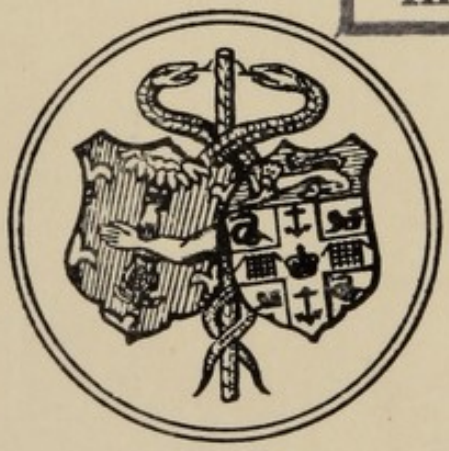
Proceedings of the Fourth International Congress of Human Genetics, Paris, 6–11 September 1971

Edited by
J. DE GROUCHY, Laboratoire de Cytogénétique, Hôpital des Enfants-Malades, Paris, France, F. J. G. EBLING and I. W. HENDERSON, Department of Zoology, The University, Sheffield, England.

INTERNATIONAL CONGRESS SERIES, No. 250

Contents

Excerpta Medica



Human genetics

Génétique humaine

Human genetics
Génétique humaine

PROCEEDINGS OF THE FOURTH INTERNATIONAL CONGRESS OF HUMAN GENETICS / COMPTES RENDUS DU QUATRIÈME CONGRÈS INTERNATIONAL DE GÉNÉTIQUE HUMAINE
PARIS, 6–11 SEPTEMBER 1971

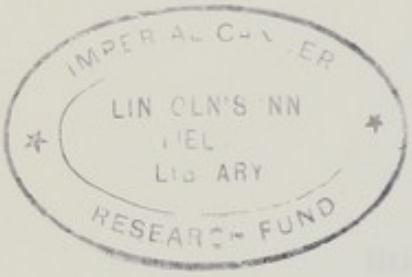
EDITORS / RÉDACTEURS

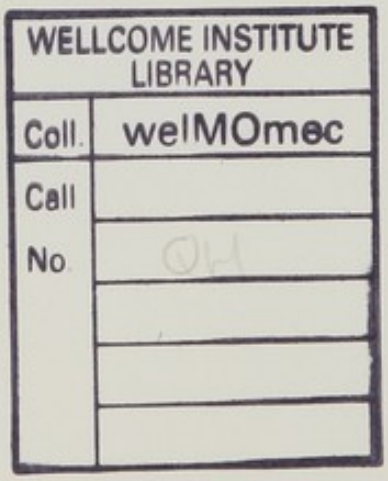
J. de Grouchy
Laboratoire de Cytogénétique,
Hôpital des Enfants-Malades, Paris

F. J. G. Ebling and I. W. Henderson
Department of Zoology,
The University, Sheffield

1972
Excerpta Medica Amsterdam

International Congress Series No. 250
ISBN 90 219 0178 1





Excerpta Medica Offices

Amsterdam Jan van Galenstraat 335
London Chandos House, 2 Queen Anne Street
Princeton Nassau Building, 228 Alexander Street

Printed in The Netherlands by N.V. Drukkerij D. Reidel, Dordrecht

Fourth International Congress of Human Genetics
Quatrième Congrès International de Génétique Humaine

Under the High Patronage of / Sous le Haut Patronage de

Monsieur Georges Pompidou	*Président de la République*
Monsieur Jacques Chaban-Delmas	*Premier Ministre*
Monsieur Maurice Schumann	*Ministre des Affaires Etrangères*
Monsieur Olivier Guichard	*Ministre de l'Education Nationale*
Monsieur François Ortoli	*Ministre du Développement Industriel et Scientifique*
Monsieur Robert Boulin	*Ministre de la Santé Publique et de la Sécurité Sociale*
Monsieur Bernard Lafay	*Secrétaire d'Etat au Développement Industriel et Scientifique*
Mademoiselle Marie-Madeleine Dienesch	*Secrétaire d'Etat à l'Action Sociale et à la Réadaptation*
Monsieur Jean Cherioux	*Président du Conseil de Paris*
Monsieur Maurice Doublet	*Préfet de la Région Parisienne*
Monsieur Robert Wagner	*Président du Conseil d'Administration du District de la Région Parisienne*
Monsieur Marcel Diebolt	*Préfet de Paris*

Fourth International Congress of Human Genetics
Quatrième Congrès International de Génétique Humaine

Honorary Committee / Comité d'Honneur

P. AIGRAIN	Délégué général à la Recherche Scientifique et Technique
J. BERNARD	Professeur à la Faculté de Médecine, Président du Conseil d'Administration de l'Institut National de la Santé et de la Recherche Médicale
C. BURG	Directeur général de l'Institut National de la Santé et de la Recherche Médicale
J. BUSTARRET	Directeur général de l'Institut National de la Recherche Agronomique
R. COURRIER	Secrétaire perpétuel de l'Académie des Sciences, Vice-Président de l'Académie de Médecine
H. CURIEN	Directeur général du Centre National de la Recherche Scientifique
R. DEBRÉ	de l'Académie des Sciences et de l'Académie de Médecine
B. EPHRUSSI	Professeur à la Faculté des Sciences
F. GALLAIS	Directeur scientifique du Centre National de la Recherche Scientifique
A. GIROUD	de l'Académie de Médecine
F. JACOB	Professeur au Collège de France, Prix Nobel
A. JOST	Professeur à la Faculté des Sciences
P. LAURENT	Directeur général des Relations Culturelles
P. LHÉRITIER	Professeur à la Faculté des Sciences
A. LWOFF	Directeur de l'Institut de Recherches Scientifiques sur le Cancer, Prix Nobel
R. MAHEU	Directeur général de l'U.N.E.S.C.O.
R. MALLET	Recteur Chancelier des Universités de l'Académie de Paris
G. MATHÉ	Professeur à la Faculté de Médecine
J. MONOD	Directeur de l'Institut Pasteur, Prix Nobel
G. RIZET	Professeur à la Faculté des Sciences
J. ROSTAND	de l'Académie française
G. TEISSIER	de l'Académie des Sciences

H. TUCHMANN-DUPLESSIS	Professeur à la Faculté de Médecine
R. TURPIN	de l'Académie des Sciences et de l'Académie de Médecine
R. DE VERNEJOUL	de l'Académie des Sciences et de l'Académie de Médecine
E. WOLMAN	Sous Directeur de l'Institut Pasteur
EMILIENNE WOLFF	Directeur de Recherches au Centre National de la Recherche Scientifique
E. WOLFF	Administrateur du Collège de France, de l'Académie des Sciences et de l'Académie de Médecine
M. ZAMANSKY	Doyen honoraire de la Faculté des Sciences

Fourth International Congress of Human Genetics
Quatrième Congrès International de Génétique Humaine

Organizing Committee / Comité d'Organisation

President/Président	Prof. Maurice Lamy
Vice-Presidents/Vice-Présidents	Prof. Jean Frézal
	Prof. Jérôme Lejeune
	Prof. Pierre Royer
General Secretary/Secrétaire général	Dr. Jean de Grouchy
Treasurer/Trésorier	Prof. Jacques Lafourcade
Secretary of foreign relations/ Secrétaire des affaires extérieures	Dr. Katherine S. Wilson

Scientific Committee / Comité Scientifique

Prof. J. L. Amiel	Prof. H. Jérôme	Prof. J. Rey
Dr. R. Berger	Dr. C. Laurent	Prof. J. M. Robert
Prof. J. Clavert	Dr. P. Maroteaux	Prof. Cl. Ropartz
Prof. J. Dausset	Dr. J. Moullec	Prof. J. Ruffié
Dr. M. Deminatti	Prof. Cl. Mouriquand	Prof. Ch. Salmon
Prof. J. Ducos	Prof. J. Poly	Prof. A. Stahl
Dr. S. Gilgenkrantz	Dr. M. O. Rethoré	Dr. E. Zuckerkandl

Permanent Committee for the International Congresses of Human Genetics

Comité Permanent des Congrès Internationaux de Génétique Humaine

Chairman/Président	Prof. M. LAMY, Paris, France
Vice-Chairmen/Vice-Présidents	Prof. E. INOUYE, Tokyo, Japan Prof. J. V. NEEL, Ann Arbor, Mich., U.S.A.
Secretary General/Secrétaire Général	Prof. L. GEDDA, Rome, Italy
Assistant Secretary General/ Secrétaire Général Adjoint	Prof. M. MILANI-COMPARETTI, Rome, Italy

Members/Membres

Prof. G. ANDERS, Groningen, The Netherlands
Prof. S. ARMENDARES, Mexico D.F., Mexico
Prof. P. E. BECKER, Göttingen, F.R.G.
Prof. J. A. BÖÖK, Uppsala, Sweden
Prof. E. CASTILLA, Argentina
Prof. R. CRUZ-COKE, Santiago, Chile
Prof. J. DE GROUCHY, Paris, France
Prof. N. DUBININ, U.S.S.R.
Prof. M. A. FERGUSON-SMITH, Glasgow, United Kingdom
Prof. J. FRANÇOIS, Ghent, Belgium
Prof. F. C. FRASER, Montreal, Que., Canada
Prof. N. FREIRE-MAIA, Curitiba, Brazil
S. GEERTS, IGF representative
Prof. D. KLEIN, Geneva, Switzerland
Prof. J. LAFOURCADE, Paris, France
Prof. H. G. SCHWARZACHER, Vienna, Austria
Prof. A. G. STEINBERG, Cleveland, Ohio, U.S.A.
Prof. C. STERN, Berkeley, Calif., U.S.A.
Prof. A. VALLES, Madrid, Spain
Prof. J. WAHRMAN, Israel

Table of contents/Table des matières

V. Mental deficiency/Arriération mentale

VI. Genetics of human enzymes and proteins/Génétique des enzymes et protéines humaines

VII. Immunogenetics/Immunogénétique

VIII. Cell genetics/Génétique cellulaire

IX. Pharmacogenetics/Pharmacogénétique

X. Chromosome mapping/Carte chromosomique

XI. Genetics – past, present and future/Génétique – passé, présent et futur

Chapter I Clinical genetics

Victor A. McKusick

Departments of Medicine, Epidemiology and Biology, The Johns Hopkins University, Baltimore, Md., U. S. A.

Introductory comments

One objective of world congresses is to review the state of the field. In this congress the symposia are designed for this particular purpose. The topic of this first symposium is the area of overlap between genetics and medicine. That this symposium is set for the first day rather than a later day as in previous congresses may reflect the central role which, it seems, clinical genetics has come to play in human genetics.

As in introducing a comparable session at the Congress in Chicago five years ago, let me quote Bradford Hill, the British biostatistician. He stated that the practice of medicine involves seeking the answer to three questions. What is wrong? The answer is diagnosis. What is going to happen? The answer is prognosis which, in our field, is the basis of genetic counseling. What can be done about it? The answer is treatment. As David Danks of Melbourne pointed out, it is the social and scientific responsibility of the physician to keep a fourth question in mind: why did it happen? Because on the answer depend scientific progress and prevention.

The speakers in this symposium will be addressing their remarks to these four or five aspects: diagnosis, prognosis (that is, genetic counseling), treatment, prevention and the contribution of the study of genetic disease to biologic knowledge.

Since the last congress, etiologic heterogeneity has become an ever more evident feature in clinical genetics with important implications in diagnosis, genetic counseling, management and search for the basic defect. Diagnosis has been aided by applied cell biology in the form of the study of cultured skin fibroblast. By extension of the cellular methods to amniotic cells, prenatal diagnosis has developed into a strong ally of genetic counseling. New techniques for staining chromosomes have found diagnostic usefulness. New modalities of therapy have been developed. Programs for prevention of recessive diseases by identification of carriers have been designed.

And throughout, there have repeatedly been examples supporting Archibald Garrod's dictum, that the study of rare diseases, especially rare genetic diseases, has much to teach us about the normal.

W. Lenz

Institut für Humangenetik, Westfälische Wilhelms-Universität, Münster, F. R. G.

Genetic diagnosis: molecular diseases and others

Precision in diagnosis of genetic disease has greatly increased in recent years. Names such as gargoylism, pituitary dwarfism, or thalassemia can no longer be considered to represent single entities. They are groups of allied disorders united by common clinical signs, but easily split into distinct entities if close attention is paid to clinical and biochemical signs. Such splitting may have important implications for individual and genetic prognosis as well as for treatment. It is important to realize that patients who have many signs and symptoms in common do not necessarily belong to the same diagnostic category. In the process of arriving at a correct diagnosis, distinctive traits should be noted as carefully as common ones. The ultimate precision in genetic diagnosis is attained in some biochemical abnormalities and in chromosomal aberrations involving loss or surplus of whole chromosomes. A word of caution is appropriate. Patients who share the same enzyme defect may still have different forms of one type of disease. I propose that, when different isoallelic mutations are involved, the conditions should be referred to as forms of a single disease, whereas if different alleles are mutated the disease should be designated as different types. The term 'varieties' would be left for various phenotypes with exactly the same underlying mutation. Thus, hemoglobin C disease and sickle cell disease would be different forms of β-hemoglobinopathies. Most hemoglobinopathies would either be of α- or of β-type, and the various clinical manifestations of homozygous Sickle cell disease would be referred to as varieties. I do not expect that my proposal will be widely accepted, but most people interested in the field would probably agree that the time has come to redefine such confusing terms as disease, type, form and variety or to replace them by more meaningful words.

A diagnosis should not be thought of as a description of the total individual phenotype, but only of that part which the individual shares with other patients with the same diagnosis. Besides having a particular disease, every patient is still an individual in the remainder of his phenotype. To describe a patient as a case of mongolism, though widely practised, is somewhat misleading. A mongol is an individual human being with many qualities which are entirely independent of the fact that he has an additional 21–chromosome. To realize that a diagnosis never characterizes the whole individual patient is even more important in conditions in which the effect of the genetic abnormality is small in comparison with normal variation, as in XYY- or XXX-individuals. It has often been stated that the variability of mongolism or other genetic diseases is great. The standard deviation in mental capacity or physical growth of patients with a given genetic abnormality, however, is usually found to be essentially the same as in the normal population, and only the distribution curves are shifted to the left. The obvious implication is that the effect of the genetic abnormality is roughly the same for all individuals affected by it, but it is superimposed on the normally distributed effects of the remaining normal genotypes. We may say then, that a surplus 21–chromosome

lowers the I. Q. or standing height by a certain measurable amount, roughly equal for all individual patients. Thus, the variability of mongols is not characteristic of mongolism, but essentially the same as the variability of normal people.

There has been much rather futile dispute about the mental traits of XYY-men. The factual evidence is still too meagre to permit definite answers, but the problem may be more clearly understood if we realize that individual variability is not levelled out by a chromosomal aberration. Let us assume that propensity to crime is normally distributed, and that there is a measurable threshold separating criminals from non-criminals.

Let us furthermore assume that the distribution curve of propensity to crime is shifted to the right, but not altered in shape by an additional Y-chromosome. We may then analyse the variance of this propensity in XYY-men on the basis of only two values: the estimate of the incidence of criminality in normal men and that in XYY-men. This would give us a better idea of the degree to which factors other than the additional Y are involved in the criminality of XYY-individuals. This type of analysis, of course, leads to the problems of multifactorial inheritance, in which one of the factors has been singled out. An essentially similar problem is the contribution of the ABO-blood groups to peptic ulcer.

Diagnosis is somewhat less precise in the deletion and translocation syndromes than in the numerical aberrations, because the exact location of the breakage points and the amount of translocation material might differ from one case to another. The new fluorescent and Giemsa techniques will certainly permit more precise studies of the correlation between phenotype and chromosomal aberration in these cases.

An exact identification of the genetic cause has only been possible in a small minority of genetic diseases, i.e. those in which an alteration in the amino acid sequence of the abnormal gene product has been demonstrated. It may, however, be accepted as a well founded dogma that all monogenic diseases are basically molecular diseases, even though it is usually unknown which gene product is defective. This does not mean, however, that diagnosis must be imprecise in these conditions. Many hereditary syndromes have such an intricate and unmistakable pattern of morphological anomalies that diagnosis is unequivocal, though, of course, the possibility remains that mutation at different loci or different mutations at the same locus may produce the same phenotype. It has only recently been realized that rare morphological syndromes of genetic origin are, as a rule, highly specific, and that the diagnostic entities of the older textbooks usually comprise large heterogeneous groups of diseases.

The specificity of syndromes within a group may be illustrated by genetic types of radial defects in man, with which I have been lately concerned when I was asked to give my opinion on more than 1000 individual cases of limb deformities, thought to be phenocopies due to thalidomide.

Three genetic syndromes, involving radius aplasia, are sufficiently documented to serve as an illustration of how an analysis of morphological traits may serve to distinguish the syndromes from one another and from thalidomide embryopathy. Figure 1 is an Euler diagram in which 4 or 5 different diagnoses are treated as sets of cases characterized by morphological symptoms. If radius aplasia is thought of as a diagnosis, then no differentiation is possible. If additional signs are used, then only 2 of 4 diagnoses partially overlap. Fanconi's anemia is clearly differentiated by panmyelopathy, chromosome breaks, fetal hemoglobin, retardation of growth, pigmentation and other features from the other groups. Yet the partial overlap between Fanconi's syndrome and thalidomide embryopathy is still so striking that it stimulates speculation. The morphological signs met with in Fanconi's anemia and also in thalidomide embryopathy are predominantly such as occur after thalidomide intake between the 35th and the 42nd postmenstrual day, that is in the first half of the thalidomide sensitive period. These are duplication of thumbs, atresia of the auditory meatus, deafness, strabism, dystopia, aplasia or fusion of kidneys. Triphalangism of the thumbs, on the other hand, which is common with thalidomide intake between the 47th and 50th day, is rarely seen in Fanconi's syndrome. In the dominant radial defects, often referred to as the Holt-Oram syndrome, the

early signs of thalidomide embryopathy, such as ear and kidney malformations, are conspicuous by their absence, whereas the typical late manifestation of thalidomide embryopathy, triphalangism of the thumbs, is very common. In the present state of our knowledge some cases of thalidomide embryopathy appear to be almost perfect phenocopies of the Holt-Oram syndrome, though, morphologically, the Holt-Oram cases are only a subset of the total set of cases falling within the morphological frame of thalidomide embryopathy. I suppose, however, that a more detailed and more critical study of more material would eventually shift the circumference of these circles so much that overlap would get considerably smaller or even disappear. Clinodactyly of the 5th fingers and mild funnel chest are often met with in dominant radial defects, but not in thalidomide embryopathy. Such minor signs may not have

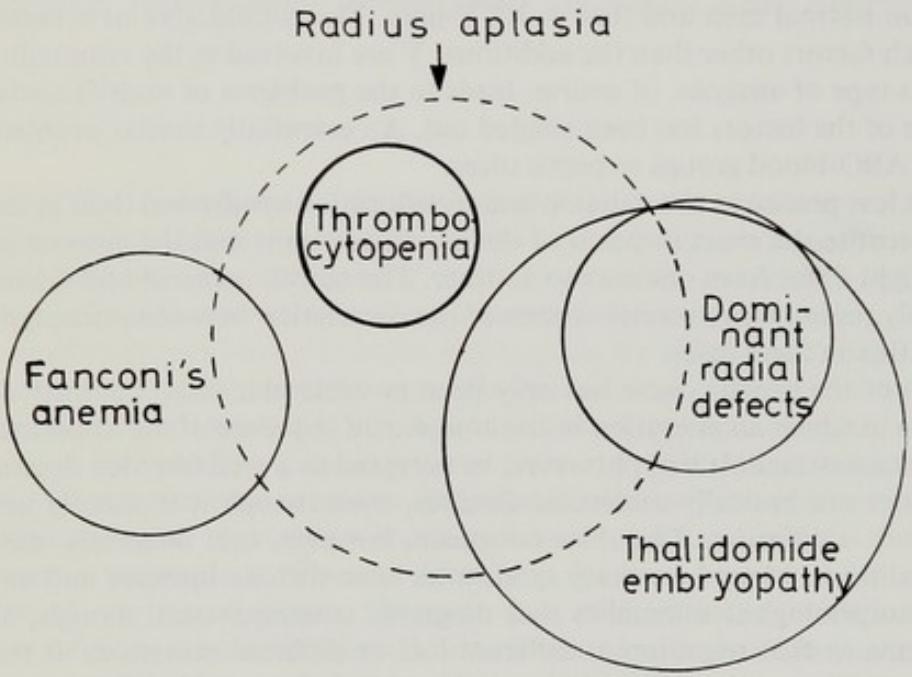


Fig. 1. Euler diagram showing morphological overlap or non-overlap of syndromes united by radius aplasia.

TABLE 1

	Fanconi's anemia	Dominant radial defect
Number of cases	133	246
Radius aplasia	11	33
Thumb aplasia or hypoplasia	44	144
Humerus aplasia or hypoplasia	0	29
Thumb triphalangism	0*	45
Index aplasia	0	19
Thumb duplication	5	0
Renal malformation	26	0
Deafness	17	0
Microphthalmos	14	0

* Dr. Wiedemann has seen triphalangism of the thumbs in a case of Fanconi's anemia (personal communication), and I have found one additional such case in the literature.

been mentioned in many published cases. Thus, their diagnostic significance may be greater than realized at present.

In the third genetic syndrome of radius aplasia, that associated with thrombocytopenia, phenotypical variability is definitely less than in the others. The radius is totally lacking on both sides in every case, and yet without exception the thumbs are nearly normal, with first metacarpals and both phalanges rather well developed. The middle phalanges of the 5th fingers, on the other hand, are always rudimentary. A comparison with thalidomide cases shows that no malformations like those caused by thalidomide only before the 40th or only after the 45th day are seen in the thrombocytopenia-radius aplasia syndrome. One might suppose, therefore, that the normal alleles of the genes underlying these 3 syndromes are active in the following time sequence: first Fanconi's anemia, then the thrombocytopenia-radius aplasia syndrome, and finally the dominant radial defects. The sensitive period of the dominant syndrome, however, appears to be spread over a somewhat longer period. A mother might have triphalangeal thumbs and her baby bilateral 3-finger phocomelia with partial defects of humerus and aplasia of radius. Similar malformations, if seen in thalidomide children, would be attributed to intake at about the 47th to 50th day for triphalangeal thumbs and at the 42nd and 44th postmenstrual day for 3-finger phocomelia. I believe that a more detailed morphological analysis will, in this as in other morphological syndromes, not only permit more precise diagnosis, but also give insight into the strategy of the genes in the development of the embryo.

A brief remark might be added in relation to thalidomide embryopathy. Though this condition has sometimes been called thalidomide syndrome, the name does not appear to be a proper one. Thalidomide embryopathy is far too variable to be called a syndrome in the usual sense. Cases of anotia with cranial nerve defects, but no limb defects, cases with amelia of the arms and normal ears, and cases with triphalangeal thumbs but otherwise normal limbs do not constitute one syndrome. Yet they are links of one chain of syndromes connected by intermediate varieties and combinations. They are steps of one teratogenic series. The explanation of this variety became obvious when the relation of morphology to time of intake of thalidomide was studied. Cases in which the embryo has been damaged by thalidomide at exactly the same developmental stage constitute a good syndrome, and the actual varieties of interconnected thalidomide syndromes are easily explained by the variable time of intake.

Diagnosis can theoretically be precise in monogenic diseases and structural chromosomal aberrations, but I doubt whether it ever can or even should be precise in multifactorial disease. Names such as schizophrenia or essential hypertension do suggest qualitative entities, where a concept of quantitative variation would be more appropriate. In multifactorial diseases, therefore, one should aim at softer diagnoses rather than at harder ones. The concept of a multifactorial disease implies that not all cases of such a disease are due to the same genes. The disease cannot be clearly separated from normal variation of the population. Multifactorial diseases might be called deviations rather than diseases and should be looked at in relation to the normal distribution of the respective traits – serum uric acid in gout, blood glucose in diabetes, blood pressure in hypertension, etc.

Diagnosis is no longer magic exorcism exerted by sticking a mysterious label on an enigmatic case. In its proper sense the process of diagnosis is understanding the very nature of the disease.

C. O. Carter

MRC Clinical Genetics Unit, Institute of Child Health, London, United Kingdom

Genetic counselling – principles and prospects

Genetic counselling is a rapidly developing branch of the medical services. The reasons for this are:

The rapid reduction in infant and childhood mortality from environmentally caused disease, throwing genetically and part-genetically determined disease into greater prominence.

The increased survival rate of patients with such disorders, putting a long-term strain on the parents and the community; for example, in England 40 years ago 7 out of 8 children with Down's syndrome died in childhood, now only 1 patient in 2 does so.

The advent of family planning, giving parents a sense of responsibility for the birth of each child.

The realisation that soon mean family size all over the world must drop to just over 2 children per parental couple, so that parents want these 2 to be healthy.

The spread of knowledge of human genetics by the new school biology syllabuses, books, articles, television and recently through active parents' associations, for example parents of children with muscular dystrophy and cystic fibrosis.

A consciousness that effective new treatments of genetically determined disease will lead to an increase, even if in most instances only slowly, in the birth frequency of these disorders unless counter measures are taken.

These influences are each likely to become stronger and offer a challenge to medical genetics which must be met and increasingly is being met. To quote from Britain's experience – the first genetic clinic was established in London, at The Hospital for Sick Children, in 1946 and now 25 years later there is a genetic clinic at each University Medical School, and this includes at least one in each of the Hospital Regions of the United Kingdom and 4 specialist clinics in London. Every family doctor in Britain has been circulated with a list of these clinics by the Ministry of Health and Social Security.

OBJECTIVES

The objectives of genetic counselling may be summarised as follows:

To give information to parents and their relatives on the risks to future children, this information to include both the risk of disease and the prognosis for an affected child.

To alert the medical profession to special risks to an as yet unborn child, thus facilitating early diagnosis and treatment.

To prevent an increase in the birth frequency of genetically and part-genetically determined disease following the introduction of effective new treatments, and in the long term to reduce the birth frequency of such diseases well below the level normally maintained by the balance of mutation and selection.

ORGANISATION

The detailed organisation of genetic counselling and the channels of referral of patients will depend on the general organisation of each country's medical services. In most instances the first enquiry will be to the family doctor, who may be able to reassure the parents at once that there is no problem, but if not will refer the parents to an appropriate specialist, for example a paediatrician, who may be able to give the parents the necessary information. Lastly, with problems of special difficulty or perhaps where specialised laboratory tests are required, referral may be appropriate to a genetic clinic. The problems referred will constantly change as medical practitioners, general and specialist, themselves become more knowledgeable about medical genetics and as new techniques which are of value in estimating genetic risks become available.

REQUIREMENTS

There are essentially two requirements for estimating genetic risks: a reliable family history and an accurate diagnosis. Just occasionally the family history itself will indicate strongly how the condition in question is inherited. The pedigree may show clearly that the condition is dominant, or autosomal recessive or X-linked recessive. More often however, in these days of small families one has to rely on an accurate diagnosis and knowledge from the literature or on one's own experience to determine just how the condition is inherited. For example, the lateral X-ray of the spine is quite characteristic in the X-linked form of spondyloepiphyseal dysplasia (named spondylo-epiphyseal dysplasia tarda by Professor Lamy and his colleagues). It is the necessity to check, and sometimes increase the precision of, the diagnosis which makes it desirable that those carrying out genetic counselling should be medically qualified and ideally with specialist medical qualifications as well. There are many aspects of medicine with which a medical geneticist does not have to be familiar and so he should be able to make himself expert in the diagnostic features of genetic conditions.

CATEGORIES OF RISK

In estimating actual risks there are essentially two basic situations, though these overlap. The first is that in which an exact risk may be given based on genetic theory, as with simple Mendelian situations – dominant, recessive and X-linked. The second is where there is no precise theoretical basis for the estimate of risk, but the estimate is based on empirical observations of a large series of families in which the condition in question has occurred. With Mendelian situations there may, of course, be complications – for example: the possibility that a sporadic case of an X-linked condition is due to a fresh mutation, and the allowance to be made for the presence of unaffected males in the family; the possibility of incomplete penetrance with dominant conditions; the allowance to be made for the life-time risk already experienced without manifestation in a condition with variable age of onset such as Huntington's chorea. In most instances, however, in such Mendelian situations the risk is either greater than 1 in 10 or there is a small risk, perhaps not much more than the random risk. Empirical risks for common conditions are usually between 1 in 10 and 1 in 100. Some geneticists find empirical risk situations unsatisfactory. They like scientific precision. They are conscious that these risk estimates are only averages and that in the particular family the actual risk may be, if one only had more information, greater or less than the average. However, the empirical risk estimates are useful. In medicine, in other applied sciences and in many other real life situations one constantly has to make decisions based on probabilities. Some examples of common conditions for which there are empirical risk estimates are several of

the common congenital malformations such as spina bifida and cleft lip, the major psychoses such as schizophrenia, idiopathic epilepsy, non-specific severe mental retardation and many others. Here again precise diagnosis is important; for example cleft lip with mucus pits of the lower lip is a dominant condition. It is important to remember also that an empirical risk estimate established for one particular population will not necessarily apply to another, particularly if the condition in the other population has a markedly different birth frequency.

RISKS IN PERSPECTIVE

Two considerations in putting estimates of risks into perspective are first to relate the additional risk in a particular pregnancy to the overall risk of malformation in any random pregnancy, and secondly to explain the prognosis for the child if it should be affected. It is well known to the medical profession, but not to the layman, that in any pregnancy there is a 2 to 3% risk of serious malformation of some kind or another. Any special risk is additional, but must be seen in this perspective. The prognosis for the child if affected is important. The follow-up that colleagues and I carried out, published last year (Carter *et al.*, 1971), showed that parents were sometimes prepared to take a high risk if the child if affected would be stillborn or die in infancy. In no case did they plan a further pregnancy where the child if affected would be expected to survive for years with a major handicap. It is also necessary to explain to a parent, who for example suffers from a relatively mild manifestation of a dominant condition, that her child if affected is likely to be much more severely handicapped.

A further aspect of putting risks into perspective involves a matter of principle about which there will be different opinions between one doctor and another. This is the degree to which the doctor providing information on genetic risks should also directly advise parents on whether to plan further children or not. In the future we are going to rely on genetic counselling to control the birth frequency of much genetically determined disease, but we will achieve little unless parents take responsible decisions on the basis of the information given them. In the United Kingdom, with the prevailing concept of the right and duty of individuals to make their own decisions, and more specifically in matters medical the trend to involve the patient in all decisions, our feeling is that the ultimate choice must rest with the parents. Therefore the question I have sometimes been asked, 'Do parents take your advice?' is inappropriate. We give information and not advice. Many patients, however, do find it helpful if the doctor does also indicate the decision he would make if faced with their particular problem. In other countries, with a more authoritarian tradition of the doctor-patient relationship, medical practitioners may well feel that they should more directly advise or instruct parents on what decisions to take. One experienced paediatrician has said that he feels he should appear to take decisions for the parents after first sensing what they themselves would like to do, so that if things go wrong they can blame him and not themselves. In practice, however, parents do take sensible and responsible decisions, provided that they are given the correct information and it is put into proper perspective.

PROSPECTS

Coming now to future prospects for counselling and the control of the birth frequency of genetically determined disease, the major new development in recent years has been the very welcome advent, as a practical possibility, of the diagnosis at the 14th to 16th week of pregnancy of a number of genetic abnormalities. This will be a great boon to those parents with a high risk of a serious condition who much want further children, but are not prepared to take the risk unless they can be offered prenatal diagnosis and a termination if the foetus should prove to be affected. Dr. Littlefield will be discussing this in more detail.

Another prospect which has been with us much longer, but is still in its infancy, is that of detecting clinically normal heterozygotes. This is important not only for autosomal recessive and X-linked conditions, but also dominant conditions of late onset. It is in fact for the latter two classes of conditions, the X-linked recessive and the dominant of late onset, that heterozygote detection will be of most value in individual families. A more precise test than plasma creatine kinase levels for the carrier state for Duchenne muscular dystrophy would be of great help to the girls in the families at risk. Similarly, a test which would distinguish between those who do not carry the gene would be of great help to young adult offspring of a man or woman with Huntington's chorea. Carrier detection is in general of less practical importance for autosomal recessive conditions. The exceptions are the few conditions which are relatively common in certain populations: sickle-cell anaemia in West Africans, thalassaemia in certain Mediterranean populations, and cystic fibrosis in North Europeans. Here carrier detection, already possible in the case of the two severe anaemias, offers the alternative prospect of substantially reducing the birth frequency of homozygous patients by informing couples both of whom are carriers of the risk before they marry. Some attempts at this have already been made, for example in Italy for thalassaemia. It is likely that more will soon be made among populations of West African descent. Many North European peoples will certainly apply the procedure to cystic fibrosis as soon as a carrier test suitable for screening whole populations is discovered. The prospects of carrier detection for a great variety of Mendelian conditions are good, but depend essentially on advances in biochemistry.

With common conditions of multifactorial aetiology the elucidation of the detailed mechanisms of the genetic predisposition and the additional environmental triggers is only at a very early stage. But there are ultimately good prospects of prevention here, since the presence of an important environmental component in their aetiology offers the opportunity for special environmental prophylaxis of those genetically at risk.

In summary the future control of genetically determined disease is likely to be:

- For chromosome anomalies, mostly prenatal screening by amniocentesis.
- For conditions due to mutant genes of large effect, genetic counselling, supplemented by prenatal diagnosis.
- For multifactorially determined conditions, the detection of those genetically at risk and special environmental prophylaxis.

REFERENCE

CARTER, C. O., FRASER ROBERTS, J. A., EVANS, K. A. and BUCK, A. R. (1971): Genetic clinic: a follow-up. *Lancet*, *1*, 281.

Charles R. Scriver

The deBelle Laboratory for Biochemical Genetics, McGill University-Montreal Children's Hospital Research Institute, 2300 Tupper Street, Montreal, Quebec, Canada

Screening and treatment for hereditary (metabolic) disease

Diagnosis, counselling and treatment of genetic disease in man, and of hereditary metabolic disease in particular, is an important component of the practical medical sciences today. Dr. McKusick asked me to speak at the 3rd International Congress on the subject of treatment for genetic disease (Scriver, 1967); at that time treatment was in many ways more potential than realized. The pace of events has been swift since the last congress and there have been several reviews written on the subject of treatment (for example Craig, 1968; Scriver, 1969; Holtzman, 1970; Dancis, 1970), describing both past achievements and new developments. Advances in the techniques of prenatal diagnosis (Milunsky *et al.*, 1970) have allowed genetic disease not responsive to treatment by environmental manipulation to be counselled more effectively than before. Changes in social structures, and particularly in abortion laws, have improved the options available to families unfortunate enough to encounter such genetic disease. The effectiveness of screening methods has been increased and broadened so that the number of patients in need of formal diagnosis, counselling and treatment has steadily increased. In this context, Dr. McKusick's 1971 request to discuss screening and treatment of hereditary metabolic disease can be met by examining four aspects of the problem:

1. The case load of genetic disease requiring facilities for diagnosis, counselling and treatment.
2. Current objectives in screening for genetic disease.
3. Progress in 'environmental engineering' as an approach to treatment; (Dr. Rogers will discuss 'genetic engineering' later in this symposium).
4. Systems for delivery of care to patients with genetic disease.

1. THE GENETIC DISEASE CASE LOAD

A clearer understanding of our commitments to genetic disease control can be achieved if the size of the case load is known. Knowledge of genetic disease is better now than it was; but what we find is, to some extent, dependent on time and place. For example, Carter's analysis of the genetic content of pediatric deaths at the Great Ormond Street Hospital (Carter, 1956) covered the years 1914, 1934 and 1954; his study preceded the flowering of clinical cytogenetics. A similar study by Roberts *et al.* (1970) was performed in the North of England during 1960–66; chromosomal disease appeared in the later study.

If one of the objectives of medical genetics is to prevent genetic disease we should know how many of its patients are actually admitted to hospitals today. We carried out a study of the admissions to the Montreal Children's Hospital for this purpose (Saginur *et al.*, 1970). We

have a typical children's hospital serving a large industrial region with its surrounding countryside. The case records were examined for a statistically representative sample of the 12,084 admissions in 1970. Discharge diagnoses were classified as: 'genetic'; 'congenital malformation'; and 'non-genetic'. A disease was 'genetic' if it was listed in McKusick's catalogue (McKusick, 1968) and it was a 'congenital malformation' if it was listed in the NIH catalogue of Hay and Tonascia (1968). The results are summarized in Figure 1. We found that genetic disease accounts for a very important fraction of our medical illness, while congenital malformation constitutes a major portion of the surgical admissions. Our findings are similar to those mentioned by Barton Childs (1970) for the Johns Hopkins Hospital pediatric medical service. No doubt there will be studies of other hospitals and of different age groups and we

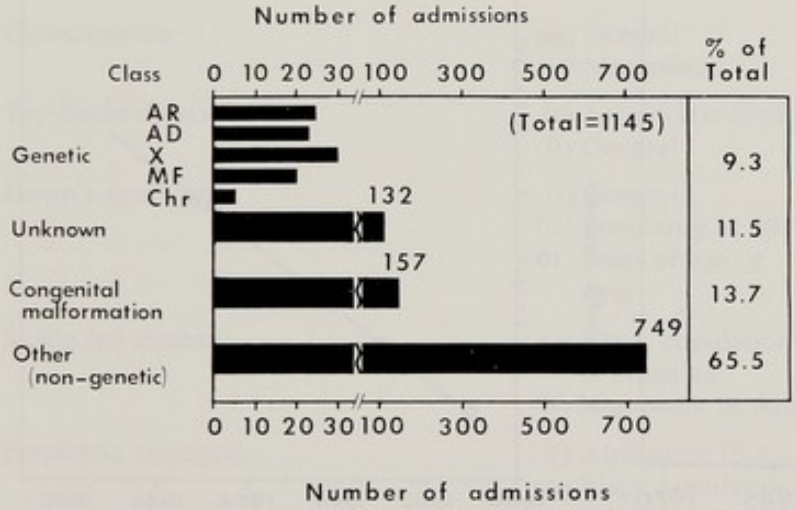


Fig. 1. Discharge diagnosis in a sample representing 12,084 admissions during 1970 to a referral hospital for children in North America (Montreal Children's Hospital). The sample was randomized. Diagnoses were classified to define genetic content of admissions (see text).

can anticipate that data will soon be available to present the general picture of genetic disease more accurately.

The growth rate of our knowledge about genetic diseases influences our awareness of it. For example, the number of recognized hereditary aminoacidopathies and G6PD mutants has increased steadily in the past two decades (Fig. 2) because of available technology, mass screening and expanding interest in such problems. These two examples of nosological growth are typical of nearly all forms of hereditary disease which now occupy our interest. The result is that Dr. McKusick's (1968) catalogue of genetic disease has grown at a frightening rate during the evolution of its three editions from 1966 to 1971.

To recognize the heterogeneity of genetic disease is one thing; to know its frequency is another. Both are necessary to calculate case loads accurately for purposes of diagnosis, counselling and treatment. The frequency of some disease varies from region to region according to the genetic make-up of the population as for example with sickle cell disease, Tay-Sachs disease, porphyria variegata and hereditary tyrosinemia (Table 1). The frequency may also change in relation to age of the population as in the case of Down's syndrome and maternal age.

Another view of the genetic disease case load is shown in Figure 3. This graph indicates the cumulative case load at the Montreal Children's Hospital in our program for diagnosis, counselling and treatment of patients with hereditary metabolic disease. The patient volume is a direct reflection of increased local interest in case finding. The increased demand on care facilities is a direct legacy of increased activity in screening for genetic disease.

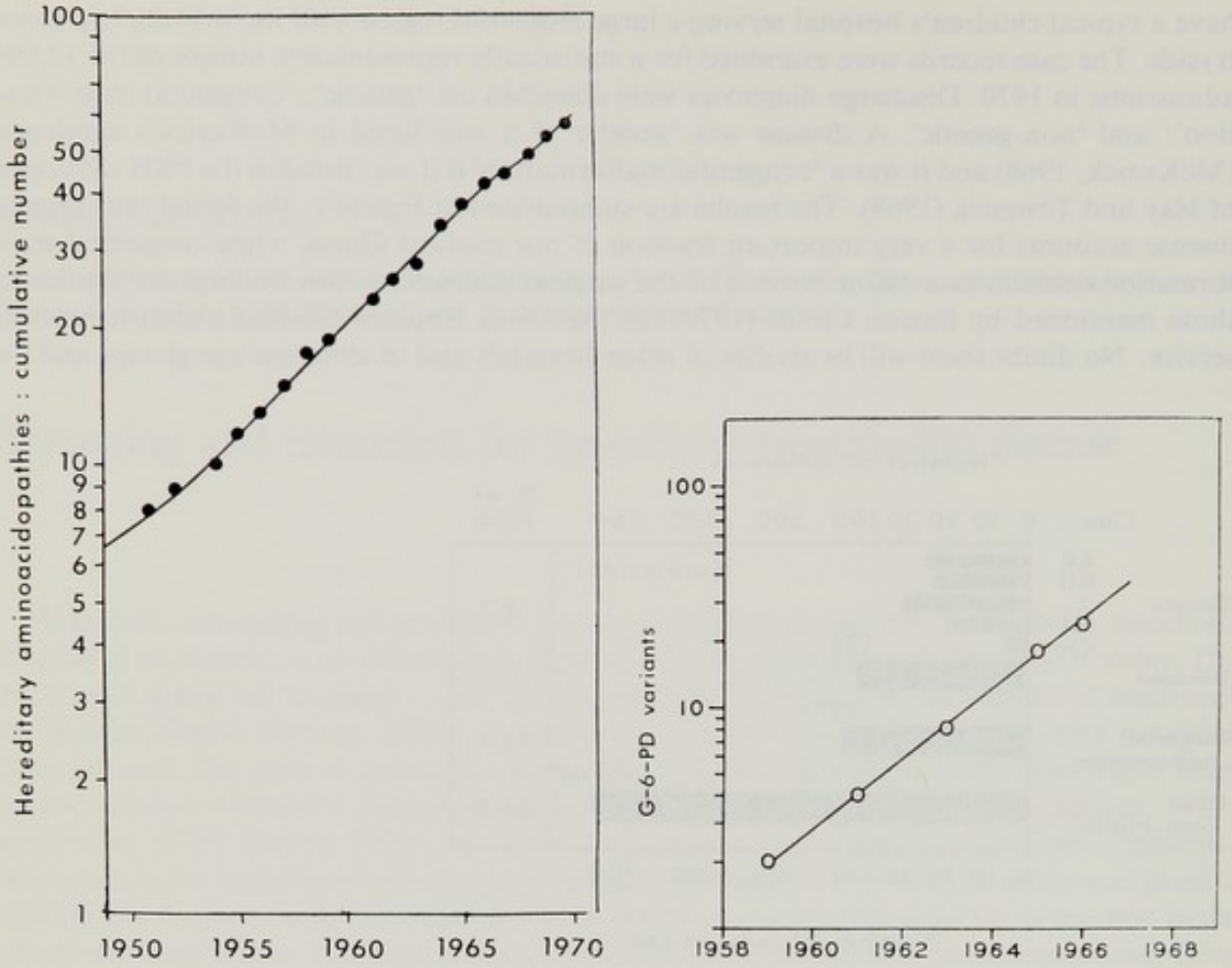


Fig. 2. Growth rate of knowledge concerning two types of genetic disease. Left: for inborn error of amino acid metabolism. Right: for hereditary variants of G6PD activity in blood. (Latter from Childs, 1967.)

2. SCREENING FOR GENETIC DISEASE

The types of genetic disease susceptible to surveillance include chromosomal anomalies, multifactorial disease and point mutations. The latter may include rare alleles with large effect or common alleles with little visible effect in the normal environment and whose significance more often than not is unknown.

Screening for genetic variation may serve one or more purposes. There is still a basic need for information about the frequency, natural history and inheritance of much genetic disease; some types of screening can help supply this information. In other situations it is now practical to establish mass screening for purposes of diagnosis, counselling and treatment of treatable genetic disease. In some circumstances specialized screening procedures or *in utero* diagnosis have widened the options available to persons at risk for untreatable genetic illness.

The World Health Organization has issued a technical report (Report of a WHO Scientific Group, 1968) on screening for hereditary metabolic disease. It recommended, amongst other things, that pilot studies gather information relevant to proposed mass screening programs. Mass screening on a public health basis was not recommended if there were no facilities for patient retrieval, diagnosis, counselling and treatment; facilities for centralized activity and expertise and for data storage were also advised. Collaborative and interdisciplinary efforts are required if the scientific and social consequences of screening programs are to be realized.

TABLE 1 *Apparent frequency of representative types of genetic disease*

Genetic problem	Constituency	Estimated frequency
Classical phenylketonuria	(*i*) General (*ii*) Ashkenazic Jews	(*i*) 0.71×10^{-4} (*ii*) Very rare
Benign hyperphenylalaninemia	General	0.34×10^{-4}
Maple syrup urine disease	General	0.30×10^{-5}
Hereditary tyrosinemia	(*i*) General (*ii*) French Canadian isolate	(*i*) 0.40×10^{-5} (*ii*) 0.30×10^{-3}
Galactosemia	(*i*) General (*ii*) Manitoba	(*i*) 0.43×10^{-5} (*ii*) 0.62×10^{-4}
Tay-Sachs disease	(*i*) Ashkenazic Jews (*ii*) General	(*i*) Approx. 0.3×10^{-3} (*ii*) Approx. 10^{-5}
Down's syndrome	(*i*) General (*ii*) Pregnancy at 40 years of age or over	(*i*) 0.15×10^{-2} (*ii*) 0.20×10^{-1}
Sickle cell disease	(*i*) Black population in America (*ii*) Non-black in America	(*i*) 0.25×10^{-2} (*ii*) Very low
Porphyria variegata	(*i*) Afrikaaner (S.A.) (*ii*) Black population (S.A.)	(*i*) 0.25×10^{-2} (*ii*) Very low

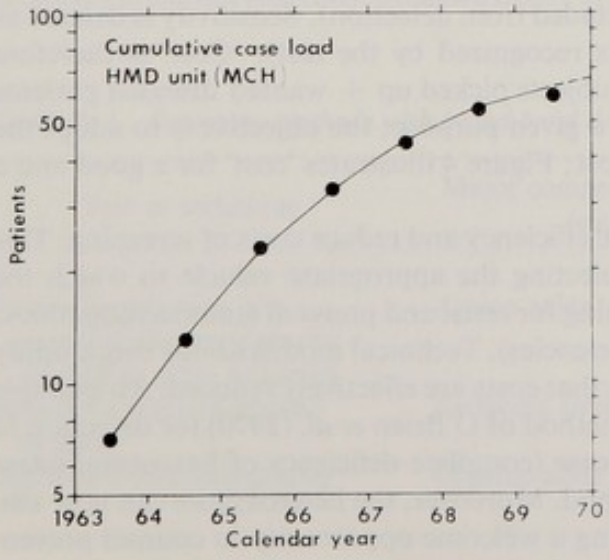


Fig. 3. Growth of case load in hereditary metabolic disease unit at the Montreal Children's Hospital. Growth reflects increasing community activity for diagnosis and treatment of genetic disease. (From Clow *et al.*, 1971*b*.)

The technical ease of the screening and its specificity all determine the real value of a screening program (Report of a WHO Scientific Group, 1968; Janeway, 1971).

Screening for genetic disease may be done at four primary levels (Scriver, 1965): (*i*) the genetic material, (*ii*) the gene product, (*iii*) the function controlled by the gene product, (*iv*) the disease caused by genetic aberration. Each level has its difficulties and merits. I will not mention the first level of screening except to note that new methods for staining human chromosomes have already recharged cytogenetic research and the field has gained renewed momentum. Screening for the disease itself (level IV) is not often desirable, since it is usually too late to help the patient; the objective of screening is to prevent pathological change or at least to recognize disease at its incipient stage. Unfortunately, this is not possible for many types of genetic illness and a proband may be irrevocably affected before a family can be counselled to avoid further risk. But, as Dr. Littlefield will discuss in his paper, it may be possible to recognize some genetic disease easily enough *in utero* to allow society to exert those options which can prevent further expression of such disease from taking its toll.

Screening methods are effective in direct proportion to their 'signal: noise' ratio, an analogy I use because it implies a relationship between the truly positive test and the background 'static' of false-positive tests, missed diagnosis and costs of the screening program (and noise from the critics!). To be more precise in this analogy, the 'signal' is the index trait sought by the screening method while 'noise' is non-disease, heterogeneity in the disease trait and other sources of a false signal. Noise is obviously a measure of 'cost' as well. The value of any screening method lies largely in its ability to yield an acceptable signal-to-noise ratio. The signal must be amplified so that it can be detected efficiently and noise must be damped to avoid extraneous signals.

A simple statistical definition of the signal is usually not adequate in mass screening. As shown in Table 2 a very large number of repeat tests would be required to find one patient with a disease whose frequency is 10^{-4} or 10^{-5}, if the investigator accepted a value more than +2SD from the mean of a normal distribution as indicative of an 'abnormal' test result. The situation can be improved by going to + 3SD as the cut-off; but 'follow-up' noise is still appreciable.

Choice of test is usually a compromise between the signal:noise ratio and the cost benefits of the screening method. Figure 4, which is adapted from Partington's discussion (Partington, 1968), summarizes the way in which *specificity* and *sensitivity* of a screening test can be used to determine its cost. Specificity is defined as (a/a+b) 100 according to the matrix described in the figure (*i.e.* per cent of healthy subjects excluded from detection). Sensitivity is defined as (d/c+d) 100 (*i.e.* per cent of diseased patients recognized by the test). 'Cost' is therefore (b+c/a+b+c+d) 100 (*i.e.* unwanted healthy subjects picked up + wanted diseased patients missed). Cost may vary with different tests for a given purpose; the objective is to adopt the test which is most useful and has the lowest cost; Figure 4 illustrates 'cost' for a good and a poor test.

Appropriate signal amplification can improve efficiency and reduce costs of screening. This can sometimes be achieved biologically, by selecting the appropriate vehicle to which the test is applied (*e.g.* urine *versus* plasma in screening for renal and prerenal aminoacidopathies; serum *versus* leukocytes for certain enzyme deficiencies). Technical modifications can amplify the signal or increase reception to such a degree that costs are effectively reduced. An example of signal amplification is found in the elegant method of O'Brien *et al.* (1970) for detection of Tay-Sachs disease. Patients with Tay-Sachs disease (complete deficiency of hexoseaminidase A) can be recognized with certainty by this method. Moreover, the heterozygote can now also be detected with great confidence, thus providing a welcome opportunity to counsel preventively for this disease.

Correct choice of the screening method can broaden the receiving band so that many signals can be received from a single test, thus improving the efficiency of screening. Table 3 lists some proven examples of this approach. Even very restricted sophisticated methods such

TABLE 2 *The problem of 'noise' in screening programs with the statistical approach*

Size of population tested: 100,000		
Criteria for a 'positive test'	Noise: signal ratio found when disease frequency is: 10^{-5}	10^{-4}
> +2SD from mean*	2250:1	225:1
> +3SD from mean*	160:1	16:1

* Assuming a normal distribution.

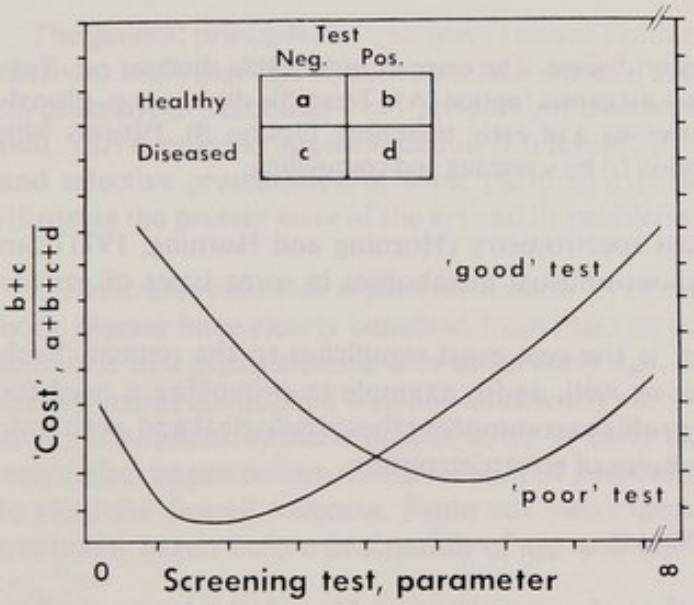


Fig. 4. Graph depicts cost in relation to the use of screening test. Matrix reflects specificity and sensitivity of the test (see text). (Adapted from Partington, 1968.)

TABLE 3 *Screening methods with broad-band detection*

Test or technique	Major compound identified (signal)	Source of signal (disease or trait)
Ninhydrin reaction plus partition chromatography applied to urine or serum	Amino acids	Amino acidopathies (over 50 types identifiable)
Benedict's reaction applied to urine	Reducing substances	Over 20 disorders of carbohydrate metabolism
Gas liquid chromatography	Organic acids	Over 18 diseases of branched chain amino acids and short chain fatty acid metabolism
Microbial inhibition assay	Natural analogue of inhibitor	Many hereditary metabolic diseases

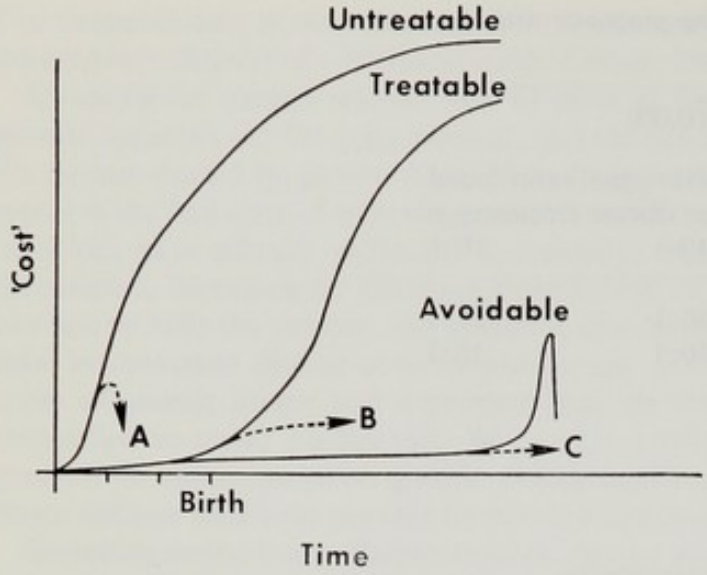


Fig. 5. Cost profiles for 3 types of hereditary disease. The costs of untreatable disease, *e.g.* Tay-Sachs disease, have been modified by prenatal diagnosis (option A). Treatable disease, *e.g.* phenylketonuria, can be modified by newborn screening and early treatment (option B). Patients with sickle cell trait can avoid being put at risk (option C) by screening and counselling.

as gas chromatography coupled with mass spectrometry (Horning and Horning, 1971) can become valued sources of information about unusual metabolites in some types of genetic disease.

The biological cost of the genetic trait is the cost most significant to the patient. Such disease usually exerts economic pressures as well, as for example in provoking a need for chronic medical care. Three types of cost profile accommodate these biological and economic considerations (Fig. 5), and influence the choice of screening option.

A. THE COST PROFILE FOR UNTREATABLE DISEASE

Such disease may exert a cost from the time of conception (*e.g.* Down's syndrome, or Tay-Sachs disease). Antenatal screening of high risk persons coupled with the option for therapeutic abortion can preclude some of the human cost and also reduce the economic costs of the disease.

B. THE COST PROFILE FOR TREATABLE DISEASE

Some mutant alleles can be neutralized by continuous postnatal environmental engineering (*e.g.* phenylketonuria). Early diagnosis at the first expression of the trait *ex utero* allows treatment to begin at the stage of incipient illness. The established pathological state is avoided and the patient can achieve a productive life.

C. THE COST PROFILE FOR AVOIDABLE DISEASE

Some mutations place the subject at risk only in certain environments. Knowledge of genotype can protect him from that risk. The case for screening high risk groups for the sickle cell trait and G6PD deficiency has been advanced (Mentzer *et al.*, 1970) in this context.

If the short-term costs of genetic disease can be improved, what of its long-term costs? Have we gained anything if female homozygotes for hereditary metabolic disease treated successfully today, harm their offspring *in utero* tomorrow? This is one of the unwanted legacies of successful screening for phenylketonuria, but it is surely a problem which can be met par-

tially with a better diet for management of this disease. If we successfully neutralize a rare mutant allele with large effect so that its probands can then readily pass it on to another generation, what will we do to the gene frequency? It takes relatively few generations to double the frequency of a rare X-linked disease, rather longer with dominantly inherited disease and a very long time (thousands of years) with an autosomal recessive trait when the heterozygote frequency is 1–2% and offspring are limited to 2–3 per family. Whether these considerations are of any real significance depends on whether treatment and control of genetic illness is effective, simple and benign, or difficult and costly. Treatment with the former attributes can convert a gene with 'bad' biological implications into a metaphysical entity of neutral significance to our world as we presently know it. The quality of treatment thus becomes an important factor.

3. TREATMENT OF HEREDITARY METABOLIC DISEASE

The general principles of treatment remain similar to those discussed at the Third Congress (Scriver, 1967) namely: (*i*) substrate restriction, if substrate accumulation is the principal cause of phenotypic pathology; (*ii*) product replacement, if depletion of a metabolite is important; (*iii*) coenzyme implementation if relevant; (*iv*) enzyme replacement if possible. A brief and selective presentation of some personal experience with these modes of treatment can illustrate the present state of the art and its problems.

i. Substrate restriction in phenylketonuria Patients with this prototype of hereditary metabolic disease have clearly benefited from mass screening and the opportunity this affords to initiate a low phenylalanine diet at an early age. There is now considerable evidence that dietary treatment applied well and sufficiently early can yield a satisfactory outcome in terms of IQ attainment. Modest deficits in IQ of early treated patients (Berman and Ford, 1970) may reflect imperfections in the delivery of medical care rather than the inability of treatment to yield the desired outcome. From our own experience (Fig. 6) there is little question that treatment begun before one month of age and not later than two months of age achieves a

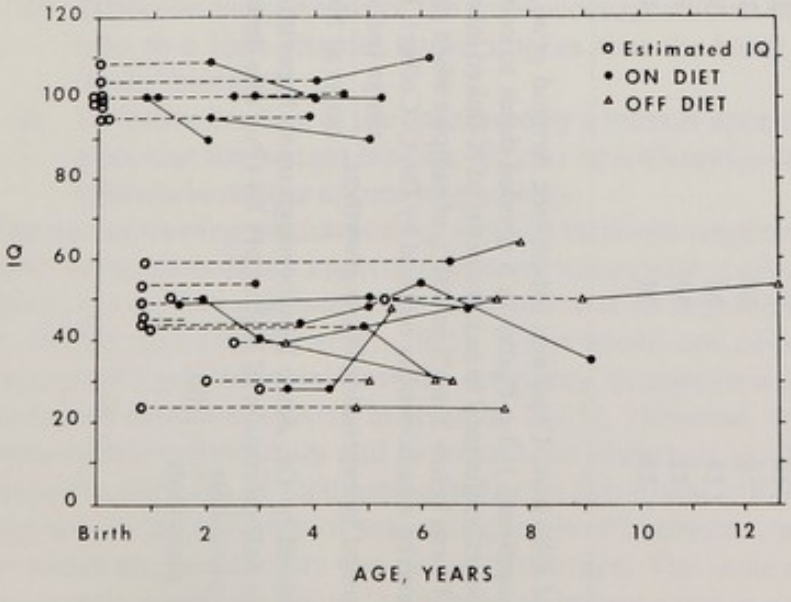


Fig. 6. Effect of dietary treatment and early diagnosis on the IQ in classical phenylketonuria. An early diagnosis and treatment prevent retardation. Late diagnosis (after 2 months) allows brain damage. Treatment may prevent further deterioration. (Adapted from data of Clow *et al.*, 1971*b*.)

TABLE 4 *X-linked hypophosphatemia: model for the 'product replacement' mode of treatment**

Patient**	Age (years)	Sex	Serum Pi achieved on therapy (mg %) (mean ± SD)***	Bone metabolism		Linear growth (per cent of normal)††	Whole blood P_{50} (per cent of untreated value)†††
				Active rickets	Bone density†		
MC	10	F	3.7 ± 1.1	0	Normal	128	108
SV	10	F	2.8 ± 0.4	0	–	125	109
JB	8	M	3.2 ± 0.6	0	–	128	109
EM	11	M	3.5 ± 1.3	0	Normal	100	111
LA	11	M	5.1 ± 1.4	0	Normal	133	110
DA	13	M	4.2 ± 1.0	0	Normal	165	118

* Data of Glorieux, Clow and others (see Arnaud *et al.*, 1971; Clow *et al.*, 1971*b*; Glorieux *et al.*, 1970). Treatment comprised phosphate salt mixture by mouth supplying 1–3 g Pi/day in 5 divided doses to compensate for loss of Pi in urine and to overcome a transport defect selective for Pi.

** All patients conformed to requirements of X-linked inheritance and exhibited an isolated defect in Pi metabolism.

*** Serum Pi was below 3 mg% in 100% of samples when patients were not treated with phosphate.

† Measured by a radiodensity method by Dr. C. Colbert, Fels Research Institute.

†† Determined by expressing linear growth as: $\frac{\text{cm/yr (patient)} \times 100}{\text{cm/yr at 50th percentile for controls}}$; the normal rate being taken from Harvard growth chart of Harold C. Stewart.

††† Determined by Dr. Anne Roseborough upon heparinized blood specimens obtained when patients were on and off Pi therapy. The oxygen pressure necessary for half saturation of Hgb at pH 7.4 (P_{50}) was determined under conditions similar except for the serum Pi value. Data are:

$$\frac{P_{50} \text{ on phosphate}}{P_{50} \text{ off phosphate}} \times 100.$$

better result than late diagnosis and treatment, especially if the screening program is coupled to an effective team for delivery of the subsequent treatment (Clow *et al.*, 1971*b*).

ii. Product replacement I will cite one disease in detail where replacement of a deficient-metabolite appears to neutralize the effect of a mutant allele. X-linked hypophosphatemia is the classic prototype of 'vitamin D resistant rickets'. We have recently identified that vitamin D 'resistance' in this X-linked disease apparently does not reflect a defect in vitamin D dependent metabolism (Arnaud *et al.*, 1971) but is rather a primary hereditary defect of phosphate transport in kidney and perhaps in other tissue (Glorieux *et al.*, 1971). Therefore, treatment with phosphate salts might correct phosphate depletion and benefit these patients. Serum phosphorus in fact rises dramatically (Table 4) when patients are treated aggressively with oral phosphate supplements every 4 hours, five times daily (Clow *et al.*, 1971*b*; Glorieux *et al.*, 1970). When serum phosphorus is raised in this manner, active rickets is abolished, normal bone density is achieved and, most important, 'catch-up' linear growth is observed (Table 4). The mechanism by which the dwarfism of this disease can be offset is probably complex, but it may involve a rise in the whole blood P_{50} (oxygen pressure at half saturation of hemoglobin) so that oxygen delivery to the peripheral tissues can be enhanced (Table 4). To achieve any success with Pi treatment in this difficult disease is gratifying, but as we have mentioned elsewhere (Clow *et al.*, 1971*b*) it requires a dedicated team to supervise patient care on a day-to-day basis; the implications of this are discussed in the final section and it is for this reason that this problem has been mentioned here in detail.

iii. Coenzyme supplementation Certain types of hereditary metabolic disease can be treated with large amounts of a particular vitamin. The controversial term 'hereditary vitamin dependencies' (Scriver, 1967; Rosenberg, 1970) is of recent origin, but it draws attention to several diseases (Table 5) where a vitamin intake at levels far exceeding the recommended daily requirement (thus the meaning of 'dependency') can correct the attendant metabolic abnormality. The 'hereditary vitamin dependencies' are currently popular because of their intrinsic biology and because they offer opportunities for dramatic treatment.

Figure 7 illustrates two of the four proposed mechanisms for an elevated vitamin requirement:

1. Defective biosynthesis of the active coenzyme form of the vitamin: clinical responsiveness to a high vitamin intake implies that the block in biosynthesis is incomplete (a 'leaky' mutant).
2. Defective binding of the coenzyme by a mutant apoenzyme: clinical responsiveness implies that the mutant binding site can be sufficiently saturated at high concentrations of vitamin to restore apoenzyme activity.

The two remaining mechanisms of vitamin responsiveness are variations on a common theme. Both bring on reduced apoenzyme inactivation, with a resultant increase in holoenzyme activity. It has been suggested that inactivation is an important mode of apoenzyme regulation in diploid cells (Barker *et al.*, 1971). When Mudd and colleagues (1962) studied vitamin B_6 responsive cystathionine-synthase deficiency (homocystinuria), they found that vitamin B_6 increased synthase activity in liver by 2–3%. However, this increase is sufficient to restore homocysteine catabolism and to correct the abnormal biochemical phenotype under normal dietary conditions of methionine intake in this disease. Reduced inactivation of the mutant enzyme, in the presence of saturating levels of coenzyme, was thought to be the mechanism by which enzyme activity was partially restored. The same mechanism of vitamin action can apparently produce a 'shunt' to bypass an inborn error in a metabolic pathway. For example, pyruvate carboxylase deficiency may produce a form of pyruvate accumulation and lactic acidosis which is thiamine responsive (Delvin *et al.*, 1971). Thiamine pyrophosphate is the coenzyme for pyruvate dehydrogenase, a key enzyme for an alternate route of pyruvate meta-

TABLE 5 *The hereditary 'vitamin dependencies'*

Disorder	Year first reported	Enzyme defect	Vitamin	Required dose/day normal patient		Proposed mechanism of defect and response
Megaloblastic anemia	1969	?	B_1	1 mg	20 mg	?
Ataxia	1969	Pyruvate decarboxylase	B_1	1 mg	60 mg	Stabilization?
Lactic acidosis	1971	Pyruvate carboxylase	B_1	1 mg	5–20 mg	Shunt?
Maple syrup urine disease (variant)	1971	Keto-acid decarboxylase	B_1	1 mg	5–20 mg	Stabilization?
Convulsions	1954	Glutamic decarboxylase (?)	B_6	<1 mg	<10–50 mg	Binding?
Cystathioninuria	1963	Cystathionase	B_6	<1 mg	100–400 mg	Binding?
Hypochromic anemia	1956	Δ-Amino levulinic acid synthetase	B_6	<1 mg	10 mg	?
Xanthurenic aciduria	1960	Kynureninase	B_6	<1 mg	5–10 mg	Binding and/or stabilization?
Homocystinuria	1967	Cystathionine synthase	B_6	<1 mg	200–500 mg	Stabilization
Ketosis and methylmalonic aciduria	1968	Methylmalonyl CoA isomerase	B_{12}	1 μg	1000 mg	Biosynthesis
Hypomet'emia with homocystinemia and MMAuria	1970	N^5-THF-methyl transferase	B_{12}	1 μg	?	Biosynthesis
Ketosis with propionic acidemia	1970	Propionyl CoA carboxylase	Biotin	100 μg	5 mg	?
Ketosis and beta-methyl-crotonylglycinuria	1971	Beta-MC CoA carboxylase	Biotin	100 μg	10 mg	?
Vitamin D dependency	1958	Synthesis of Ca transport protein (?)	D_2 D_3	400 units	50,000 to 100,000 units	Biosynthesis or binding

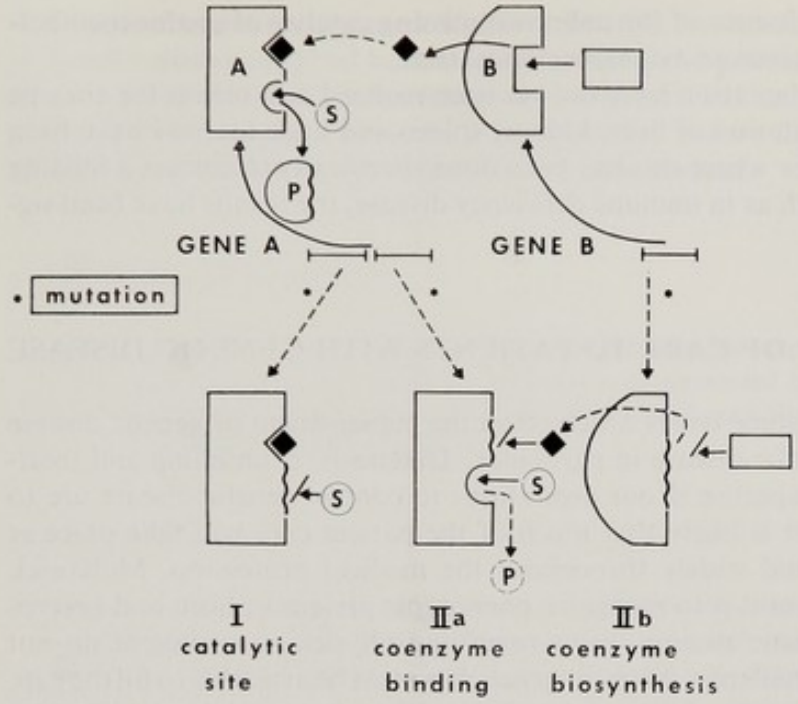


Fig. 7. Mechanisms underlying hereditary vitamin dependency. Gene A codes for apoenzyme A. Gene B codes for apoenzyme B. Enzyme B converts vitamin to coenzyme. Coenzyme then interacts with apoenzyme A to form holoenzyme. Mutation at locus A or B can modify vitamin requirements for normal activity of enzyme A.

bolism. We found that pyruvate dehydrogenase activity was abnormally high in the patient's cells suggesting that thiamine restored pyruvate metabolism by maintaining a 'shunt' for its oxidation.

Table 5 shows that the so-called hereditary vitamin dependencies of man are being discovered at an impressive rate. Our understanding of the relation of vitamin to enzyme activity has benefited from study of these experiments of nature. Equally important, awareness of these phenomena has improved the opportunities for treatment available to some patients with hereditary metabolic disease.

iv. Enzyme replacement, and other approaches Some progress has been made with enzyme replacement. For example, Chang and Poznanski (1968) reported the beneficial but temporary effect of intraperitoneal microcapsules loaded with catalase in an acatalasemic strain of mouse. The same group has designed a cartridge for enzyme-loaded microcapsules which can be used with an arteriovenous shunt; this approach might benefit patients for example with life threatening hyperammonemia due to a defect in the urea cycle, provided the relevant enzymes can be obtained and encapsulated.

A number of interesting studies suggest the potential of enzyme replacement. Neufeld's group (Fratantoni *et al.*, 1969) and Porter *et al.* (1971) have reported *in vitro* correction of mutant biochemical phenotypes in cultured fibroblasts obtained from patients with mucopolysaccharidosis and metachromic leukodystrophy, respectively. However, the transition from these *in vitro* models to treatment of living patients seems to involve formidable obstacles, one of which is the hazard of immune intolerance to the administered enzyme if it is not encapsulated.

Goldman and colleagues (Goldman *et al.*, 1970; Aaron *et al.*, 1971) examined a different approach to modify cystine accumulation in cystinosis. They found that cystinotic fibroblasts exposed to the thiol reducing agent dithiothreitol lost their cystine. This particular

chemical approach to offset the absence of the unknown missing catalyst of cystine metabolism in cystinosis has not yet been given an extensive clinical trial.

The prospects for organ transplantation have not yet been realized as a means for enzyme replacement. Successful transplantation of liver, kidney, spleen and bone marrow have been reported, but the occasions are few where this has been done specifically to correct a missing genetic function. When it has, such as in immune deficiency disease, the results have been significant and promising.

4. SYSTEMS FOR DELIVERY OF CARE TO PATIENTS WITH GENETIC DISEASE

I would like to mention briefly some issues which affect the management of genetic disease in general, and hereditary metabolic disease in particular. Diagnosis, counselling and treatment of genetic disease require expertise if our aspirations to control genetic disease are to find fulfilment. For this reason, it is likely that much of the patient care will take place at centers until this resource is found widely throughout the medical profession. McKusick (1971) has discussed how important it is to recognize phenotypic pleiomorphism and heterogeneity when counselling for genetic disease; most practising physicians at present do not have the experience to meet this challenge. Medical geneticists must be available to fill the gap, and networks of genetic centers are emerging to make a limited resource available to the many who need it.

Genetic centers are essential if the complex treatment programs for hereditary metabolic disease are to be carried out effectively. It is of a little value for a community to establish a screening program if the patient, once found, must spend much of his life in hospital or under arduous surveillance. For these reasons comprehensive systems of genetic disease management have developed which recognize the social and human needs of the patient and family. The recent report by Clow and colleagues (1971*b*) describes one approach using non-medical personnel and emphasizing home care, which has achieved very satisfying results in the management of a dozen different forms of hereditary metabolic disease, including the very difficult problem of X-linked hypophosphatemia (see above). Their program of continuous counselling and treatment in the home reduced the in-hospital admission rate (the expensive component of care) while maintaining a high standard of disease control (*viz.* Fig. 6 and Table 4 for data from this program). The cost of this type of care is not high (Clow *et al.*, 1971*b*).

A further advantage of regionalized programs in genetics is the opportunity they offer to serve large geographic regions. Since patients with hereditary metabolic disease appear at low frequency in the population, their geographic distribution is likely to be widespread in most communities. An effective treatment network must be established if patients are to be effectively managed in their normal social milieu. A description of a regional program serving six million people in Quebec province and adjacent regions is forthcoming (Clow *et al.*, 1971*a*) and the more broadly based effort of the National Genetics Foundation to provide a complete spectrum of diagnostic procedures and follow-up to patients in North America has been described elsewhere at this Congress.

I shall close with some comment about our omissions. Our mutants with hereditary metabolic disease are consumers with special needs (Scriver, 1971) and their reliance on chemical manipulation and environmental control places special emphasis on the subtle qualities of these controls. Yet we have made little or no progress in correcting the poor esthetic quality of their special diets for example. We have not seen much progress in the problems of organ transplantation or in enzyme synthesis and patients who might benefit from advances in these difficult areas are still waiting. Perhaps the gap between theory and practice reflects the feeling expressed all too often that there are so few of 'them' (the mutants) and so many of us, and with many other important health problems on our minds the effort and cost of genetic dis-

ease control seem unwarranted. If that is the case, we should dismantle our screening programs and our beliefs in applied human genetics now, and get on with something better – if we can agree what that is. But if geneticists each took personal action at the level of their own experience on behalf of their patients, I imagine that before long our mutants would find their special needs being met more effectively than at present.

ACKNOWLEDGEMENTS

I am grateful to my wife and Mrs. Huguette Ishmael for help in preparing this paper. The original work in our laboratory described in the paper was supported by grants from the Medical Research Council of Canada, the Department of National Health and Welfare, the Quebec Genetics Network and the National Genetics Foundation.

REFERENCES

AARON, K., GOLDMAN, H. and SCRIVER, C. R. (1971): Cystinosis: new observations. 1. Adolescent (Type III) form. 2. Correction of phenotypes *in vitro* with dithiothreitol. In: *Inherited Disorders of Sulphur Metabolism*. Editors: N. A. J. Carson and D. N. Raine. J. and A. Churchill Ltd., London.

ARNAUD, C., GLORIEUX, F. and SCRIVER, C. R. (1971): Serum parathyroid hormone in X-linked hypophosphatemia. *Science*, *173*, 845.

BARKER, K. L., LEE, K. L. and KERNEY, F. T. (1971): Turnover of tyrosine transaminase in cultured hepatoma cells after inhibition of protein synthesis. *Biochem. biophys. Res. Commun.*, *43*, 1132.

BERMAN, J. L. and FORD, R. (1970): Intelligence quotients and intelligence loss in patients with phenylketonuria and some variant states. *J. Pediat.*, *77*, 764.

CARTER, C. O. (1956): Changing patterns in the causes of death at the Hospital for Sick Children. *Great Ormond Street J.*, *11*, 65.

CHANG, T. M. S. and POZNANSKI, M. J. (1968): Semipermeable microcapsules containing catalase for enzyme replacement in acatalasaemic mice. *Nature* (*Lond.*), *218*, 243.

CHILDS, B. (1967): Genetics and child development. *Amer. J. Dis. Child.*, *114*, 467.

CHILDS, B. (1970): Personal communication.

CLOW, C. L., FRASER, F. C., LABERGE, C. and SCRIVER, C. R. (1971*a*): The medical genetics centre. Role in diagnosis, counselling and treatment. *Progr. med. Genet.*, in press.

CLOW, C. L., READE, T. M. and SCRIVER, C. R. (1971*b*): Management of hereditary metabolic disease. The role of allied health personnel. *New Engl. J. Med.*, *284*, 1292.

CRAIG, J. W. (1968): Present knowledge of nutrition in inborn errors of metabolism. *Nutr. Rev.*, *26*, 161.

DANCIS, J. (1970): Nutritional management of hereditary disorders. *Med. Clin. N. Amer.*, *54*, 1431.

DELVIN, E., SCRIVER, C. R., GAGNAN-BRUNETTE, M. and HAZEL, B. (1971): Mechanism for thiamine responsiveness in pyruvic acidemia due to pyruvate carboxylase deficiency: a proposal. *Proc. Canad. Fed. biol. Sci.*, *14*, 168 (abstr. no. 656).

FRATANTONI, J. C., HALL, C. W. and NEUFELD, E. F. (1969): The defect in Hurler and Hunter syndromes. II. Deficiency of specific factors involved in mucopolysaccharide degradation. *Proc. nat. Acad. Sci. USA*, *64*, 360.

GLORIEUX, F., ARNAUD, C., CLOW, C., GOLDMAN, H., READE, T. and SCRIVER, C. R. (1970): Phosphate therapy for X-linked hypophosphatemic rickets. *Clin. Res.*, *18*, 726.

GLORIEUX, F., SCRIVER, C. R. and ARNAUD, C. (1971): The defect in renal transport of phosphate in X-linked hypophosphatemia. *Proc. Canad. Fed. biol. Sci.*, *14*, 102 (abstr. no. 390).

GOLDMAN, H., SCRIVER, C. R., AARON, K. and PINSKY, L. (1970): Use of dithiothreitol to correct cystine storage in cultured cystinotic fibroblasts. *Lancet*, *1*, 811.

HAY, S. and TONASCIA, S. (1968): *A Classification of Congenital Malformations*. U.S. Department of Health, Education and Welfare, Public Health Service, Dental Health Center, San Francisco, Calif. (For use in coding birth certificates in the national cleft lip and palate intelligence service.)

HOLTZMAN, N. A. (1970): Dietary treatment of inborn errors of metabolism. *Ann. Rev. Med.*, *21*, 335.

HORNING, E. C. and HORNING, M. G. (1971): Human metabolic profiles obtained by GC and GC/MS. *J. Chrom. Sci.*, *9*, 129.

JANEWAY, C. A. (1971): Screening for inherited disease. *New Engl. J. Med., 284*, 787.

MCKUSICK, V. A. (1968): *Mendelian Inheritance in Man. Catalogs of Autosomal Dominant, Autosomal Recessive and X-linked Phenotypes, 2nd ed.*, p. 222. The Johns Hopkins Press, Baltimore, Md.

MCKUSICK, V. A. (1971): The nosology of genetic disease. *Hosp. Pract., 6*, 93.

MENTZER, W. C., LUBIN, B. H. and NATHAN, D. G. (1970): Screening for sickle cell traits and G-6-PD deficiency. *New Engl. J. Med., 282*, 1155.

MILUNSKY, A., LITTLEFIELD, J. W., KANFER, J. N., KOLODNY, E. H., SKIH, V. E. and ATKINS, L. (1970): Prenatal genetic diagnosis. Parts I, II and III. *New Engl. J. Med., 283*, 1370, 1441 and 1498.

MUDD, S. H., EDWARDS, W. A., LOEB, P. M., BROWN, M. S. and LASTER, L. (1970): Homocystinuria due to cystathionine synthase deficiency: the effect of pyridoxine. *J. clin. Invest., 49*, 1762.

O'BRIEN, J. S., OKADA, S., CHEN, A. and FILLERIP, D. L. (1970): Tay-Sachs disease. Deletion of heterozygotes and homozygotes by serum hexoseaminidase assay. *New Engl. J. Med., 283*, 15.

PARTINGTON, M. W. (1968): Case finding a phenylketonuria. III. One-way chromatography of the amino acids in blood. *Canad. med. Ass. J., 99*, 638.

PORTER, M. T., FINHARTY, A. L. and KIHARA, H. (1971): Correction of abnormal cerebroside sulfate metabolism in cultured metachromatic leukodystrophy fibroblasts. *Science, 172*, 1263.

REPORT OF A WHO SCIENTIFIC GROUP (1968): *Screening for Inborn Errors of Metabolism.* WHO Technical Report Series No. 401, p. 5.

ROBERTS, D. F., CHAVEZ, J. and COURT, S. D. M. (1970): The genetic component in child mortailty. *Arch. Dis. Child., 45*, 33.

ROSENBERG, L. E. (1970): Vitamin-dependent genetic disease. *Hosp. Pract., 5*, 59.

SAGINUR, R., CLOW, A. and SCRIVER, C. R. (1970): Unpublished data.

SCRIVER, C. R. (1965): Screening newborns for hereditary metabolic disease. *Med. Clin. N. Amer., 12*, 807.

SCRIVER, C. R. (1967): Treatment in medical genetics. In: *Proceedings, III International Congress of Human Genetics, Chicago, Sept. 1966*, p. 45. Editors: J. F. Crow and J. V. Neel. The Johns Hopkins Press, Baltimore, Md.

SCRIVER, C. R. (1969): Treatment of inherited disease: realized and potential. *Med. Clin. N. Amer., 53*, 941.

SCRIVER, C. R. (1971): Mutants: consumers with special needs. *Nutr. Rev., 29*, 155.

John W. Littlefield, Aubrey Milunsky and Lee B. Jacoby

Genetics Unit, Children's Service, Massachusetts General Hospital, Department of Pediatrics, Harvard Medical School, Boston; and Eunice Kennedy Shriver Center at Walter E. Fernald State School, Waltham, Mass., U. S. A.

Prenatal genetic diagnosis – present and future developments*

It is now possible to diagnose *in utero* almost all chromosome disorders and many inborn errors of metabolism by sampling the amniotic fluid at about 16 weeks of pregnancy, followed by examination of the fluid and fetal cells therein. This procedure constitutes a major advance in medical genetics because for counseling it is much better to know with certainty if a fetus is affected or not, rather than just the statistical risk involved. Further, the frequency of complications of amniocentesis appears so far to be very low, and very few mistakes have been made in determining the sex, karyotype or enzymic constitution of the fetus.

In preparation for this program, we asked a number of the genetic clinics in the USA for their experience with prenatal diagnosis. Table 1 shows that up to this summer, 677 pregnancies were studied in 14 centers, and of course there have been more elsewhere as well. Three-quarters of the cases (509 cases) have involved the question af a chromosome disorder; in Table 2 this group is separated into 3 main indications, familial translocations, pregnancy in an older woman, and previous child with trisomy 21, plus a miscellaneous group. Thus, as

TABLE 1 *Partial listing of US experience with prenatal diagnosis*

Disorders	Total cases	Major centers	
Chromosomal	509	Bloom, A. D.	Ann Arbor
		Epstein, C.	San Francisco
Sex-linked	49	Hirschhorn, K.	New York
		Hug, G.	Cincinnati
Metabolic	119	McIntyre, N.	Cleveland
		Milunsky, A.	Boston
		Nadler, H. L.	Chicago
		New, M.	New York
		O'Brien, J. S.	San Diego
		Rattazzi, M.	Buffalo
		Robinson, A.	Denver
		Rosenberg, L. E.	New Haven
		Scott, R.	Seattle
		Volk, B. W.	Brooklyn
	677		

* Studies mentioned in this article were supported by U.S.P.H.S. grants AM 13655 and HD 05515 and a U.S.P.H. S. research fellowship GM 32919.

TABLE 2 *Prenatal diagnosis for chromosomal disorders*

Indication	Cases	Affected fetuses	Aborted	Diagnosis confirmed	Delivered
Translocation carrier	50	9	9	9	39
Maternal age 35 years	234	6	5	5	107*
Previous trisomy 21	182	3	2	1	103**
Miscellaneous	43	1	1	–	31***
	509	19	17	15	280

* Spontaneous abortion; ** Stillborn; *** Therapeutic abortion.

would have been predicted, the most common indication has been older maternal age (234 cases); we have estimated (Milunsky *et al.*, 1970) that the woman pregnant at age 40 or older runs a 1 in 40 chance that her offspring will have a serious chromosome disorder such as trisomy 13, 18 or 21, XXY or XXX. So far, the risk of amniocentesis at 16 weeks appears so low that we feel all women pregnant at 40 or older should be offered this test, and perhaps those over age 35 as well. Indeed since serious chromosome disorders occur overall at a frequency of 1 in 200 live infants, it may become wise to offer amniocentesis to *all* pregnant women when and if data are obtained establishing more definitely the low frequency of immediate and/or long-term complications of amniocentesis. Note in Table 2 that for 1 of the 6 affected fetuses in this group, the parents did not wish the pregnancy terminated. This may represent a change of mind, as many centers feel that amniocentesis should not be done unless parents are prepared to take appropriate action. Other geneticists feel differently on this point, however.

Three fetuses with trisomy 21 were discovered in the 182 pregnancies in women who had previously had an offspring with the same disorder. Probably the main reason for amniocentesis in this group had been to allay anxiety in the mother, but this relatively high frequency is an added indication if confirmed in a larger series of cases. Again, one family decided against termination. In this and the miscellaneous group, one abortus was unfortunately not examined in order to confirm the diagnosis, but otherwise all diagnoses were correct, including the normal infants delivered to date. This was true also for the translocation carrier group of 50 amniocenteses and 9 affected fetuses. These latter families are, of course, particularly appropriate for amniocentesis, and we feel strongly that geneticists should search out potential translocation carriers to advise them. Of course, it should be widely appreciated that prenatal diagnosis is possible only if both chromosomes participating in the translocation can be identified. The new chromosome stains may help in this regard.

Another caution concerning the prenatal diagnosis of chromosome disorders is the frequent occurrence of tetraploidy in amniotic fluid cell cultures (Milunsky *et al.*, 1971), as shown in Table 3, which includes our recent experience. Skin fibroblast cultures usually contain about 3% tetraploid cells. The surprisingly high frequency in Table 3 perhaps indicates that some of cells derive from the amnion, since tetraploidy is common in this organ.

Forty-nine amniocenteses were done for X-linked disorders, with abortion of 20 male fetuses out of 27, and confirmation of male sex in the 19 abortions examined. One hundred and nineteen amniocenteses were done for 21 different inherited metabolic disorders, especially Tay-Sachs disease (52 cases), Pompe's disease (24 cases), the mucopolysaccharidoses (11 cases) and maple syrup urine disease (6 cases). Thirty-three affected fetuses were diagnosed, 27 aborted, with one error recognized (a misdiagnosis of Tay-Sachs).

The list of inherited metabolic disorders diagnosed or potentially diagnosable *in utero* con-

TABLE 3 *Tetraploidy in cultured amniotic fluid cells*

Cases	Per cent tetraploid cells
22	less than 10
19	10–25
12	25–50
3	50–75
0	75–100

tinues to grow, as shown in Table 4. Recent additions include fucosidosis, mannosidosis, and more of the glycogen storage diseases, various gangliosidoses, and several new amino acid disorders. All but 2 of these disorders (Morquio's and Chediak-Higashi syndromes) are evidenced by the deficiency of a specific enzyme or another specific biochemical feature. We have emphasized elsewhere the importance for these tests of choosing as controls cultured amniotic fluid cells of comparable gestational age, total age in culture, and position in the lag-log-stationary growth cycle (Littlefield, 1971). Cystic fibrosis is still conspicuously absent from Table 4, but will probably be added in a year or so.

Prenatal diagnosis has been reviewed repeatedly in recent months (Nadler and Gerbie, 1970; Emery, 1970; Milunsky *et al.*, 1970; Nadler, 1971; Milunsky and Littlefield, 1972) and we want to concentrate here upon only a few developments.

First for the inherited metabolic disorders, there is a great need for miniaturized enzyme or kinetic assays to shorten the time required for prenatal diagnosis. At the invitation of Dr. R. S. Wilroy, we were recently given the opportunity to diagnose the genotype of a fetus in a family at risk for recurrence of argininosuccinic aciduria. This time the amniotic fluid cells grew slowly, and it was only after 7 weeks in culture, as the cells were becoming senescent, that we had enough for Dr. V. Shih to carry out her argininosuccinase microassay, which established that the fetus was unaffected. It seemed likely that an earlier diagnosis might have been obtained if the cells were exposed to C^{14}-citrulline, which would be converted to C^{14}-argininosuccinic acid and in cells containing argininosuccinase to C^{14}-arginine for incorpora-

TABLE 4 *Inherited metabolic disorders diagnosable prenatally*

Acatalasemia
Argininosuccinic aciduria
Chediak-Higashi syndrome
Citrullinemia
Congenital erythropoietic porphyria
Cystinosis
Fabry's disease
Fucosidosis
Galactosemia
Gaucher's disease
Glucose-6-PO_4 dehydrogenase deficiency
Glycogen storage diseases (Types 2, 3 and 4)
Gm_1 gangliosidoses (Types 1 and 2)
Gm_2 gangliosidoses (Types 1, 2 and 3)
Homocystinuria
Hyperlysinemia
Hypervalinemia
I-cell disease
Ketotic hyperglycinemia
Krabbe's disease
Lesch-Nyhan syndrome
Lysosomal acid phosphatase deficiency
Mannosidosis
Maple syrup urine disease
Metachromatic leukodystrophy
Methylmalonic aciduria
Mucopolysaccharidoses (Types 1, 2, 3, 5, and 6)
Niemann-Pick disease
Ornithine-α-keto acid transaminase deficiency
Orotic aciduria
Pyruvate decarboxylase deficiency
Refsum's disease
Xeroderma pigmentosum

tion into protein. Therefore, skin fibroblasts from a normal control, a patient with citrullinemia (Tedesco and Mellman, 1967) and a patient with argininosuccinic aciduria were exposed to C^{14}-citrulline in arginine-deficient medium for 24 hours, and then the counts in protein determined on each culture. H^3-leucine was included too, so that the results could be expressed as the ratio of the incorporation of C^{14}-citrulline/H^3-leucine, if variation in growth rate occurred between the cultures. The results are shown in Figure 1. The incorporation of H^3-leucine was linear over the 24-hour period and similar in all the cultures, although other experiments showed that the growth of the cells from the patients with citrullinemia and argininosuccinic aciduria subsequently slowed in arginine-deficient medium. C^{14}-citrulline incorporation paralleled that of H^3-leucine in the control culture, but did not occur at all in the cells from the patient with citrullinemia and only gradually in the cells from the patient with argininosuccinic aciduria. In other experiments similar 'leakiness', as expected

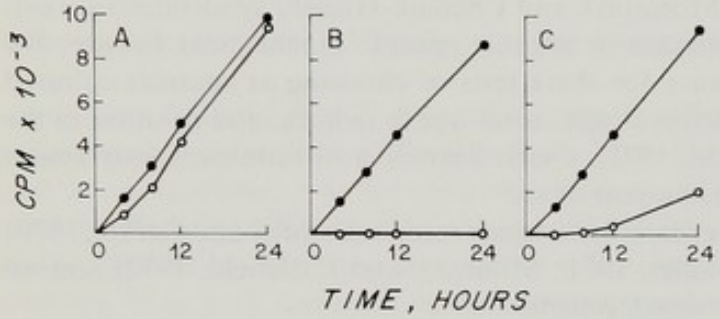


Fig. 1. Incorporation of H^3-leucine (•) and C^{14}-citrulline (o) into trichloroacetic acid-precipitable material in cells from a normal control (A), a patient with citrullinemia (B), and a patient with argininosuccinic aciduria (C). One hundred thousand cells in Eagle's medium were inoculated into 30 mm plastic Petri dishes containing 22 mm cover slips. Forty-eight hours later when the cells were growing exponentially, they were refed with arginine-deficient medium containing the radioisotopes. After 4 or more hours of incubation at 37° C, cover slips were washed in saline and several times with cold acid, dried and counted. All assays were performed in duplicate, and iced controls subtracted. (From Jacoby *et al.*, 1972.)

clinically and theoretically, occurred in cells from patients in 3 other kindreds with argininosuccinic aciduria. Several heterozygotes gave nonoverlapping intermediate values. Next, with a 4-hour incubation period for maximal discrimination, we examined amniotic fluid cells cultured from 3 pregnancies terminated for various reasons, and from the fetus in question. The average of 2 assays on the latter cells was 59% of the average of the control values, indicating the fetus to be a heterozygote (Jacoby *et al.*, 1972). This was confirmed subsequently by argininosuccinase assay of cord blood. Of course, considerably more experience with this technique is needed, but it requires only a total of 10^5 cells in contrast to 10^7 needed for the enzyme microassay, and it should be possible about 2 weeks earlier. The same assay could be used for the prenatal diagnosis of citrullinemia. Similar miniature assays would be most helpful for other inborn errors.

But probably in the long run the best method to screen for many metabolic diseases at once will not be multiple individual enzyme or kinetic microanalyses as this one, but rather a simultaneous analysis of the concentrations of many metabolites. Automated technology to determine this sort of profile of body fluid or cell constituents is now becoming available. Probably these analyses would best be done not on amniotic fluid itself but on cultured amniotic fluid cells, which are removed from the correcting influence of the mother.

Next, we want to discuss for a moment the status of prenatal diagnosis for 3 common hematological disorders, sickle cell anemia, hemophilia, and glucose-6-phosphate dehydrogenase deficiency. Only the latter can now be diagnosed directly in cultured amniotic fluid

cells, and generally this deficiency is not serious enough to warrant amniocentesis. In regard to hemophilia, human skin fibroblasts contain a clotting factor similar if not identical to Factor VIII, fibroblasts cultured from hemophiliacs contain an equal amount of the same factor, and so do cultured amniotic fluid cells (Zacharski *et al.*,1969; Green *et al.*, 1971; Milunsky and Colman, unpublished). Therefore, it seems unlikely that it will be possible to distinguish *in utero* between an affected and a normal male fetus. However, the locus for Factor VIII is closely linked on the X-chromosome to that for glucose-6-phosphate dehydrogenase. It has recently been emphasized by several geneticists that the prenatal diagnosis of hemophilia is now possible with a high degree of accuracy for women who are heterozygous at the dehydrogenase locus and who have had one son with hemophilia or are known to be carriers of hemophilia from other linkage relationships.

It seems quite likely that the prenatal diagnosis of sickle cell anemia will become practical within the next year or so. Hollenberg *et al.* (1971) have now confirmed earlier reports of the presence of measurable amounts of adult hemoglobin in the peripheral blood of 9- to 18-week human fetuses, and have focused on this hemoglobin by examining its rate of synthesis and by blocking the synthesis of fetal hemoglobin. The technique is so sensitive that a fetus with sickle cell trait could be distinguishd from one with sickle cell anemia with only 10 microliters of fetal blood. To obtain this amount of blood from a 16-week fetus may well be possible from the placenta, which can be localized at this point of pregnancy through ultrasound (Miller, 1971). Since about 1 in 500 black infants in the USA has sickle cell anemia, the ability to diagnose this disorder *in utero* would markedly stimulate the initiation of sickle trait screening programs, as well as put a strain on our current facilities for prenatal diagnosis.

Looking further into the future, one wonders if it will ever be possible to diagnose *in utero* serious autosomal dominant conditions such as neurofibromatosis, tuberous sclerosis, retinoblastoma, and Huntington's chorea. As with hemophilia the discovery of close linkage to markers manifest in amniotic fluid cells would be of great help (Renwick, 1969). More precise would be detection of the effect of the gene itself, but what would one look for in an amniotic cell? Perhaps these cells could be coupled somehow to differentiated 'indicator' cells, or alternatively to cells which already manifest cytopathology characteristic of the disorder. Thus, it is possible that amniotic fluid cells from a fetus affected with a dominant disorder such as retinoblastoma might cause neoplastic growth of normal retinal cells in culture, or conversely fail to suppress the neoplastic growth of retinoblastoma cells. This effect might occur if the two cells were in contact, or might require cell fusion. Possibly the best indicator in some instances would actually be a whole mouse embryo. In this case the human amniotic fluid cell could be fused with a fertilized mouse egg (Graham, 1969), or injected into a mouse blastocyst (Gardner, 1968). Then the egg or blastocyst would be reimplanted, and after a few weeks' further development, the embryonic or newborn mouse examined for some sort of characteristic histopathology.

It would be unfair to end on this optimistic and speculative note without emphasizing that there are a number of problems with prenatal diagnosis at this moment. These include ethical considerations about abortion, societal issues and legal concerns, as well as genetic aspects such as effective methods for counseling, and the population genetic effects of prenatal diagnosis. It is essential to define as precisely as possible the immediate and long-term risks of amniocentesis to the fetus and mother, before we can begin to agree on the indications for this procedure; fortunately, the United States Public Health Service has now established prenatal diagnosis registries in several genetic clinics to collect this sort of data. Hopefully the risks can be lessened, assays improved, and more disorders detected, as mentioned earlier.

ACKNOWLEDGEMENTS

We are grateful to the individuals listed in Table 1 for the information which they supplied

REFERENCES

EMERY, A. E. H. (1970): Antenatal diagnosis of genetic disease. In: *Modern Trends in Human Genetics, Vol. I*, Chapter 9, pp. 267–296. Editor: A. E. H. Emery. Appleton-Century-Crofts, London.

GARDNER, R. L. (1968): Mouse chimeras obtained by the injection of cells into the blastocyst. *Nature (Lond.), 220*, 596.

GRAHAM, C. F. (1969): The fusion of cells with one- and two-cell mouse embryos. In: *Heterospecific Genome Interaction, Wistar Institute Symposium Monograph No. 9*, pp. 19–35. Editor: V. Defendi. The Wistar Institute Press, Philadelphia, Pa.

GREEN, D., RYAN, C., MALANDRUCCOLO, N. and NADLER, H. L. (1971): Characterization of the coagulant activity of cultured human fibroblasts. *Blood, 37*, 47.

HOLLENBERG, M. D., KABACK, M. M. and KAZAZIAN, H. H., Jr (1971): Synthesis of adult hemoglobin by reticulocytes from the human fetus at midtrimester. *Science*, in press.

JACOBY, L. B., LITTLEFIELD, J. W., MILUNSKY, A., SHIH, V. E. and WILROY, R. S., Jr (1972): A microassay for argininosuccinase in cultured cells. *Amer. J. hum. Genet., 24*, 321.

LITTLEFIELD, J. W. (1971): Problems in the use of cultured amniotic fluid cells for biochemical diagnosis. In: *Intrauterine Diagnosis*; *Birth Defects*: *Original Article Series, Vol. VII, No. 5*, pp. 15–17. Editors: D. Bergsma and A. Motulsky. The National Foundation, New York, N. Y.

MILLER, D. J. (1971): Discussion of symposium papers. In: *Intrauterine Diagnosis; Birth Defects: Original Article Series, Vol. VII, No. 5*, pp. 33–34. Editors: D. Bergsma and A. Motulsky. The National Foundation, New York, N. Y.

MILUNSKY. A., ATKINS, L. and LITTLEFIELD, J. W. (1971): Polyploidy in prenatal genetic diagnosis. *J. Pediat., 79*, 303.

MILUNSKY, A. and COLMAN, R. W.: Unpublished observations.

MILUNSKY, A. and LITTLEFIELD, J. W. (1972): The prenatal diagnosis of the inborn errors of metabolism. *Ann. Rev. Med., 23*, 57.

MILUNSKY, A., LITTLEFIELD, J. W., KANFER, J. N., KOLODNY, E. H., SHIH, V. E. and ATKINS, L. (1970): Prenatal genetic diagnosis. *New Engl. J. Med., 283*, 1370, 1441, and 1498.

NADLER, H. L. (1971): Indications for amniocentesis in the early prenatal detection of genetic disorders. In: *Intrauterine Diagnosis; Birth Defects: Original Article Series, Vol. VII, No. 5*, pp. 5–9. Editors: D. Bergsma and A. Motulsky. The National Foundation, New York, N. Y.

NADLER, H. L. and GERBIE, A. B. (1970): Role of amniocentesis in the intrauterine detection of genetic disorders. *New Engl. J. Med., 282*, 596.

RENWICK, J. H. (1969): Widening the scope of antenatal diagnosis. *Lancet, 2*, 386.

TEDESCO, T. A. and MELLMAN, W. J. (1967): Argininosuccinate synthetase activity and citrulline metabolism in cells cultured from a citrullinemic subject. *Proc. nat. Acad. Sci. USA, 57*, 829.

ZACHARSKI, L. R., BOWIE, E. J. W., TITUS, J. L. and OWEN, C. A., Jr (1969): Cell-culture synthesis of a Factor VIII-like activity. *Mayo Clin. Proc., 44*, 784.

Elizabeth F. Neufeld, Hans M. Kresse, Clara W. Hall, Michael J. Cantz** and Robert W. Barton****

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md., U. S. A.

The mucopolysaccharidoses – an illustration of genetic heterogeneity and prospects for therapy

When this symposium was being organized, the second part of the title of this paper seemed an optimistic projection into the future. In the intervening time, treatment by plasma infusion or by transfusion of lymphocytes has been reported (DiFerrante *et al.*, 1971; Knudson *et al.*, 1971) and is being tested in a number of hospitals.

These recent attempts at therapy are based on findings made in cell culture. Fibroblasts grown from the skin of patients affected with a mucopolysaccharidosis accumulate excessive amounts of sulfated mucopolysaccharide, primarily dermatan sulfate (Danes and Bearn, 1966; Matalon and Dorfman, 1969). It was subsequently shown by exposing the cells to $^{35}SO_4$ and following the fate of the radioactive mucopolysaccharide formed, that the accumulation is the result of impaired degradation (Fratantoni *et al.*, 1968).

The impaired degradation, in turn, is due to the absence of specific protein factors. The factors can be supplied exogenously, and are detected by the 'correction' of abnormal mucopolysaccharide metabolism of the deficient fibroblasts – that is, by the reduction in the accumulation and shortening of the turnover time of mucopolysaccharide-$^{35}SO_4$. If sufficient factor is supplied, the patient's fibroblasts are corrected fully and can no longer be distinguished from normal cells. The factors can be found in fibroblast secretions, in the fibroblasts themselves, and in urine – provided the donor of fibroblasts or urine does not have the same genetic mucopolysaccharidosis as the cells being tested (Neufeld and Cantz, 1971). For example, the accumulation of mucopolysaccharide-$^{35}SO_4$ in Hunter fibroblasts is markedly reduced by secretions from normal fibroblasts, and from fibroblasts of patients with other mucopolysaccharidoses or unrelated genetic disorders, but not by secretions from fibroblasts of other Hunter patients (Fratantoni *et al.*, 1969). When the factor which corrects Hunter cells is purified, it is found to be quite specific, and to have no effect on cells from patients of other mucopolysaccharidoses or on normal cells (Cantz *et al.*, 1972).

By pooling data obtained in cross-correction tests and in the determination of specificity of purified factors, we arrived at a biochemical classification of the mucopolysaccharidoses

* Present address: Physiologisch-chemisches Institut, University of Münster, Münster, Federal Republic of Germany.

** Present address: Kinderklinik der Christian-Albrechts Universität, Kiel, Federal Republic of Germany.

*** Present address: Department of Medicine, Washington University School of Medicine, St. Louis, Mo. 63110, U. S. A.

TABLE 1 *Biochemical classification of the mucopolysaccharidoses*

Mucopolysaccharidosis*	Deficiency	Reference
I, Hurler	Specific protein	Barton and Neufeld, 1971*a*
II, Hunter	Specific protein	Cantz *et al.*, 1970, 1972
III, Sanfilippo	Specific protein A or specific protein B	Kresse *et al.*, 1971 Kresse and Neufeld, 1972
IV, Morquio	Not studied	
V, Scheie	Same as Hurler	Wiesmann and Neufeld, 1970 Barton and Neufeld, 1971*a*
VI, Maroteaux-Lamy	Specific protein	Barton and Neufeld, 1971*b*

* Clinical classification of McKusick (1966).

(Table 1) which corresponds very well to the clinical classification developed by Maroteaux and Lamy (1965) and by McKusick (1966). Two differences are seen, however. The Sanfilippo syndrome is biochemically heterogeneous in that fibroblasts from some patients are deficient in one protein factor and some in another; we have arbitrarily designated these as A and B. On the other hand, the clinically very different Hurler and Scheie syndromes share a deficiency of the same protein. To understand these observations, it is essential to consider the nature and function of the corrective factors.

Three factors, thus far, have been extensively purified: Hurler, Hunter and Sanfilippo A. These are heat-labile molecules, optimally stable at a pH somewhat below neutrality, anionic above pH 6 and cationic below pH 4. There is no doubt that they are proteins. When supplied to deficient fibroblasts, they accelerate mucopolysaccharide-$^{35}SO_4$ degradation in the recipient cells. As an example, Table 2 shows the stimulatory effect of Hunter factor on the degradation of radioactive proteodermatan sulfate pinocytosed by Hunter cells (Cantz *et al.*, 1972). Normal cells, which already have adequate amounts of Hunter factor, are not affected by the addition.

The degradative function of the factors suggests that they are lysosomal enzymes. (We assume, though this has yet to be rigorously proved, that in fibroblasts the mucopolysaccharide is stored in lysosomes, as occurs in other tissues (Van Hoof and Hers, 1964; Wallace *et al.*, 1966), and that the factors are delivered to the substrate by pinocytosis.)

The factors cannot be identified with the known lysosomal enzymes that are testable with chromogenic substrates. We stress that the three purified factors have no β-galactosidase activity, in view of numerous proposals of β-galactosidase deficiency as the primary defect in the mucopolysaccharidoses (Van Hoof and Hers, 1968; Öckerman, 1968; Gerich, 1969; Mac-

TABLE 2 *Effect of Hunter factor on mucopolysaccharide degradation by fibroblasts*

Cells	Factor addition	Degradation (%)
Hunter	−	13
	+	76
Normal	+ or −	75

Both mucopolysaccharide-$^{35}SO_4$ (proteodermatan sulfate) and factor were supplied to the cells for 24 hours. Degradation is expressed as the percentage of the mucopolysaccharide taken in by pinocytosis which becomes degraded.

Brinn *et al.*, 1969). It is unfortunate that the normal catabolic pathways for dermatan sulfate and heparan sulfate (the substances accumulated in the mucopolysaccharidoses) are not known, for it is the enzymes on those pathways that are relevant to this problem.

We have reasoned that if the factors are degradative enzymes, then the materials which accumulate in their absence should be the substrates. We therefore mixed purified factors with the mucopolysaccharide-$^{35}SO_4$ prepared from the deficient cells. This approach was successful in the case of the Sanfilippo A factor: preliminary experiments implicate that factor as a novel kind of sulfatase, which acts on the mucopolysaccharide fragments (heparan sulfate) accumulated by Sanfilippo A cells (Kresse and Neufeld, 1972).

In the case of the Hurler and Hunter factors, we did not observe factor-dependent release of sulfate, and postulate that these factors may be glycosidases rather than sulfatases. We are now attempting to prepare mucopolysaccharides labeled in the carbohydrate chain in order to test that possibility.

Findings in a case of an atypical mucopolysaccharidosis (a child with roentgenographic signs of the Hurler syndrome but no psychomotor retardation) encouraged us in the belief that we are dealing with lysosomal enzymes (Quinton *et al.*, 1971). Fibroblasts from this patient are deficient in β-glucuronidase, and show abnormality in mucopolysaccharide metabolism similar to that found in the classified mucopolysaccharidoses. Exogenously added β-glucuronidase functions as a corrective factor in these fibroblasts (Hall *et al.*, 1971).

The concept of the corrective factors as enzymes in a catabolic pathway of dermatan sulfate and heparan sulfate metabolism allows us to consider the question of heterogeneity. One possible reason for heterogeneity is that the factors may be sequential enzymes in a multienzyme pathway. This would be analogous to the heterogeneity one finds in the glycogen storage diseases or in the glycolipidoses. We think that such is the difference between mucopolysaccharidoses I, II, III A and B, and VI, for the factor proteins deficient in these various disorders are all different from each other. Clinical consequences of different blocks in a pathway may or may not be different, depending on the metabolic effects of the products that accumulate.

Another level of heterogeneity would be due to different mutations at the same locus, resulting in different alterations of the same polypeptide chain. The many mutational changes of hemoglobin or glucose-6-phosphate dehydrogenase are well-known examples of this phenomenon. By analogy, we can anticipate heterogeneity within the biochemical classes delineated in Table 1, when we have the opportunity to look at the factors at a molecular level (*e.g.*, with antibodies and amino acid sequencing). We suspect that the difference between the clinically mild Scheie syndrome and the clinically severe Hurler syndrome is due to a different modification of the same protein. The Scheie mutation would be such as to leave enough residual factor activity *in vivo* for good intelligence and longevity.

There are many variations possible on this general theme, such as alteration of different subunits of one enzyme. One can even predict that if a mutation merely reduces the catalytic activity of a factor, addition of large quantities of this defective factor to the cells from which it originated would cause correction.

Suggestion of the variety that we are likely to encounter is evident from the observations of Danes and Bearn, who found cross-correction between some Hunter lines and between some Scheie lines (Danes and Bearn, 1971).

We very much hope that correction by factors is not limited to fibroblasts, but can be applied to the patient. Introduction of an enzyme (particularly of a lysosomal enzyme) to replace that absent or inactive because of mutation, is not a new idea. It has been specifically enunciated by Hers and colleagues (Baudhuin *et al.*, 1964) when they attempted to introduce α-amylase into the liver of a patient with Pompe's disease. Production of sufficient quantities of purified factors for therapeutic trials is primarily a question of effort; we foresee no major technical obstacles, though there may be some economic ones.

But the patients may not have to wait for our preparations of pure proteins. DiFerrante *et*

al. (1971) have been infusing large amounts of plasma into Hurler and Hunter patients with promising results. We are not convinced that the beneficial effects are due to the corrective factor content of plasma, which we find to be very low. It is possible that other enzymes in plasma, such as hyaluronidase, help to purge the mucopolysaccharide stores by alternative routes.

Transfusion of lymphocytes has been tested, also with promising clinical results, on the assumption that these cells produce corrective factor (Knudson *et al.*, 1971). Yet other approaches have been suggested, such as tissue transplantation, or activation of the defective factor. Though these proposals may not be currently realizable, we can anticipate that with work and ingenuity, ways will be found to undo some of the damage of the mutations.

Note added in proof

The factor deficient in the Hurler and Scheie syndromes has been identified as the enzyme α-L-iduronidase (Bach *et al.*, 1972; Matalon and Dorfman, 1972), and the Sanfilippo B factor as the enzyme N-acetyl-α-D-glucosaminidase (O'Brien, 1972; Von Figura and Kresse, 1972).

REFERENCES

Bach, G., Friedman, R., Weissmann, B. and Neufeld, E. F. (1972): *Proc. Nat. Acad. Sci. USA*, in press.

Barton, R. W. and Neufeld, E. F. (1971*a*): The Hurler corrective factor. Purification and preliminary studies. *J. biol. Chem., 246/24*, 7773.

Barton, R. W. and Neufeld, E. F. (1971*b*): A distinct biochemical deficit in the Maroteaux-Lamy syndrome (mucopolysaccharidosis VI). *J. Pediat., 80/1*, 114.

Baudhuin, P., Hers, H. G. and Loeb, H. (1964): An electron microscopic and biochemical study of type II glycogenosis. *Lab. Invest., 13/9*, 1139.

Cantz, M., Chrambach, A. and Neufeld, E. F. (1970): Characterization of the factor deficient in the Hunter syndrome by polyacrylamide gel electrophoresis. *Biochem. biophys. Res. Commun., 39/5*, 936.

Cantz, M., Chrambach, A., Bach, G. and Neufeld, E. F. (1972): The Hunter corrective factor. Purification and preliminary characterization. *J. biol. Chem.*, in press.

Danes, B. S. and Bearn, A. G. (1966): Hurler's syndrome, a genetic study in cell culture. *J. exp. Med., 123/1*, 1.

Danes, B. S. and Bearn, A. G. (1971): Correction of cellular metachromasia in cultured fibroblasts in several inherited mucopolysaccharidoses. *Proc. nat. Acad. Sci. USA, 67/1*, 357.

DiFerrante, N., Nichols, B. L., Donnelly, P. V., Neri, G., Hrgovcic, R. and Berglund, R. K. (1971): Induced degradation of glycosaminoglycans in Hurler's and Hunter's syndromes by plasma infusion. *Proc. nat. Acad. Sci. USA, 68/2*, 303.

Fratantoni, J. C., Hall, C. W. and Neufeld, E. F. (1968): The defect in Hurler's and Hunter's syndromes: faulty degradation of mucopolysaccharide. *Proc. nat. Acad. Sci. USA, 60/2*, 699.

Fratantoni, J. C., Hall, C. W. and Neufeld, E. F. (1969): The defect in Hurler and Hunter syndromes. II. Deficiency of specific factors involved in mucopolysaccharide degradation. *Proc. nat. Acad. Sci. USA, 64/1*, 360.

Gerich, E. (1969): Hunter's syndrome: β-galactosidase deficiency in skin. *New Engl. J. Med., 280/15*, 799.

Hall, C. W., Cantz, M. and Neufeld, E. F. (1971): β-Glucuronidase as a corrective factor in fibroblasts from an atypical mucopolysaccharidosis patient. In preparation.

Knudson, A. G., Jr, DiFerrante, N. and Curtis, J. E. (1971): Effect of leukocyte transfusion in a child with type II mucopolysaccharidosis. *Proc. nat. Acad. Sci. USA, 68/8*, 1738.

Kresse, H. and Neufeld, E. F. (1972): The Sanfilippo A corrective factor. Purification and mode of action. *J. biol. Chem., 247/7*, 2164.

Kresse, H., Wiesmann, U., Cantz, M., Hall, C. W. and Neufeld, E. F. (1971): Biochemical heterogeneity of the Sanfilippo syndrome: preliminary characterization of two deficient factors. *Biochem. biophys. Res. Commun., 42/5*, 892.

MacBrinn, M., Okada, S., Woollacott, M., Patel, V., Ho, M. W., Tappel, A. L. and O'Brien, J. S. (1969): β-Galactosidase deficiency in the Hurler syndrome. *New Engl. J. Med., 281/7*, 338.

Maroteaux, P. and Lamy, M. (1965): Hurler's disease, Hunter's disease, and related mucopolysaccharidoses. *J. Pediat., 67/2*, 312.

Matalon, R. and Dorfman, A. (1969): Acid mucopolysaccharides in cultured human fibroblasts. *Lancet, II*, 838.

Matalon, R. and Dorfman, A. (1972): *Biochem. biophys. Res. Commun., 47/4*, 959.

McKusick, V. (1966): The mucopolysaccharidoses. In: *Heritable Disorders of Connective Tissue, 3rd ed.*, Chapter 9, pp. 325–399. C. V. Mosby, St. Louis, Mo.

Neufeld, E. F. and Cantz, M. J. (1971): Corrective factors for inborn errors of mucopolysaccharide metabolism. *Ann. N. Y. Acad. Sci., 179*, 580.

O'Brien, J. S. (1972): *Proc. Nat. Acad. Sci. USA*, in press.

Öckerman, P. A. (1968): Acid hydrolases in skin and plasma in gargoylism. Deficiency of β-galactosidase in skin. *Clin. chim. Acta, 20/1*, 1.

Quinton, B. A., Sly, W. S., McAlister, W. H., Rimoin, D. L., Hall, C. W. and Neufeld, E. F. (1971): β-Glucuronidase deficiency: a new mucopolysaccharide storage disease. In: *Abstracts of Pediatric Meetings, Atlantic City, N. J.*, p. 198. American Pediatric Society, Inc.

Van Hoof, F. and Hers, H. G. (1964): L'ultrastructure des cellules hépatiques dans la maladie de Hurler (gargoylisme). *C. R. Acad. Sci. (Paris), 259/5*, 1281.

Van Hoof, F. and Hers, H. G. (1968): The abnormalities of lysosomal enzymes in mucopolysaccharidoses. *Europ. J. Biochem., 7/1*, 34.

VonFigura, K. and Kresse, H. (1972): *Biochem. biophys. Res. Commun.*, in press.

Wallace, B. J., Kaplan, D., Adachi, M., Schneck, L. and Volk, B. W. (1966): Mucopolysaccharidosis type III. *Arch. Path., 82/5*, 462.

Wiesmann, U. and Neufeld, E. F. (1970): Scheie and Hurler syndromes: apparent identity of the biochemical defect. *Science, 169/3940*, 72.

Stanfield Rogers

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A.

Genetic engineering*

Many of the metabolic deficiency diseases of man are caused by significant alterations in the structures of single genes. The result of such an alteration may be either a relatively ineffective gene product, such as a specific enzyme, or in some instances the complete loss of a gene product. Either can produce a variety of abnormalities. Since the discovery of transformation made by Avery (Avery *et al.*, 1944) scientists have wondered whether specific genetic information can be transferred in animals, including man; and this speculation has been increased greatly by the discovery of transduction (Morse *et al.*, 1956).

It has been known for some years that viruses are simply tiny pieces of genetic information (RNA or DNA) covered by a protein coat, and that some viruses act as passengers, producing no cytopathic effects in the cells they inhabit (Rivers and Pearce, 1925). We have been working with one such passenger virus, the Shope rabbit papilloma virus, which produces warts upon infection of the squamous epithelium of the skin of rabbits. Several years ago we were fortunate in discovering that the Shope virus induces the synthesis of an arginase (Rogers, 1959). The squamous epithelium of the rabbit, in contrast to that of most other animals, contains no arginase, so the enzyme is new to the virus-infected tissue. At once we became interested in determining whether the induced arginase is synthesized from viral genetic information or induced from information contained in the rabbit cell genome.

Purified virus-induced arginase was compared with arginase from normal rabbit liver and found to differ in several chemical and physical parameters: molecular weight, buoyant density, sedimentation velocity, and antigenicity (Rogers and Moore, 1963). In relation to the latter, it is noteworthy that virus-induced arginase purified from an individual rabbit tumor acts as an antigen when it is tested against the same rabbit's serum, indicating that the rabbit reacts to the enzyme as to a foreign protein. This does not prove, however, that the enzyme is derived from virus information, as the virus may simply unmask a normally unexpressed arginase of the rabbit. As another approach to the problem, the amino acid compositions of the two purified arginases were investigated, and it was found that there is a much larger percentage of basic amino acids in normal rabbit liver arginase than in the virus-induced enzyme.

A more direct approach to the question of whether the enzyme is synthesized directly from virus information was provided in 1954, when Dr. Richard Shope discovered a mutant line of the Shope papilloma virus (Selbie *et al.*, 1948). This mutation is associated with the ability of the virus to be recovered from tumors and passed sequentially in domestic rabbits, an operation that is impossible with the wild-type virus line. Should the mutation also involve a change in the structure of the induced enzyme, it would provide direct evidence that the syn-

* Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.

thesis is controlled by the genetic information of the virus. The elution characteristics of wild-type virus arginase, mutant-line virus arginase, and rabbit liver arginase on carboxymethyl cellulose were studied (Rogers, 1970*a*) and shown to be widely separated. From the amino acid composition mentioned above, it is of interest that the virus-induced arginases are more acidic than liver arginase, containing smaller amounts of basic amino acids and thus eluting earlier from the cellulose column. When molecular weight studies were made it was found that the wild-type virus arginase has a molecular weight of about 43,000, while its mutant counterpart exhibits almost exclusively a molecular weight of about 400,000. A small amount of mutant arginase of molecular weight 43,000 came off the column at the same point as the 400,000, so it is plain that the degree of polymerization alone does not explain the change in its elution characteristics from those of the wild-type arginase. The lighter enzyme probably is a monomer.

We thought at first that the virus-induced enzyme differed critically from normal rabbit liver arginase in not requiring manganese activation; however, with the mutant line enzyme, which is highly polymerized, we found that manganese activation is required. Crude wild-type virus arginase, before carboxymethyl cellulose elution, when it is still in a relatively polymerized state, also requires manganese activation; in fact, the requirement was first described by Orth *et al.* (1967) who were working with crude arginase.

In studies of the optical rotary dispersion characteristics of the three arginases, the mutant virus-induced enzyme shows significantly less specific rotation at 233 nm and about 50% less specific arginase activity than that induced by the wild type. Examination of the free lysine, ornithine, and arginine pools in papillomas induced by wild-type and recoverable virus indicated that there is a relative increase in the amount of arginine in the mutant-line papillomas, which in all probability is related to the loss of specific activity and the change in the structure of the enzyme associated with the mutation (Rogers and Moore, 1963).

Looking at the serum of rabbits infected with the virus, both those with papillomas and those injected intravenously and showing no tumors, we noted that the arginine level dropped significantly within two or three months after inoculation with the virus. Thus, the virus-induced arginase has a systemic effect, lowering the blood arginine through infection of cells which it does not otherwise harm. This same effect can be seen in mice, rats, and monkeys, although injection of the virus apparently produces other effects in these animals. About this time, we decided to assay the serum levels of arginine in people who had worked with the virus as compared to controls taken at random for a general hospital, who were sick with a variety of diseases. It was clear that about half of the people who had worked with this virus had significantly lower blood arginine levels than controls. When serum samples from these people were run blind, individuals who had worked with the virus could be detected by their blood arginine levels alone (Rogers, 1966). In none of these individuals has any adverse effect possibly attributable to the virus been found. It appeared at this time that we had a rather ideal passenger virus, carrying information for an arginase, but nothing to do with it. How we wished then that it was carrying information for phenylalanine hydroxylase, as we might have been able to do something for phenylketonuria.

A short time later, however, I received a note from Dr. Joshua Lederberg bringing a paper by Drs. H. G. Terheggen, A. Schwenk, A. Lowenthal, M. van Sande, and J. P. Colombo, published in *Lancet*, to my attention. It concerned the discovery in Germany of two children with argininemia (Terheggen *et al.*, 1969). Studies had been made of the mother, father, and siblings of these two sisters. It was clear that the parents and two of the siblings were heterozygotes. Their arginine blood levels were somewhat elevated but did not approach those of the children with the disease. It has turned out that this disease is progressive, associated with problems in handling ammonia, epilepsy, spasticity, and mental retardation (Terheggen *et al.*, 1970). With much trepidation but great hope, Terheggen and his associates and I decided to try to stop the progression of the disease with the Shope virus. The effectiveness of the virus in inducing arginase in cell cultures taken from one of the children was tested. Arginase activity was

represented as counts of ^{14}C-ornithine measured after incubation of the cells with ^{14}C-arginine, and the amount of ornithine was found to increase after the cultures were inoculated with the virus. The results were similar when extracts of the cells were tested after Shope virus infection. The specific arginase activity per milligram of protein in the cells, with and without Shope virus infection, was measured. Immunofluorescent studies revealed that about half of the cells of the cultured preparation were infected with the virus, as measured by an anti-arginase conjugated serum. Significant amounts of arginase were induced. Normal human cells show about twice as much arginase as was induced with the virus in the cells of the child who was treated with the virus, but when the number of cells infected is taken into account, the amount of arginase is roughly the same. Upon infection of normal cells with the virus, an increase in activity is also found. (Crude horse liver extracts show a three- to fourfold increase in arginase activity per milligram of protein.) It is still too early to say anything about the *in vivo* tests (Rogers, 1970*b*).

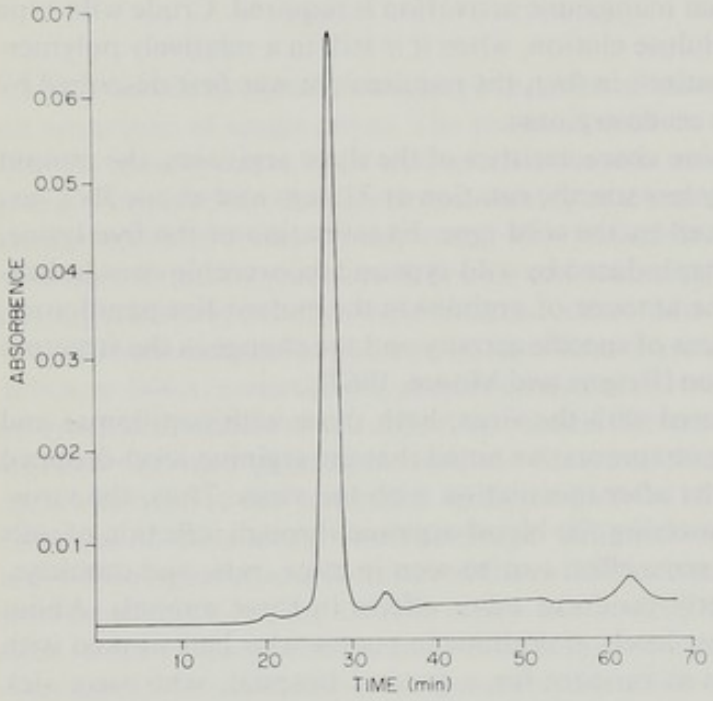


Fig. 1.

Of course, we are very excited about the therapeutic possibilities of the Shope virus for this monogenic deficiency disease. However, one can hardly hope to find passenger viruses carrying information for synthesis of all the specific enzymes that are deficient in the variety of diseases of this sort. It seems clear that the specific information needed will have to be added chemically to the end of such passenger virus DNA's or RNA's. Shope virus DNA is a continuous cyclic helix, so it has not been feasible to use it as a test system for adding information; instead, we have been using tobacco mosaic virus RNA. In one experiment, adenosine diphosphate was incubated with the virus RNA in the presence of polynucleotide phosphorylase, which adds sequences of poly(A) to the end of the RNA primer. The RNA with poly(A) added was tested in tobacco plants, and a number of polylysines were found to be induced (Rogers and Pfuderer, 1968). In addition, the modified virus can be isolated from infected plants and passed through new plant generations. One of the polylysines, which in its elution pattern from carboxymethyl cellulose appeared to be a decalysine and upon hydrolysis proved to be wholly lysine, continued to be synthesized through four plant passages (Fig. 1). This indicates that such added information is replicated and may be passed in a stable way.

We would like to be able to add information which has more biological significance than

the induction of polylysine. One way in which information can be isolated is the extraction of messenger RNA. Figure 2* shows peaks of mRNA extracted from papillomas and separated in a centrifuge after top loading on a cesium chloride gradient of density 1.70. All five of the peaks appear to be RNA, as they disappear following mild alkaline hydrolysis or treatment with ribonuclease. After extraction, the RNA was specifically hybridized with the virus DNA on a nitrocellulose column. We are in the process of trying to add these mRNA's to the tobacco mosaic virus RNA by chemical methods, to find out whether we can induce Shope virus protein coat or arginase in tobacco plants. Bacteria provide another possible source of genetic information, and Roozen has worked out methods for isolating single genes from *Escherichia coli*, using his minicell systems (Roozen *et al.*, 1971). Such DNA's could be readily attached to a passenger virus DNA, using the methods worked out by Richardson (1969) and by Goulian *et al.* (1967). That E. *coli* genes can be read in mammalian cells has been found

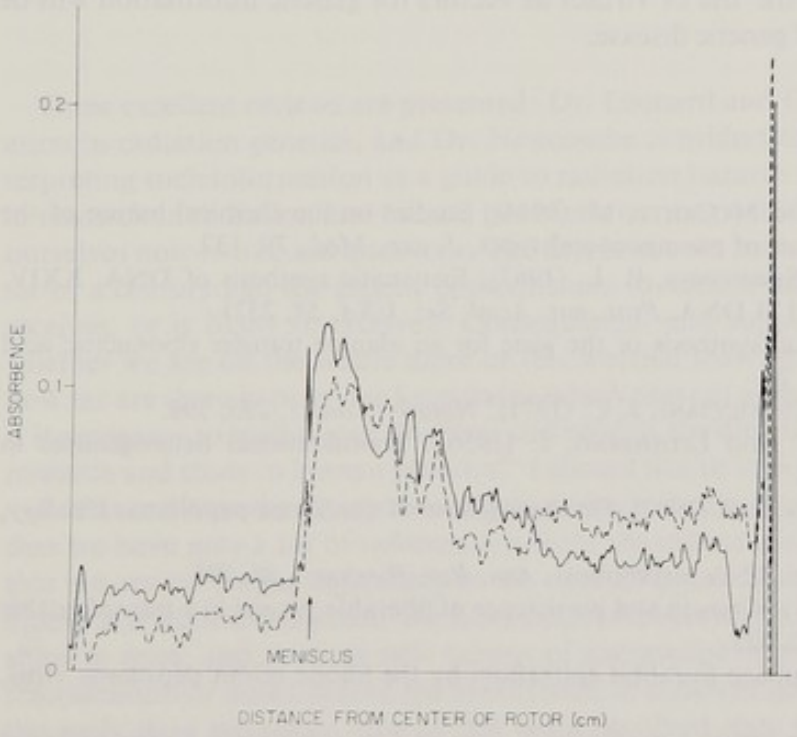


Fig. 2.

TABLE 1 *Plaques counted 6 days after inoculation of tobacco plants with tobacco mosaic virus (TMV)*

	Plant nr 1	Plant nr 2	Plant nr 3	Plant nr 4	Total	Average
TMV RNA	44	90	60	30	224	56
TMV RNA with ribonuclease	0	1?	1	0	1	0.25
TMV RNA with polylysine	1	5	3	3	12	3.0
TMV RNA with polylysine + ribonuclease	3	4	2	2	11	2.7
TMV RNA with polyornithine	0	6	8	1	15	3.7
TMV RNA with polyornithine + ribonuclease	2	3	3	0	8	2

* Experiments done with the cooperation of Stewart Rigsby and William Breillatt.

recently by Merril and his associates, who were able to use a lambda phage to transduce an *E. coli* gene in tissue culture cell (Merril *et al.*, 1971).

Clearly, however, the best way to get the information is to synthesize it directly *in vitro*. Khorana *et al.* (1970) have synthesized such DNA sequences, one of which is large enough to code for a transfer RNA. Through such synthetic methods, codes could be made for the human enzymes desired, greatly reducing potential immunological problems. The reassembly problem, that is the coating of modified nucleic acid with protein to protect it against nucleases, may prove to be less difficult than it appears. For example, with such simple materials as polylysine or polyornithine the tobacco mosaic virus RNA can be protected against ribonuclease (Table 1). Its infectivity is greatly reduced, however, as if the tobacco plant were having difficulty removing the polybasic amino acid. On the other hand, J.B. Bancroft (personal communication) has found recently that double-stranded nucleic acid can be protected with subunits derived from the Brome mosaic virus of barley.

In any event, it appears that the use of viruses as vectors for genetic information will be of great value in future therapy of genetic disease.

REFERENCES

Avery, O. T., MacLeod, C. M. and McCarty, M. (1944): Studies on the chemical nature of the substance including transformation of pneumococcal types. *J. exp. Med.*, *79*, 137.

Goulian, M., Kornberg, A. and Sinsheimer, R. L. (1967): Enzymatic synthesis of DNA. XXIV. Synthesis of infectious phage ϕX174 DNA. *Proc. nat. Acad. Sci. USA*, *58*, 2321.

Khorana, H. G. *et al.* (1970): Total synthesis of the gene for an alanine transfer ribonucleic acid from yeast. *Nature (Lond.)*, *227*, 27.

Merril, C. R., Geier, M. R. and Petricciani, J. C. (1971): *Nature (Lond.)*, *233*, 398.

Morse, M. L., Lederberg, E. M. and Lederberg, J. (1956): Transductional heterogenotes in *Escherichia coli*. *Genetics*, *41*, 758.

Orth, G., Vielle, F. and Changeux, J. P. (1967): On the arginase of the Shope papilloma. *Virology*, *31*, 729.

Richardson, C. (1969): Enzymes in DNA metabolism. *Ann. Rev. Biochem.*, *38*, 795.

Rivers, T. M. and Pearce, L. (1925): Growth and persistence of filterable viruses in a transplantable rabbit neoplasm. *J. exp. Med.*, *42*, 523.

Rogers, S. (1959): Induction of arginase in rabbit epithelium by the Shope rabbit papilloma virus. *Nature (Lond.)*, *183*, 1815.

Rogers, S. (1966): Shope papilloma virus: a passenger in man and its significance to the potential control of the host genome. *Nature (Lond.)*, *212*, 1220.

Rogers, S. (1970*a*): Separation and characterization of an arginase induced by a mutant line of Shope virus. *Reports of the Xth International Cancer Congress, Houston, Texas, May 22–29, 1970.*

Rogers, S. (1970*b*): Manuscript in preparation.

Rogers, S. and Moore, M. (1963): Studies of the mechanism of action of the Shope rabbit papilloma virus. *J. exp. Med.*, *117*, 521.

Rogers, S. and Pfuderer, P. (1968): Use of virus as carriers of added genetic information. *Nature (Lond.)*, *219*, 749.

Roozen, K. J., Fenwick, R. G., Jr and Curtiss, R. III (1971): Isolation of plasmid and specific chromosomal segments from *Escherichia coli* K-12. In: *Informative Molecules in Biological Systems*, pp. 249–266. Editor: L. Ledoux. North Holland Publishing Co., Amsterdam.

Selbie, F. R., Roberson, R. H. and Shope, R. E. (1948): Shope papilloma virus: reversion of adaptation to domestic rabbit by passage through cottontail. *Brit. J. Cancer*, *2*, 375.

Terheggen, H. G., Schwenk, A., Lowenthal, A., Van Sande, M. and Colombo, J. P. (1969): Argininaemia with arginase deficiency. *Lancet*, *2*, 748.

Terheggen, H. G., Schwenk, A., Lowenthal, A., Van Sande, M. and Colombo, J. P. (1970): Hyperargininämie mit Arginasedefekt. Eine neue familiäre Stoffwechselstörung. *Z. Kinderheilk.*, *107*, 298.

Chapter II Radiation genetics

Alan C. Stevenson

MRC Population Genetics Unit, Old Road, Headington, Oxford, United Kingdom

Radiation genetics

Three excellent reviews are presented: Dr. Léonard and Dr. Searle discuss the present situation in radiation genetics, and Dr. Newcombe considers the long-standing difficulties of interpreting such information as a guide to radiation hazards in man. After the immense efforts in research in radiation and human genetics over the past 25 years it is inevitable that we ask ourselves now two broad questions. The first is 'are we better able to predict now than a quarter of a century ago the genetic consequences to man of such radiation exposure as he now receives, or is likely to receive?' Consequential and supplementary to this is the question whether we are on the whole more or less worried than we were. The second question is 'in how far are there gaps in our knowledge which prevent a better understanding and in how far is it necessary to realign our thinking and modify the direction and emphasis of experimental research and study in human genetics?' I should like to look briefly at these questions.

It is clear that in the experimental field perhaps the most important single advance has been that we have now a lot of information from the mouse and a little from other mammals, so that we are no longer dependent as we were 25 years ago almost entirely on evidence from plants and from Drosophila. We have accumulated much information on dose effect relationships in mice, and a whole new corpus of knowledge of the effects of speed of delivery, of fractionation of doses, and of the sensitivities of different germ cell stages in the two sexes. In the early days relatively little of the work involved tests for mutational effects induced in spermatogonia and in young oocytes. Most was in later germ cell stages. This is understandable in view of the time and effort required for such experiments relative to those involving testing of later germ cell stages, but in human populations under all circumstances it must be these early germ cell stages which receive most of the damage which will be transmitted.

It seems to be held as a fairly general opinion that information on the sensitivity of spermatogonia may be considered, with reasonable confidence, as applicable to man. The situation is less clear as regards oocytes in view of known differences in the precise resting stage of oocytes in the two species, and of sensitivity to cell killing at different stages in mice and in primates. Nevertheless, it seems to be agreed that it is most unlikely that irradiation of young oocytes in man would determine as much transmitted damage in zygotes and offspring as identical irradiation of spermatogonia. In a recent very helpful review Lüning and Searle (1971) have made use of the linear relationship between single rapidly delivered doses to spermatogonia in mice and all the specific effects which could be scored in first generation zygotes and mature progeny. They have shown that, for these rapidly delivered single exposures, the mean doubling doses for specific recessive visibles, recessive lethals, dominant visibles, semi-sterility and small dominant effects on the skeleton fall within the small range of 16 rads to 51 rads. For unknown reasons the doubling dose for dominant lethals is much higher.

If it is considered justifiable to apply such values directly to man, then they are useful as

guides to upper limits for anticipated specific radiation effects. We can estimate reasonably well existing total frequencies of dominant and recessive visibles in man. We can probably safely assume that recessive lethals in man determine peri-implantation losses which are of no social consequence and there is no evidence that later losses are more frequently associated with parental consanguinity. We can, having reasonable and improving estimates of the frequencies of unbalanced translocations in abortion material and in the newborn, anticipate from semi-sterility data and the associated cytological information in mice the likely effects in man. We can also, as indicated by Dr. Newcombe, make appropriate allowances for sex and types of small doses received by man. Considering all this work we may conclude that damage in man of these specific types which is likely to result from present or anticipated levels of exposure is not a cause for very serious anxiety.

Over the same time as this experimental work has been proceeding we have also acquired much relevant knowledge in human genetics. A huge corpus of knowledge has accumulated on the types of chromosomal abnormalities which occur in man and their frequency. We know more about the frequencies of single gene traits and of the consequences of inbreeding. We are recognising the very high proportion of loci where there are large series of alternative alleles and beginning to relate these to our new found knowledge at a molecular level.

What is important is that we know more about types of detriment in man which are comparable with those which the experimental geneticists have been using for scoring radiation effects. In so far as *that range of effects* is concerned, we can probably be reassured and need not have major anxieties for the future unless the radiation situation should change drastically.

However, as has been pointed out repeatedly from the earliest days when there were anxieties about radiation these tangible effects probably only represent a fraction of all the results of mutational changes. We have some reassurance from the extensive work using multigeneration exposure of mice and other mammals in that there is no evidence of accumulation of deleterious effects which could be attributed to small effect dominants. Perhaps we also have some reassurance from the work on induction of small skeletal changes in first generation offspring carried out by Ehling (1966). Further, Searle's (1964) work on multigeneration exposure of mice and search for skeletal changes did not reveal any more variance in the exposed than in the control lines. We may also perhaps take some comfort from the absence of evidence of induced non-disjunction in mice, and the very high doses of radiation which are needed to induce a detectable frequency of aneuploidy in Drosophila. We may also remember that sex chromosome loss resulting in XO mice appears to be a phenomenon resulting entirely from exposure of late germ cell stages in the female.

Finally, it is important that none of the types of malformation comparable to those in man resulting from failures of mid-line closures such as the lip and palate clefts, and the neural tube defects, ever appear to have occurred more commonly in offspring of irradiated mice than in controls. This is reassuring in view of the high frequencies of these and other so-called 'congenital malformations' in man. We must still worry about individually small effect deleterious mutations which have not been detected in mouse experiments. We can probably now, with confidence, abandon an extreme hypothesis of population structure whereby with few exceptions any heterozygosity is determined by actively or potentially harmful mutations persisting temporarily in a population and eliminated by selection. Nevertheless, the evidence from man of lesser early viability in the offspring of consanguineous marriages has to be explained.

In short, then, the answer to the first question is a qualified 'yes', we do understand more and we are less worried as we realise that the kind of exposures to which human populations are exposed is less mutagenic than we supposed. We can probably predict with some confidence and within limits the effects of radiation in man in respect of certain traits and characteristics whose frequencies are directly mutation-dependent. Nevertheless, we are little more knowledgeable than we were 25 years ago as to other detrimental effects of mutation.

Some of these remarks are clearly part of the answer to the second question which I posed.

There are huge gaps in our knowledge about the underlying factors determining most of the harmful variation in man which apparently has some genetic component in aetiology. There are one or two specific areas where the amounts and precision of knowledge in mouse and man are very different. When it became possible to accumulate data on the frequency of chromosomal aberrations at birth in man, it proved to be much higher than was expected on evidence from other species and there was a very large contribution from autosomal and sex chromosome trisomies. However, very little is known of the birth frequencies of chromosomal aberrations in mice. This is mainly because of the problems of getting satisfactory lymphocyte cultures in rodents, and the labour in scoring the small acrocentric chromosomes. It is believed that most aneuploidies in mice behave as dominant lethals and are eliminated in the peri-implantation stages. If this is so, then we can take comfort from the high doubling dose for dominant lethals in the mouse. Then we have very little information about phenotypic effects of unbalanced translocations in newborn mice, and although the so-called centric fusion type of translocation has been described there is no evidence that it has been induced by radiation. It does seem that a strong case can be made for lymphocyte studies in mice. The technical difficulties cannot be insuperable. It would be invaluable to know the amount and type of chromosomal damage occurring in the offspring of normal and of irradiated mice. It will be remembered, also, that a great deal of information has been accumulated in man as to the type and frequency of damage to lymphocytes following whole and part body irradiation, and by irradiation of lymphocytes immediately after removal from the body. Comparable studies in mice would be of great value in providing cross-reference points as to sensitivity. No doubt there would be many difficulties in making exact comparisons by reason of the differences in numbers and sizes of chromosomes in these two species, but these are not insurmountable, particularly with the aid of parallel experiments in other species. Such experimental work is relatively simple and economical. Scoring, although tedious, is not particularly difficult and after adequate training can be done by relatively unskilled observers.

We also need more information of the effects of consanguinity in man, not only to get better estimates of the load of recessives but to clarify the associations of inbreeding with stillbirths and with childhood deaths. It also seems important to get data which will support or will not support the findings in the World Health Organization Congenital Malformations Study (Stevenson *et al.*, 1966) where in the two maternity hospitals where the frequency of consanguinity was highest, there was a significant association between consanguinity and neural tube defects. Further, the same direction of association was shown in other hospitals, although the findings were not technically significant, possibly because the levels of consanguinity were so low. It is hoped that a current study in progress in Ankara where the frequency of cousin marriages of mothers is about 20% will yield useful information. It is really remarkable that this type of information has not been collected on a larger scale. It could be done in selected hospitals over long periods all over the world and, provided there was adequate checking of the validity of the consanguinity recording, and preferably if this was done by observers who knew nothing else about the mothers and their babies, then the information could be of a high degree of accuracy.

The answer to the second question then must be that we can probably afford to be less worried than we were 25 years ago. Specific harm which varies directly as the frequency of mutation seems likely to be induced only at very low frequencies by present levels and types of radiation doses.

Even if developmental defects and disorders where the genetic components are not just single mutational changes are much commoner and more socially important it seems likely that the frequencies of these traits would increase, if at all, only at a very low rate with a rise in mutation levels.

Finally, I should like to stress my agreement with Dr. Newcombe's plea for re-thinking of experimental research, probably in the direction of considering more empirically designed experiments not too limited in design by existing theory.

REFERENCES

EHLING, U. (1966): Dominant mutations affecting the skeleton in offspring of X-irradiated mice. *Genetics*, *54*, 1381.

LÜNING, K. G. and SEARLE, A. G. (1971): Genetic risks from ionizing radiation. *Mutation Res.*, *12/3*, 291.

SEARLE, A. G. (1964): Effects of low-level irradiation on fitness and skeletal variation in an inbred mouse strain. *Genetics*, *50*, 1159.

STEVENSON, A. C., JOHNSTON, H. A., GOLDING, D. R. and STEWART, M. I. P. (1966): Congenital malformations: a report of a study of series of consecutive births in 24 centres. *Bull. Wld Hlth Org.*, *34*, *Suppl.*

Howard B. Newcombe

Biology and Health Physics Division, Atomic Energy of Canada Limited, Chalk River, Ontario, Canada

Effects of radiation on human populations

The two other speakers in this symposium will discuss the induction of gene mutations and gross alterations of chromosome structure by exposure to ionizing radiation. It remains to be considered, however, what importance should be attached to such artificially induced changes when they occur in the human germ plasm.

Herein lies the weakest part of the chain of information linking the initial physical exposure to radiation with the eventual consequences of such exposures. There exists substantial knowledge of the variables which alter the yields of the different sorts of genetic changes resulting from a given level of radiation, but little about the magnitudes of the harm that may result from them.

It will be my task to discuss some of the problems of attempting to draw practical guidance from studies such as Drs. Searle and Léonard will describe, with respect to the levels of radiation to which society may reasonably expose its members in order to provide certain benefits for them. In particular I shall emphasize the areas of ignorance in human and mammalian genetics, and in genetic theory, with which one is confronted when seeking to estimate population risks.

First let me pose a problem for the social conscience of geneticists. They have made the point, albeit somewhat unquantatitively, that the human germ plasm is society's most precious possession, to be jealously guarded against erosion by its temporary custodians in each generation. And they have warned that radiation-induced genetic changes constitute one such kind of erosion. There have been sceptics, but by-and-large the geneticists have been listened to.

Over the past quarter of a century these warnings have had two effects. One has been the widespread popularization of our ideas about the importance of the human gene pool, initially in connection with concern over the effects of exposures to exceedingly small amounts of radiation from nuclear weapon testing, and more recently in connection with the development of nuclear power. I should add parenthetically that much larger genetically significant exposures of the population result from medical radiology, amounting in some countries to a substantial fraction of the levels of natural background radiation, but these have provoked much less controversy. (For details of the magnitudes of such exposures see UNSCEAR, 1958, 1962, 1964, 1966, 1969.)

The second effect of these warnings has been substantial long-term monetary support, drawn largely from atomic energy budgets, for research in radiation genetics and cytology and in human genetics. The investment has presumably been made in part because of enlightened policies of support for basic research which would expand our knowledge of radiation effects of all kinds. But if qualitative insights were sought first, it was because quantitative insights might be needed later.

A time has now come when the development of nuclear power must be accelerated if society is to meet the needs of an expanding population, the growth rate of which is unlikely to decline rapidly in the foreseeable future despite the increasing emphasis on family planning programs around the world. Power will be basic to the control of pollution, the production of food, the substitution of readily available materials for those which are in limited supply (*e.g.* aluminum for copper), and only with abundant power will it ever become possible to provide for the developing countries a standard of living comparable to that of the more industrialized parts of the world. Thus there is a special requirement at this time for practical guidance concerning the quantitative importance of radiation effects in man, so that the standards of protection against radiation injury will be appropriately balanced against the future needs of society for nuclear power.

This implies a social obligation on the part of geneticists to re-examine the manner in which the investment of effort and resources in their science, for such purposes, has been used. I will propose that some of the most needed kinds of information have received the least emphasis because they have somehow failed to excite scientific interest among geneticists, but I will suggest to you that this need not be true for the future.

The point will become clearer when we consider some of the procedures for estimating quantitatively the magnitude of the genetic consequences of a given level of radiation exposure of a human population.

THE CASE FOR ATTEMPTING TO ESTIMATE GENETIC RISKS TO MAN

Before describing some of the ways which have been used to estimate genetic risks, I should perhaps deal with the argument that we do not yet know enough, and should not attempt such an exercise. There have been many who have felt that we ought to limit ourselves to making general statements about the importance of the gene pool, and the need to keep any radiation exposures of human germ cells 'as low as possible' (a phrase so vague as to be virtually useless for the purpose of drafting legally enforceable safety regulations).

In this connection I recall the early experiences of two national committees which subsequently produced reports on the subject. In one case these were described to me, and in the other I witnessed some of them myself. The geneticists on one of these committees took only a short time to agree that they could draw no quantitive conclusions at all about the effects in man. At this point they were reminded that if practical guidance really could not be extracted from the currently available data, then future safety standards would have to be set by administrators and politicians who had no knowledge of the biological effects of radiation.

In the other committee which I mentioned, geneticists were urged to make their point clearly if they wished people to continue to listen to them. 'Suppose the mutation rate were doubled, do you think the effect on human health would be important or would it be trivial?'

Such forms of exhortation have been applied many times since. And geneticists have had no choice but to listen and to respond if they wished to have a voice in deciding how much protection ought to be afforded to the human gene pool.

MUTATIONS AND 'GENETIC DEATHS'

One line of reasoning, advanced initially by Muller (1950) and Haldane (1937), equated directly the numbers of radiation-induced mutations with the eventual numbers of deaths and failures to reproduce due to the mutated genes. Collectively these were known as 'genetic deaths.'

For a population to remain in a state of genetic equilibrium it was argued that each new mutation must be balanced by the elimination of a mutant gene. The alternative was genetic deterioration through the accumulation of harmful hereditary changes. If a population is to

avoid such deterioration, any increase in the mutation rate must thus result in an increase in deaths and failures to reproduce.

One of the simplifications inherent in the argument has made it especially attractive. The reasoning applies equally to mutations which have severely harmful effects and to those which produce only slight harm. The former will tend to be eliminated within a few generations and will each harm a few people severely, whereas the latter will be transmitted over many generations before they are eventually eliminated, and will thus each harm more people but less severely. In the long run the accumulated detriment resulting from a mutation will be similar, independent of whether its expressions are severe or slight.

It is not a difficult matter to estimate the numbers of mutations likely to be induced by exposing a population of a given size to a certain level of radiation. Data from irradiated mice are frequently used, but information on spontaneous mutation rates in man are also helpful. The answer is obtained in terms of the numbers of future genetic deaths, and these are regarded as being associated with genetically caused handicaps and losses of fitness of less specific kinds.

Despite its simplicity, the approach is no longer in vogue. The reason has little to do with the uncertainty of extrapolating from animal data to man, because this can never be wholly avoided. Instead, it is the difficulty of determining what fraction of the gene eliminations is associated with socially important events. In fruitflies many of the mutant genes are lost through deaths during pupation, but it is not certain how to apply this observation to man. If such deaths in flies corresponded, for example, to failures of implantation they would tend to pass unnoticed, but if their human counterparts were deaths at the time of weaning these would be regarded as tragedies in our society.

The uncertainty arises because of a major area of ignorance concerning the genetics of man. We know little indeed about the range of phenotypes associated with a random sample of newly mutated genes, or about the stages in the life cycle at which selection operates to eliminate these phenotypes.

The results of mammalian studies, far from settling the problem, have seemingly served to deepen the mystery. A number of empirical studies with mice, rats and pigs, have failed to show either the expected mortality at any stage in the life cycle, or the expected reduction in physical fitness. A few have revealed minor effects on such quantitative traits as body weight, but for the most part no changes have been observed and in particular no harmful trends (for reviews see Green, 1968*a*; Newcombe, 1971).

The extent of our ignorance and the inadequacy of current concepts are emphasized especially by studies of this kind in which mammalian populations have been exposed to repeated heavy irradiation extending over many generations. In such circumstances generally accepted theory would lead us to expect an accumulation of small-effect deleterious mutations in the gene pool. This should continue until the deaths and failures to reproduce, resulting from the combined harmful effects, selectively eliminate the mutant genes at a rate which equals that at which they are being introduced.

Some experiments of this kind have involved many generations of exposure to doses of radiation which raise the mutation rate to a number of times its natural level. Assuming that naturally occurring mutations do contribute substantially to mortality and morbidity under normal circumstances, it is difficult to believe that in such heavily irradiated populations the increased contribution due to the artificially induced mutations could escape detection. And yet, to take one example, lines of mice which were given 200 rad per generation over as many as 35 generations by Spalding and his co-workers at Los Alamos, have shown no measurable change in body characteristics, no decline in fertility and no increase in mortality (Spalding *et al.*, 1969).

This confllict between theory and empiricism may be one of the most relevant developments of recent years. It has served to emphasize a basic lack of understanding of the two major processes in organic evolution, namely mutation and selection. It suggests that more

of the mutations may have neutral or nearly neutral effects than was previously suspected, and that many of the eliminations of mutant genes may occur early in the life cycle. But curiously little scientific glamour surrounds the kinds of experiments that yield such negative or nearly negative results, and only limited financial support has been given to them despite their relevance to the practical problem.

It continues to be more popular to work within accepted genetic models, rather than to develop new theories with which to account for the unpredicted experimental findings and new animal experiments with which to test the new theories.

MUTATIONS AND HUMAN DISEASE

For these and other reasons estimates of genetic risks tend increasingly to make use of data on the incidence and prevalence of known hereditary diseases of man. But such approaches likewise expose areas of fundamental ignorance that are currently receiving only modest attention.

The reasoning usually starts with an estimate of the total identifiable burden of ill health from hereditary and partially hereditary causes. An attempt is then made to single out the fraction of this burden which is believed to be maintained by the pressure of recurrent mutations of natural origin. It is only this latter fraction which would be expected to increase with an artificially induced rise in the mutation rate.

Both the assessment of the importance of the hereditary component in human disease, and the partitioning of this into a mutation-maintained and a selection-maintained fraction, are fraught with difficulties which have attracted only limited attention.

THE AMOUNT OF GENETIC DISEASE

Although geneticists tend to regard the contribution of hereditary factors to human pathology as important, there have been exceedingly few serious attempts to estimate quantitatively the total impact of hereditary variations on man's health.

In fact, the only well-documented summations of the required breadth stem from a survey of Northern Ireland carried out by Stevenson (1959). The resulting list of hereditary diseases, with their frequencies among newborn children and among the living population, has been used by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR, 1958, pp. 198–200, Tables XI–XIV), and the subsequent revised estimates by that Committee have had this list as their starting point (UNSCEAR, 1966, p. 7 para. 4, p. 8 para. 11). For some reason human geneticists have shown surprisingly little interest in knowing the overall importance of genetic disease in man. The conclusion drawn from such data is that about 6 children out of every 100 born alive are more or less seriously affected at some time in their lives by conditions which are largely or in part determined by hereditary causes. The total may be broken down as indicated in Table 1.

The choice of conditions for inclusion in these totals necessarily involves an arbitrary cut-off, and many diseases which are recognized as having some substantial genetic component have been excluded, among them coronary heart disease, and duodenal and gastric ulcers. However, this manner of assessing the importance of hereditary ill health is accepted as convenient even if it is theoretically untidy. We are apt to be reminded that most human diseases must have some genetic component in as much as they tend to be peculiar to humans, or at least to mammals. The comment misses the main point, however, because what we are interested in is the contribution to ill health from the genetic variability within the human race, not that which exists outside it. For the most highly heritable diseases, genetic variation clearly contributes much more than do any of the environmental variations; and at the other end of the spectrum the reverse is true.

What is needed is not so much a list of genetic diseases, truncated at some point in the spectrum where the genetic contribution has dropped below an arbitrarily chosen level. Instead, the most informative sort of answer would be in terms of the relative importance of the genetic and environmental variables in determining diseases, summed over the total to human ill health. The units could be chosen to represent the numbers of seriously affected people, or the economic burden to society, or in other ways.

Taking a concrete example, one might ask by what factor would the total dollar cost for health care delivery be increased if the contribution from the genetic variation were doubled? Is 50% of the current bill attributable to genetics, or is the correct figure closer to 5%, or 0.5%? Is the genetics of man as important as many of us subjectively feel? And if so, can we place numerical values on the degree of importance, in some reasonably objective fashion that will stand up to scrutiny?

Few human geneticists are conditioned to ask questions of this kind, and the concepts involved have seemed quite foreign to their usual way of thinking. The unavoidably imprecise answers that would be obtained, even after the expenditure of considerable effort, may seem to lack usefulness or value to those who have not been thinking in terms of specific needs for such information. Only when geneticists are asked for guidance concerning an important social application of their science, in this case the protection of the human germ plasm from exposure to radiation, does it become apparent what widely divergent views they hold. We are, in fact, still profoundly ignorant about the magnitude of the impact of hereditary variation on public health.

TABLE 1 *Combined frequencies of hereditary and congenital diseases of different kinds*

Kind of disease	Per 100 liveborn individuals
Single gene	1
Chromosomal	1
Congenital	2.5
Constitutional and degenerative	1.5
Total	6.0

Based on estimates by Stevenson (1959) and UNSCEAR (1958, 1966).

In case you may feel that I should attempt some such estimate here, I will suggest that about one-sixth of the cost of the delivery of health care is attributable to hereditary variation. The figure is based on Stevenson's (1959) estimate that just over 25% of hospital beds and 6–8% of the physician's consulting time in Northern Ireland are used by persons with hereditary diseases. Since the premiums for hospital insurance and for medical care insurance in Canada are roughly equal, I have assigned equal weights to the two sorts of percentage given by Stevenson and simply averaged them. The figure of 1/6th, for the fraction of our health care costs attributable to genetics, may be crude but at least it can serve as a basis for discussion.

THE AMOUNT OF ILL HEALTH THAT IS MAINTAINED BY MUTATION

More difficult still is the problem of partitioning all hereditary illness into a mutation-maintained and a selection-maintained fraction. The problem is best tackled by considering separ-

ately (*a*) the simple dominantly inherited diseases, (*b*) the non-mendelian hereditary and partially hereditary diseases, including the relevant continuously distributed and quasi continuous traits, and (*c*) the chromosomal diseases. The recessive diseases, affecting about 0.15% of liveborns, are unlikely to respond rapidly to a change in mutation rate, and so may be excluded without greatly affecting the conclusion reached, at least as applied to the next half dozen generations or so.

I will suggest that only the dominant diseases and a small fraction of the chromosome diseases should be regarded as mutation-maintained and likely to increase in frequency with exposure to radiation. The suggestion is made with the knowledge that other geneticists regard mutations as contributing substantially to the less simply inherited diseases, and even to non-specific differences in well being and resistance to bacterial and other diseases. I would like to emphasize this diversity of opinion, not because any of us is in a position to be dogmatic but because the studies needed to resolve the uncertainty are more likely to be carried out if the extent of our ignorance is made apparent.

A. DOMINANT DISEASES

That the more serious dominant diseases of man affect perhaps as many as 1% of liveborn individuals and are probably maintained at their current frequencies in the population by the 'pressure' of recurrent mutations has been accepted by the UN Committee. This does not mean, however, that their frequency has been accurately estimated or that the evidence is adequate to rule out entirely the possible action of a selective mechanism in maintaining the causal genes, except for a rather small number of these traits.

The frequency of 1% is certainly an upper limit, and the original list on which it is based (UNSCEAR, 1958, p. 197) has not been revised since its compilation. Included among the most common conditions are some which are still of uncertain etiology and others that are only occasionally harmful. For this reason some geneticists would estimate the frequency of severe dominant disease as closer to 0.1%.

Also, local situations exist in which affected individuals have been shown to be reproductively more active than their normal siblings and than other members of the same communities. An example of this relates to the disease, aniridia, in an economically depressed community in eastern Canada (Gove *et al.*, 1961). In this community 77 individuals were identified with bilateral aniridia, all descended from one aniridic woman born in 1824. The aniridics showed approximately a 20% elevation of reproductive activity as compared with the rest of the community, and this community was in turn nearly twice as fertile as the rest of Canada.

I do not intend to review here the limited documentation and anecdotal evidence indicating that handicapped individuals may in certain circumstances possess a reproductive advantage. The point has been made elsewhere in sufficient detail to keep the question open (see Clarke, 1959). Sufficient for present purposes is the thought that, in a heterogeneous environment, opposing selective forces may be operating to maintain the frequencies of some part of the harmful dominant traits in equilibrium. The possibility requires continued exploration.

In the meantime it is prudent to assume that the frequencies of such traits will vary in direct proportion to the rates of mutation of the responsible gene loci. It is their current collective frequency which is most in question here.

B. DISEASES WITH COMPLEX INHERITANCE

Much more difficult is the question of the contribution from mutations to the genetic and partially genetic diseases that are not inherited in a simple mendelian fashion. These diseases include many of the congenital malformations such as club foot, cleft palate and pyloric stenosis. And they also include such constitutional and degenerative diseases as idiopathic epi-

lepsy, schizophrenia, and diabetes. Collectively the non-mendelian hereditary traits constitute a far more important source of ill health (*i.e.* at least 2/3 of the 6%) than those determined by single genes, and many of them result in prolonged hospitalization.

Such diseases are, in general, individually more common and less heritable than the single gene traits. In fact, the two sorts of disease form discrete clusters with almost no overlap when plotted on a two-dimensional graph in which the axes represent respectively the frequency in the population and the increased risk among siblings of cases (Newcombe, 1964 and Fig. 1). Thus the irregularly inherited diseases as a class appear to have little in common with the single gene traits.

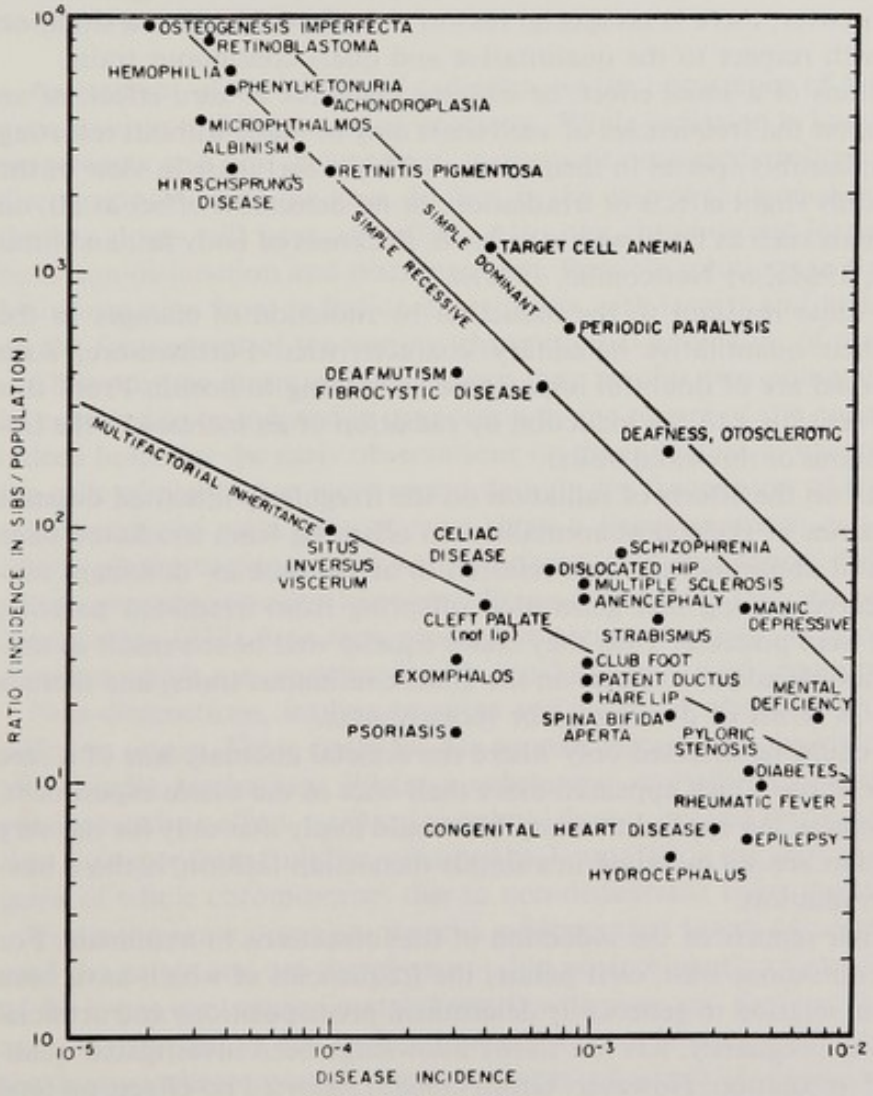


Fig. 1. Comparison of the frequencies of hereditary diseases in the population, with the increased risks among siblings of cases, showing the relatively discrete clusterings of the single gene traits and of the irregularly inherited diseases. (From Newcombe, 1964.)

A multifactorial and quasi continuous or threshold type of inheritance is now well established for certain of these diseases, notably pyloric stenosis, cleft palate and coronary heart disease, and probably also for diabetes and club foot. In fact, for the irregularly inherited diseases in general, the risks among siblings of cases tend to be close to those predicted on the multifactorial threshold model. Thus the role of single genes with large effects in causing such diseases appears to be small.

The model which I would favour is thus one in which (*a*) a multiplicity of loci contribute to the likelihood of occurrence of the disease, (*b*) large-effect genes are the exception rather

than the rule, (*c*) alleles which influence the underlying quantitative variable in a positive, and in a negative, direction do not differ greatly in frequency, and (*d*) the alleles at the different loci contribute in a simple additive fashion with little dominance or recessiveness.

Simple theory might seem to indicate that the genetic diversity which is responsible for quantitative and quasi continuous variation will increase with an increase in mutation rate. However, the extent of the increase in the quasi continuous traits must be limited in two ways. It could not be great unless mutation acted to raise the frequencies of the rarer alleles. And even then, the extent of the increase in variation would be further restricted to situations in which the rare alleles that do exist have effects which are different in direction and magnitude from those of the common alleles at the other loci. This point has tended to be overlooked in the past. Moreover, there is no special reason to believe that such a situation is other than quite usual with respect to the quantitative and quasi continuous traits.

Thus, theoretical predictions of a small effect, or in some instances of zero effect, of an increase in mutation rate upon the frequencies of such traits may be made without resorting to assumptions that are implausibly special in their natures. This is fortunate in view of the numerous observations of only slight effects of irradiation, or no detectable effect at all, on quantitative traits of mammals such as body weight at birth, thickness of body fat, and litter size (see reviews by Green, 1968*a*, *b*; Newcombe, 1971).

Mammals appear to be quite resistant to the induction by radiation of changes in the means and variances of their quantitative hereditary characteristics. Furthermore, such changes as have been observed are of doubtful importance as relating to health. From this might be inferred a similar resistance to the induction by radiation of an increase in the frequencies of the quasi continuous or threshold traits.

The best mammalian data on the effects of radiation on the irregularly inherited diseases have to do with the frequencies of skeletal abnormalities in offspring from irradiated mice (Ehling, 1965). These induced abnormalities were referred to at the time as 'dominant mutations', because they appeared among first generation offspring from irradiated parents; but no tests for dominance were possible. Thus they could equally well be the result of mutations to alleles that have simple additive effects on the quasi continuous traits, and there is no special reason to think in terms of dominance or recessiveness.

The effect of irradiation could be detected only where the skeletal anomaly was of a rare type. For all other types, *i.e.* those which appeared more than once in the whole experiment, there was no measurable increase. As applied to man, this would imply that only for the very rare diseases, among those that are not inherited in a simple mendelian fashion, is there likely to be an effect due to irradiation.

There are virtually no other reports of the induction of malformations in mammals. For example, a classical quasi continuous trait, cleft palate, the frequencies of which have been studied extensively in mice in relation to genetically determined predispositions and artificial induction by cortisone during pregnancy, has not to my knowledge been investigated quantitatively following parental irradiation. However, Green (1968*b*) reported no effects on fetal abnormality in his investigations of irradiated mouse populations.

In view of the importance of the multifactorial diseases of man, the apparent lack of relevant mammalian studies is noteworthy. The reason presumably has to do with the failure of such results as have been obtained to conform with current theory, and a lack of scientific interest in experiments that yield negative results. This is unfortunate, because conflicts between theory and empiricism ought correctly to be regarded as more scientifically challenging than agreements of data with theory.

There is also a relative lack of emphasis on genetic studies of the less regularly inherited diseases of man in comparison with their importance to health. For purposes of investigation such traits offer a number of special advantages: (*a*) they tend to be common, (*b*) they are more apt to be correctly diagnosed and recorded than the rarer conditions, and (*c*) there exists a substantial battery of tests with which to identify and describe them.

The current lack of detailed knowledge of the genetics of this group of diseases makes it difficult to draw conclusions which are as firm as one would wish, about the likely effects of an increase in mutation rate. However, the available evidence indicates quite strongly that the effects would probably be small in comparison with the increases expected among the single gene traits. I will therefore suggest, somewhat arbitrarily, that to double the frequencies of the irregularly inherited traits might require a 20-fold increase in the mutation rate. The figure is highly debatable, but the experimental evidence is so strongly against any large radiation effect in this group of conditions that it would be difficult to defend the assumption of a more pessimistic value.

C. CHROMOSOMAL DISEASES

Assessment of the effects of radiation on the occurrence of the chromosomal diseases of man involves a different set of problems. While radiation is known to cause structural rearrangements and non-disjunctions, it does not necessarily follow that the yields will vary in direct proportion to the dose. In fact, in the case of the non-disjunctions it is not clear that the low doses will have any effect. Also, the chromosome losses may occur as a result of both non-disjunction and rearrangement. Possible solutions to these problems are suggested by information from radiation experiments with insects and vertebrates, together with data on the frequencies of the various chromosomal anomalies of man.

Chromosome rearrangements, since they involve two points of breakage, or 'hits', might be expected to be induced in proportion to the square of the radiation dose. This prediction indeed holds for the early observations on the induction of chromosomal rearrangements in the cells of plants, but more recent data on the frequencies of translocations induced in the spermatogonial stem cells of mice show a linear response curve (Léonard and Deknudt, 1969). Malformations of fish embryos from irradiated sperm, believed to be due to induced chromosomal anomalies, likewise follow a linear dose-response relationship (McGregor and Newcombe, 1972). It is thus prudent to assume linearity as applied to those chromosomal diseases which are associated with breakage and rearrangement.

Non-disjunctions, leading to gains and losses of whole chromosomes, may have a quite different origin. These could be due to malfunction of a multimolecular portion of the protein spindle mechanism. Where a substantial multiplicity of radiation 'hits' is required to produce such an effect, a critical threshold dose is implied below which the radiation is ineffective. Evidence from fruitflies, obtained by Traut (1970), supports this view and indicates that for gains of whole chromosomes due to non-disjunction there is a threshold at about 1000 rads.

From the same organism there is evidence that losses of whole chromosomes greatly exceed the gains, and are therefore not due predominantly to non-disjunction. Since the yields of the losses vary approximately linearly with dose and show no threshold, this type of change may be regarded as due mainly to chromosome breakage (Traut and Scheid, 1969). Among the human chromosomal diseases, losses of whole chromosomes are less common than gains instead of the reverse as indicated by the fruitfly data, but the discrepancy may be attributed to the inability of most human monosomics to survive.

For present purposes, the conclusion which can be drawn from all of this is that the monosomies and chromosome structural changes in man (including translocations, deletions, and the partial trisomies arising out of the translocation process) will be increased in frequency by exposures to low doses of radiation, in proportion to the dose, but that the simple trisomies will not.

To estimate the risk, assumptions must also be made as to whether the current frequencies of the chromosomal anomalies of man are mutation-maintained or selection-maintained. In fruitflies many chromosome structural variations are known to be maintained in populations by selection pressure. Nevertheless, it is prudent to assume that in man the troublesome ones are all maintained by mutation.

Only a small fraction of the chromosomal diseases, however, are caused by the kinds of anomaly thought to be produced by low doses of radiation, *i.e.* by losses of whole chromosomes and by translocations, partial deletions and other rearrangements. Although translocations in the balanced state are relatively common (*i.e.* about 5 per 1000 liveborn infants) the diseases caused by translocations are very much rarer.

Only about one-tenth of the chromosome diseases of liveborn children would appear to be associated with chromosome breakage, and therefore susceptible to increase as a result of exposure to low doses of radiation. The basis of this estimate is given in Table 2 (see also UNSCEAR, 1966, p. 127, Table IV, and 1969, p. 131 paras 277-283).

TABLE 2 *Frequencies of diseases due to trisomies as compared with chromosomal anomalies involving loss or rearrangement*

Diseases due to		Per 1000 liveborn individuals	
Trisomy	XX (X) Y	1.8	
	XXX (X)	1.5	
	XYY	1.5	
	21	1.5	
	18	0.3	
	13	0.2	
	combined		6.8
Monosomy	XO	0.3	
Deletion	5	0.25	
Translocation	21	0.05	
	combined		0.6

(Based on UNSCEAR 1966, p. 127, Table IV, and 1969 p. 131 paras 277–283.)

If spontaneously aborted fetuses are taken into account the numbers will, of course, be higher. But considering only the liveborn infants, an estimate of 1 child per 1000 with a chromosomal disease of a kind due to breakage and rearrangement would seem to lean, if anything, on the high side.

THE EXTENT OF THE RADIATION-INDUCED INCREASES

The discussion up to this point would suggest that all of the single-gene diseases, and some small parts of the multifactorial and chromosome disease, which I have taken as 1/20th and 1/10th respectively, are maintained by genetic changes of kinds that will increase with exposure to low doses of radiation. Collectively these may affect seriously about 0.4 to 1.3 children per 100 born alive (see Table 3).

It remains to consider the extent to which this mutation-maintained part of man's hereditary troubles would increase with a given level of radiation exposure. A convenient approach makes use of the results of mouse experiments which indicate the amount of radiation required to produce as many mutations as occur naturally in a single generation, and then applies this mutation rate 'doubling dose' directly to the mutation-maintained diseases of man.

TABLE 3 *Estimates of the mutation-maintained components in hereditary disease*

Diseases	Per 100 liveborn individuals	Fractions maintained by mutation	Mutation-maintained (per 100)
Single gene	1.0*	all	1.0*
Multifactorial	4.0	1/20	0.2
Chromosomal	1.0	1/10	0.1
Combined	6.0	–	1.3**

* Possibly overestimated by as much as 10-fold, *i.e.* 0.1 to 1.0.
** Possibly overestimated by as much as 3-fold, *i.e.* 0.4 to 1.3.

The available information on the doubling doses for genetic changes in mice has been reviewed recently by Lüning and Searle (1971). Those for gene mutations of various kinds, and for semisterile mutations which are presumably associated with chromosome breakage leading to translocation, were all remarkably similar and close to 30 rad for exposures of males to acute irradiation. Since we are primarily interested in chronic irradiation of both sexes, the figure of 30 rad must be increased by a factor of 4 to allow for the lower effectiveness of the chronic irradiation, and also by a further factor of 2 to allow for the almost complete insensitivity of females to induced mutation when exposed at low dose rates.

Thus we may infer that it would take an exposure of 240 rad per generation to double the 0.4 to 1.3 cases per 100 liveborn, which are believed to be maintained by naturally occurring mutations. With a level of 1 rad per generation the number of seriously affected individuals due to the artificially induced mutations would, by this reasoning, be in the vicinity of 0.4 to $1.3 \times 1/240 = 0.0017$ to 0.0054 per hundred, or 17 to 54 per million live births due to a level of radiation of 1 rad per generation. The natural level of radiation of about 3 rad per generation presumably accounts for 3 times this many cases of hereditary disease.

I do not wish to emphasize these numerical values which serve mainly to illustrate the kinds of reasoning that may be involved. Instead it is the limited nature of our current understanding of the collectively more important elements in the genetic diseases of man to which I would like to direct attention.

The questions on which there is still the greatest doubt have to do with:

a. how important we think the genetic component in human disease is, and

b. how important mutation is in maintaining the prevalence of hereditary ill health.

These questions will be asked repeatedly in the future, not just with respect to radiation but in relation to environmental pollutants, industrial chemicals, drugs, and food additives, whenever there is concern over suspected mutagenicity.

Studies of the quasi continuous traits are relevant and so also are studies of selection. It is to facilitate the carrying out of such studies on an appropriate scale that we have devoted so much effort at Chalk River to developing computer methods for linking very large files of routine vital and health records into family histories for whole populations (Newcombe, 1968).

ASSESSING THE TOTAL COST OF MUTATION

The opinions of geneticists concerning the importance of protecting the human gene pool from induced mutations need to be expressed in a tangible form if they are to carry weight

and to influence practical decisions. Attempts to assess the harm quantitatively in terms of the burden to individuals or to society satisfy few but must continue.

We have, for example, no adequate scale of human suffering which can be applied to disease, but it is possible to speak in terms of the numbers of sick and chronically handicapped people. Similarly, we may not be able to judge the total cost of ill health but we can look at the financial burden to the individual, and to society, of the delivery of health care. It is in such terms that practical decisions in health matters are often made, and geneticists may have to think increasingly in this fashion if they wish to influence practical decisions affecting the hereditary basis of health.

A tentative answer to the question 'How much would the cost of physicians' services and hospital beds be increased if the *de novo* rate of occurrence of troublesome genes and chromosome anomalies were artificially raised by some small fraction of a per cent?' may be possible now.

According to Stevenson's (1959) estimates for Northern Ireland the single gene traits, about which we are here primarily concerned, do not contribute greatly to the use of hospital beds and institutional places or to the numbers of consultations with physicians, even when all cases of hereditary and congenital blindness and deafness are included with these. Using his figures (*i.e.* from his Table VIII), and reasoning as before, one may draw the conclusion that only about 2% of the cost of health care delivery is associated with the single gene diseases even when so defined.

Thus, if 1 rad per generation is regarded as producing 17 to 54 cases of hereditary disease per million live births, *i.e.* an increase from 60,000 to between 60,017 and 60,054 per million, this would be equivalent to a 0.03 to 0.1% increase in the cost of health care. In Canada, or rather in Ontario, the combined premiums for hospital insurance and medical care are in the vicinity of $150-$200 per year per person. The additional cost to the individual from a 0.03 to 0.1% increase would thus be about 5 to 20c per year.

Again, I place no great emphasis on the particular numerical estimate. Its purpose is to illustrate in some detail a social application of genetics and to emphasize the importance of some of the missing kinds of information.

BALANCING SOCIAL COSTS AND SOCIAL NEEDS

The problem is important, however, because major issues are at stake. The demands which are being imposed increasingly on our society by the expansion of the population and the rising expectations of its members cannot be met without a rapidly advancing technology. It is quite unlikely that the population growth will be checked or that the aspirations of people will become more modest, in the near future. Thus there is a need to 'play for time' while society adjusts to the realization that we live in a finite environment.

In the meantime, *medical technology* will continue to expose us to relatively large amounts of artificial radiation in the process of meeting our demands for health care, *nuclear technology* will make modest population exposures in order to provide the energy that will be indispensable for our continued existence, and *chemical technology* will produce for our use an increasing array of organic molecules of which a proportion will be mutagenic.

Those who urge putting the brakes on technological advances are inclined to forget: That there is no simple and quick way of bringing population growth to a halt. That the prevention of pollution will require energy. That the substitution of common raw materials for the more readily depletable materials may depend on an abundance of available power. And that in the meantime it would be unthinkable to deliberately deny food, medical care, and material comfort to an expanding population.

Thus the social cost of a relatively small increase in mutations, through limited exposures

to radiation and mutagenic chemicals, must be balanced against the social costs of restricting technologies that we will increasingly need in the future, even with the most energetic sponsorship of family planning programs around the world.

If geneticists regard these matters as important they will be increasingly consulted. And if they regard the hereditary and mutational components of human ill health as substantial, they will be asked to assess their magnitudes. I would suggest to you that such current areas of ignorance as are exposed in the attempts to do this constitute a challenge for geneticists, as great as is offered by the tidier and more circumscribed problems of familiar and conventional kinds.

REFERENCES

CLARKE, C. A. (1959): The relative fitness of mutant human genotypes. In: *Natural Selection in Human Populations*, pp. 17–34. Editors: D. F. Roberts and G. A. Harrison. Pergamon Press, London.

EHLING, U. H. (1965): The frequency of X-ray induced dominant mutations affecting the skeleton of mice. *Genetics*, *51*, 723.

GOVE, J. H., SHAW, M. W. and BOURQUE, G. (1961): A family study of aniridia. *Arch. Ophthal.*, *65*, 81.

GREEN, E. L. (1968*a*): Genetic effects of radiation on mammalian populations. *Ann. Rev. Genet.*, *2*, 87.

GREEN, E. L. (1968*b*): Body weight and embryonic mortality in an irradiated population of mice. *Mutation Res.*, *6*, 437.

HALDANE, J. B. S. (1937): The effect of variation on fitness. *Amer. Nat.*, *71*, 337.

LÉONARD, A. and DEKNUDT, GH. (1969): Dose-response relationships for translocations induced by X-irradiation in spermatogonia of mice. *Radiat. Res.*, *40*, 276.

LÜNING, K. G. and SEARLE, A. G. (1971): Estimates of the genetic risks from ionizing irradiation. *Mutation Res.*, *12*, 291.

MCGREGOR, J. F. and NEWCOMBE, H. B. (1972): Dose-response relationships for yields of major eye malformations following low doses of radiation to trout sperm. *Radiat. Res.*, *49*, 155.

MULLER, M. J. (1950): Our load of mutations. *Amer. J. hum. Genet.*, *2*, 111.

NEWCOMBE, H. B. (1964): Panel discussion of the Session on Epidemiological Studies. In: *Proceedings, II International Conference on Congenital Malformations*, pp. 345–349. International Medical Conferences Ltd., New York, N.Y.

NEWCOMBE, H. B. (1968): Products from the early stages in the development of a system of linked records. In: *Record Linkage in Medicine*, pp. 7–33. Editor: E. D. Acheson. E. & S. Livingstone, Edinburgh.

SPALDING, J. F., BROOKS, M. R. and TIETJEN, G. L. (1969): Lifetime body weights and mortality distributions in mice with 10 to 35 generations of ancestral X-ray exposure. *Genetics*, *63*, 897.

STEVENSON, A. C. (1959): The load of hereditary defects in human populations. *Radiat. Res., Suppl. 1*, 306.

TRAUT, H. (1970): Non-disjunction induced by X-rays in oocytes of *Drosophila melanogaster*. *Mutation Res.*, *10*, 125.

TRAUT, H. and SCHEID, W. (1969): The dose-dependence of X-chromosome loss induced by X-rays in mature oocytes of *Drosophila melanogaster*. *Mutation Res.*, *7*, 471.

UNSCEAR (1958): Report of the United Nations Scientific Committee on the Effects of Atomic Radiation. *General Assembly Official Records, Thirteenth Session, Supplement No. 17 (A/3838)*. United Nations, New York, N.Y.

UNSCEAR (1962): *Seventeenth Session, Supplement No. 16 (A/5216)*.

UNSCEAR (1964): *Nineteenth Session, Supplement No. 14 (A/5814)*.

UNSCEAR (1966): *Twenty-First Session, Supplement No. 14 (A/6314)*.

UNSCEAR (1969): *Twenty-Fourth Session, Supplement No. 13 (A/7613)*.

A. G. Searle

Medical Research Council, Radiobiology Unit, Harwell, Didcot, United Kingdom

Chromosome damage and risk assessment

In recent years, most work on the induction of chromosomal aberrations in experimental mammals has been concerned, directly or indirectly, with reciprocal translocations. In this paper I want to discuss to what extent we can assess possible radiation risks associated with these, on the basis of experimental data and our increasing knowledge of human translocations.

Let us first compare how translocations are ascertained in mice and man. The original method of diagnosis in mice was based on the phenomenon of 'semi-sterility'. It was found that heterozygotes for known translocations produced litters which were only about half the normal size. Dissections in late pregnancy of females mated to such heterozygotes usually show that a number of embryos have died and diagnosis is based on this fact (Carter *et al.*, 1955). Death usually takes place at implantation, but some later embryonic death, often associated with exencephaly, is characteristic of certain translocations. The mean frequency of extra embryonic death associated with translocation heterozygotes has tended to be slightly over 50%. This indicates that the 50% unbalanced gametes expected from normal (adjacent-1 and alternate) disjunction of a translocation heterozygote are all transmitted as efficiently as balanced gametes but are lethal to the resultant zygotes. It also suggests that there is a small proportion of adjacent-2 disjunction, or non-disjunction of homologous centromeres, which leads to 100% unbalanced gametes, also showing normal transmission but lethal in zygotes. A recent study (Searle *et al.*, 1971) confirmed that unbalanced gametes are transmitted as well as balanced ones and showed that the frequency of adjacent-2 disjunction was about 13% in the three mouse translocations used.

Recent technical advances (Evans *et al.*, 1964; Meredith, 1969) have led to the increased use of cytological methods for diagnosing the presence of the translocations in the germ cells of male mammals. First, these methods have been used to confirm the presence of translocations by the examination of air-dried testicular preparations (usually cell suspensions) from presumptive heterozygotes. Secondly, they are used to give an estimate of the rate of translocation induction in treated spermatogonia. Spermatocytes descended from treated gonia are examined at diakinesis to metaphase I and the frequency of multivalent arrangements of chromosomes, characteristic of translocation heterozygotes, is determined.

It may be wondered whether these methods do in fact successfully detect all the translocations carried by the population of individuals or cells under scrutiny. There is some evidence, from a cytological survey of F_1 males after spermatogonial irradiation (Ford *et al.*, 1969), that the 'semi-sterility' method of diagnosis based on the amount of embryonic death is reasonably efficient in diagnosing male translocation carriers. However, Lyon and Meredith (1966) found a new category of autosomal mouse translocations which caused complete sterility in heterozygous males but semi-sterility in females. This was known to occur in

X-autosome translocations, in which there are some deleterious effects on viability of heterozygous females though not of the sterile males (Russell and Montgomery, 1969).

The autosomal translocations causing male sterility are recovered after treatment of post-meiotic male germ cells with radiation (Lyon and Meredith, 1966; Léonard and Deknudt, 1968*b*) or with chemical mutagens (Cattanach *et al.*, 1968). One has also been found in offspring of irradiated female mice (Searle and Beechey, 1971). Some of these translocations give rise to partially trisomic but viable progeny (Lyon and Meredith, 1966). When male these are also sterile, and overt phenotypic abnormalities are slight or absent. Thus, unbalanced translocation products are not always lethal in the mouse, but only a small additional fragment of chromosome is normally present in these examples.

Turning to the cytological method of estimating translocation frequencies in the treated males themselves, Ford *et al.* (1969) concluded that these frequencies are underestimated by approximately 17% because some translocations fail to form diagnostic multivalent associations in some spermatocytes. Thus, on present evidence, it seems unlikely that many translocations go undetected in radiation experiments on their induction.

Let us now consider methods of ascertainment for human translocations. In these, as in mouse translocations, the expected 1:1 ratio of carriers to normal individuals is generally obtained. So far, the largest group of propositi have been children with congenital malformations (Ford and Clegg, 1969). In most of these the translocation has been in unbalanced form, but in some it has been balanced. In families where the propositus carried an unbalanced form, Lejeune *et al.* (1970) found that the risk of a female carrier giving birth to a child with an unbalanced translocation was around 15%, while that for a male carrier was about 8%. Only occasionally have propositi been women with a history of spontaneous abortion.

In recent years a number of chromosome surveys have been carried out on populations of newborn babies (for references, see Jacobs *et al.*, 1970), with the examination of somatic chromosomes usually obtained from leukocyte cultures. The translocations identified have been mainly of two types: (1) Robertsonian, involving whole arms to give a metacentric from two acrocentrics, (2) reciprocal translocations in a balanced form, where the exchange of chromosomal material has been sufficiently unequal to allow recognition. So far, no infants with unbalanced reciprocal translocations seem to have occurred in these population surveys. Moreover, Jacobs *et al.* (1970) found no unbalanced translocation carriers in 12 families they had ascertained through a balanced heterozygote, nor in 14 of the same type studied by others. Thus, recent evidence suggests that rather a small proportion of human reciprocal translocations results in chromosomally unbalanced zygotes surviving to birth. If so, they resemble mouse translocations more than was previously thought.

Of course, even the population surveys are not free of bias, which favours Robertsonian translocations. These should all be visible in the somatic cells examined, while only a certain proportion of the reciprocal translocations can be detected. This proportion is not accurately known at present, but H. J. Evans has estimated that the efficiency of scoring symmetrical rearrangements in cultured human lymphocytes following radiation may be as low as 20% (see Court Brown and Smith, 1969). This suggests that the overall frequency of balanced reciprocal translocations in the newborn population may be up to 5 times the frequency of about 0.6 per thousand actually detected in recent surveys.

On this basis we can compare human and mouse translocations by splitting them into three groups as follows:

1. Reciprocal translocations in which the genetically unbalanced products all cause death *in utero*. This is probably the commonest type. In the mouse most of the resultant embryonic death is around the time of implantation, but some is later. If the same is true in man then this would explain the rather small increase in the frequency of abortions in this group (Jacobs *et al.*, 1970), since death at the time of implantation would at most result in a missed menstrual period for the mother. For reciprocal translocations, this seems much more

likely than other possible explanations mentioned by Jacobs *et al.* (1970), namely that the unbalanced forms of many translocations do not arise during meiosis or that they are eliminated by gametic selection. All the evidence with respect to mouse reciprocal translocations is against the intervention of these factors.

2. Robertsonian translocations, with an overall frequency of about 1 per thousand births in man. Some of these are known to produce a small proportion of viable unbalanced progeny with trisomy, especially of chromosome 21 (Down's syndrome), but population surveys suggest that most do not, neither is there evidence for extra foetal death (Jacobs *et al.*, 1970). With this type of translocation there is much greater likelihood that meiotic segregation will only give a low frequency of unbalanced gametes.

Robertsonian translocations have been found in the laboratory mouse (Evans *et al.*, 1967; Léonard and Deknudt, 1967), and the recent discovery of a wild population (*Mus poschiavinus*) with no less than 7 pairs of metacentrics (Gropp *et al.*, 1970) shows that they may have evolutionary importance. However, there is little evidence for their induction in germ cells. None seem to have been recognized in spermatocytes after spermatogonial irradiation, among thousands of undoubted reciprocal translocations, but recognition might well be difficult. Neither have any been reported in the F_1 progeny after irradiation of spermatogonia (Lyon *et al.*, 1964; Ford *et al.*, 1969), oocytes (Searle and Beechey, 1971) or post-meiotic stages in the male (Lyon and Meredith, 1966; Léonard and Deknudt, 1968*b*).

3. Reciprocal translocations, in which some of the resultant genetically unbalanced zygotes survive until birth, but are abnormal. Clearly this group is the one associated with the greatest social load. Recent evidence suggests that its frequency is lower than that for the other groups, but cannot be estimated with any accuracy at present. As mentioned earlier, this group appears to be rare among translocations recovered after irradiation of mice. Since the female mouse tends to eat her stillborn or grossly malformed liveborn offspring it might be difficult to find them in unirradiated stocks.

Let us now consider the kinetics of translocation induction in mouse spermatogonia, as revealed by the examination of descendant spermatocytes, so that we can obtain an estimate of likely frequencies of induction at low dose levels of radiation. Léonard and Deknudt (1967, 1968*a*) reported linear dose-responses between 100R and 600R acute X-irradiation and, later, between 25R and 100R. Results of other authors confirmed this finding, between 50R and 800R by Evans *et al.* (1970) and between 50R and 700R by Muramatsu *et al.* (1971). This linearity was surprising since a concave dose-response curve is generally expected for translocation induction, unless the two separate lesions interacting to produce the translocation always arise from the same ionizing track, which seemed very unlikely when sparsely ionizing radiations (of low linear energy transfer or LET) were used. Further work showed that there was a marked decline in translocation frequency at high X-ray exposures (Léonard and Deknudt, 1969; Lyon and Morris, 1969; Savković and Lyon, 1970). This is generally regarded as being a secondary distortion of the primary dose-response curve, probably caused by (1) the induction of many asymmetrical changes and other potentially lethal chromosome aberrations in the same cells as reciprocal translocations, so that both are eliminated together, or (2) a correlation between a high radiosensitivity to killing and a high genetic radiosensitivity for certain germ cell stages in the heterogeneous spermatogonial population (Oftedal, 1968; Lyon and Morris, 1969; Evans *et al.*, 1970). It seemed likely, therefore, that the linear response at lower exposures might also be a secondary one, especially since many cell generations elapse between induction of the lesions in gonia and their observation in spermatocytes. Further work has tended to confirm the belief that there is really a two-track component in the initial response. Thus Lyon *et al.* (1970*a*, *b*) found that fractionation of a large X-ray or γ-ray dose into small daily doses led to a marked reduction in translocation yield. Although the authors found evidence (Lyon *et al.*, 1970*b*) that a change in the susceptibility of the spermatogonial cell

population after repeated irradiation was an important factor in reducing the yield, it seems unlikely that all the effect was due to this. Reductions in γ-ray dose rate also lead to a marked reduction in translocation yield (Searle *et al.*, 1968) by a factor of 9 when one proceeds from acute to chronic exposures. A similar though less pronounced dose rate effect has recently been obtained for X-rays also (Searle *et al.*, 1972), in contrast to earlier results (Searle *et al.*, 1968).

It seems reasonable to suppose that the rate of induction of translocations after spermatogonial irradiation of human populations would likewise decrease if the exposure was protracted. Another important factor affecting mutation frequency is radiation quality. Fission neutron irradiation, both chronic and acute, is much more effective than X- and γ-irradiation in inducing translocations, except at high acute exposures (Searle *et al.*, 1969). The same is probably true for other types of high LET radiation.

Table 1 gives different estimates of how many translocation-carrying cells and how many

TABLE 1 *Expected yields of translocation-carrying cells and of translocations per million spermatocytes after a 1R acute X-ray exposure to spermatogonia of mice. (Based on regression coefficients given by authors cited)*

Authors	Affected cells	Translocations
Léonard and Deknudt (1967)	171	–
Léonard and Deknudt (1968*a*)	189	–
Evans *et al.* (1970)	225	290
Muramatsu *et al.* (1971)	195	214

translocations would be expected per million spermatocytes after a spermatogonial X-ray exposure of 1R, on the assumption of a linear response. The second measure (translocations per cell) used by Evans *et al.* (1970) allows for the fact that two or more translocation configurations may be observed in a single spermatocyte, especially at high exposures. One approach to the problem of how far these estimates are likely to be applicable to man is to study frequencies of translocation induction in other mammals also. Lyon and Smith (1971) have recently shown that translocation frequencies in guinea-pig and rabbit spermatocytes after spermatogonial X-ray doses of 300 rads or less were not greatly different from those in the mouse, but declined sharply at higher doses. Results so far from the Syrian hamster suggest a rather lower frequency than in the mouse.

On present evidence, therefore, it seems reasonable to assume that translocation frequencies in human spermatocytes after a spermatogonial radiation exposure will not differ greatly from those found in the mouse. However, what we are specially concerned with is the detrimental effect in the F_1 and subsequent generations. Studies on mice heterozygous for translocations have shown, as already noted, that about half the gametes behave as if unbalanced and kill the resultant zygote, while half of the balanced gametes carry the translocation. On this basis one would expect that if a frequency p of spermatocytes carry a translocation, then p/2 of the spermatozoa will have unbalanced translocation products and p/4 will carry the balanced translocation. If zygotes carrying the unbalanced products are lethal, then $p/4 \div (1 - p/2)$ of survivors will carry the balanced translocation (Lyon *et al.*, 1964).

A comparison by Ford *et al.* (1969) of the frequency of translocations in the spermatocytes of exposed mice with the frequency of translocation heterozygotes in offspring of the same males has shown that only about half the expected number of offspring do in fact carry balanced translocations. The results of other experiments suggest that the amount of embryonic lethality is reduced below expectation to about the same extent. The most likely explanation for this reduced transmission is that certain translocations prevent the successful completion of gametogenesis in germ cells carrying them. As already discussed, male-

sterile autosomal translocations are recovered from the products of irradiated post-meiotic germ cells; they have not been found after spermatogonial irradiation (Ford *et al.*, 1969). This sterility results, not from any lack of libido, but from a failure of gametogenesis, so that few or no spermatozoa are produced. It is logical to assume, therefore, that the reason why translocations of this type are not recovered after spermatogonial irradiation is that the spermatogonia in which they are induced would first have to pass successfully through all the remaining stages of gamete formation, but cannot do so. The reason for the effect of these balanced translocations on gametogenesis is still unknown, but it is interesting to note that those reaching meiosis are often associated with high frequencies of chain rather than ring quadrivalents and thus lack chiasma formation in one of the four arms (Lyon and Meredith, 1966; see also Searle, 1971).

Figure 1 summarizes our present beliefs about the behaviour of balanced translocations

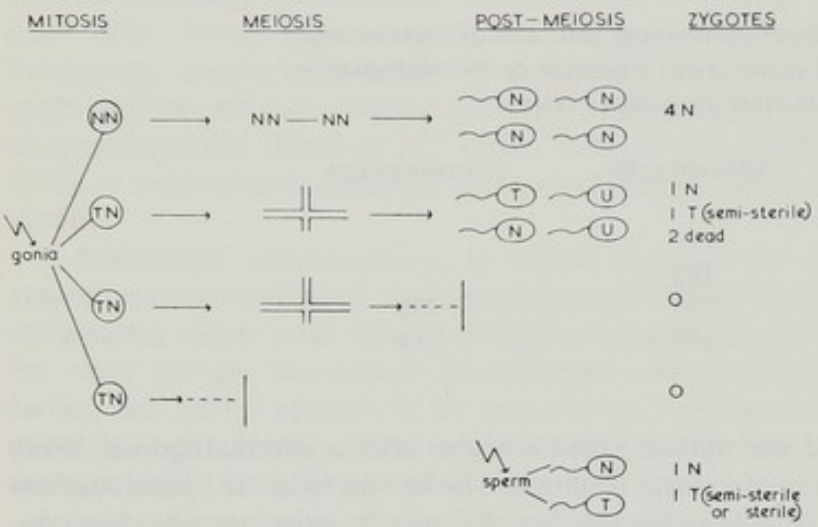


Fig. 1. Possible fates of reciprocal translocations induced in spermatogonia and spermatozoa of mice. N = normal, T = translocation-carrying, and U = unbalanced genome.

in the mouse. Since the results of spermatozoal irradiation show that a number of these cause failure of gametogenesis already at the spermatogonial stage in heterozygotes, it seems likely that the overall reduction in the transmission of balanced translocations, between spermatogonial induction and the next generation, must be greater than that between meiosis and the next generation, estimated as around 50% by Ford and co-workers. Recent findings by Chandley (1971) on chromosome abnormalities in subfertile and sterile men suggest that balanced translocations affecting male gametogenesis exist also in man.

The expected frequency of translocation heterozygotes in F_1 mice after paternal spermatogonial irradiation can be derived from the published data on frequencies in paternal spermatocytes by using appropriate reduction factors, but it seems more appropriate to derive it directly from tests on the F_1 progeny themselves. On this basis, Lüning and Searle (1971) estimated that the spontaneous mutation frequency to heritable semi-sterility was about 1 per thousand in mice, while the induced rate, on the assumption of linearity, was 34 per million per R. On this basis, an exposure of 31R acute X-rays should induce as many semi-steriles as occur spontaneously.

In the mouse, as we have seen, heritable semi-sterility can be equated with translocation heterozygosity, with a fair degree of confidence when dealing with spermatogonial irradiation. The detrimental effect of translocation induction will mainly show up, not in the transmitted translocations themselves, but in the unbalanced products of translocations. These will appear in the F_1 generation from translocations induced in meiotic or pre-meiotic germ cells

of an exposed population, and in the F_2 and subsequent generations from translocations reaching the F_1 generation in a balanced form.

Thus, we would expect that if 34 gametes per million would carry an induced balanced translocation, then at least 68 per million would carry an induced unbalanced translocation product, the exact figure depending on the extent of adjacent-2 disjunction. In the mouse these unbalanced genomes are generally lethal; independent estimates of the magnitude of this lethal effect come from experiments on the frequency of induced dominant lethality after spermatogonial irradiation. Lüning and Searle (1971) have analysed recent data, which lead to an estimated mean rate of induction of 86 dominant lethals per million genomes per R. Nearly all of the dominant lethality is now regarded as the secondary result of translocation induction (Ford *et al.*, 1969). There will, of course, be additional detriment in the F_2 and later generations, because heterozygotes for the balanced translocations which are inherited will produce about 50% unbalanced gametes, and will also transmit the translocations to about half their phenotypically normal progeny.

So far we have only considered effects in males, but information on females is also necessary if we are to assess the effects of population exposure. Work on these is difficult in mice, since the immature mouse oocyte is exceptionally sensitive to radiation killing. Fairly large X-ray doses can be given to the maturing oocyte, however, and F_1 offspring can be tested for fertility. By this method, L. B. Russell and Wickham (1957) found a very low incidence of semi-sterility in sons of irradiated females. Recently, however, we carried out similar tests on both sons and daughters of irradiated females. We found no evidence for semi-sterility in the sons but substantial evidence for it in the daughters (Searle and Beechey, 1971). The reason for this surprising sex difference is unknown, but may be due to chance. Further experiments are necessary, but these results raise the possibility that the overall rate of induction of F_1 semi-sterility in female mice (after irradiation of maturing oocytes) is not greatly different from that in the male after spermatogonial irradiation. If so, this may help to explain the fairly high incidence of embryonic dominant lethality induced by irradiation of late dictyate oocytes in the mouse (Russell and Russell, 1956; Edwards and Searle, 1963). Because translocations induced in oocytes would presumably be chromatid rather than chromosome exchanges, it can be calculated that an egg developed from an affected oocyte would be 6 times more likely to have an unbalanced form of the translocation (acting as a dominant lethal) than the balanced one. Evidence from female mice heterozygous for translocations shows that the unbalanced gametes are transmitted just as readily as the balanced ones.

In order to gain some idea of the possible risks to man from translocation induction, let us assume that a 1 rad dose of acute X-rays to mouse spermatogonia or oocytes does induce an extra 34 balanced translocations per million gametes, and that the same rates apply to man. Table 2 shows the expected numbers per million progeny with induced balanced translocations and with unbalanced translocation products. The expected figure of 272 for the latter in the F_1 generation is composed of 68 per million unbalanced genomes associated with the balanced translocations transmitted in male gametes and 204 associated with the balanced

TABLE 2 *Expected numbers, per million progeny, of balanced and unbalanced translocation carriers after acute exposures of 1R X-rays to parental population, if the rate of induction of balanced translocations is 34 × 10^{-6} per male and female gamete per R*

Generation	With balanced genome	With unbalanced genome
F_1	68	272
F_2	34+	68

translocations transmitted in female gametes. At least half the gametes produced by the unaffected balanced translocation carriers would be expected to have unbalanced genomes, giving the 68 per million zygotes with unbalanced genomes in the next generation. About one quarter of the gametes from the balanced translocation heterozygotes would have the translocation in balanced form, so that at least 34 per million of the F_2 generation would be expected to carry the balanced translocation, but the actual number would depend on the times of death of progeny with unbalanced genomes. The situation in the F_3 and subsequent generations is too uncertain for exploration at this time.

The next problem is to try and assess how much of a detriment, or social load, would result from the estimated 340 carriers of unbalanced genomes in the F_1 and F_2 generation. It seems likely, from our present knowledge of human translocations, that most would die in early embryonic life (as in the mouse) and that few would survive to become malformed infants. It seems doubtful, however, whether there is sufficient information yet to allow one to estimate the actual proportion likely to survive until birth. This would partly depend on whether the Robertsonian type of translocation is readily inducible by radiation, which seems unlikely on the basis of mouse data (see also Hecht and Kimberling, 1971).

When spontaneous mutation frequencies for human translocations can be more accurately estimated, then it will become possible to estimate rates of induction by using the appropriate doubling dose (Lüning and Searle, 1971).

Let us now consider briefly how one would expect these or other risk estimates based on acute X-irradiation to be altered under different conditions:

1. With chronic γ-irradiation, reduced by a factor of about 9, on the basis of results in the male (Phillips and Searle, 1964; Searle *et al.*, 1968).
2. With acute γ-irradiation, reduction by a factor of about 1.6 (Searle *et al.*, 1971).
3. With fission neutron irradiation, increase by a factor of about 4, based on results in the male (Searle *et al.*, 1969).

Risk estimates for low acute exposures to X-rays or γ-rays would also be reduced if it was definitely established that the yield of translocation from single small doses was less than expected from high doses, because of more efficient repair or for other reasons. This problem is being actively studied (Lyon *et al.*, 1970*a*, *b*). However, risk estimates might be more than doubled if all translocations in their balanced form were fully transmissible in man.

Unless the frequency of non-disjunction in man can be markedly increased by irradiation at low doses, it seems likely that the induction of translocations is the most important of the chromosomal hazards resulting from irradiation. It will be appreciated that the risk estimates given here are probably not even first approximations to the true situation, but perhaps they may help to define the areas in which more information is most needed. One of the most important of these concerns the oocyte, especially the problem of whether the genetic radiosensitivity of the human resting oocyte more closely resembles that of the maturing dictyate oocyte (as assumed in previous calculations here) or the immature dictyate oocyte, from which virtually no mutations can be recovered (W.L. Russell, 1967). There are many other problems of extrapolation from mouse to man with respect to cytogenetic radiosensitivity, but various attempts to bridge the gap are being made (see Searle, 1971). In addition, the recent exciting technical advances which reveal new landmarks on mammalian chromosomes will help us to revise our ideas on the overall incidence of reciprocal translocations in man and give us a better picture of their spontaneous frequency and likely detrimental effect.

REFERENCES

Carter, T. C., Lyon, M. F. and Phillips, R. J. S. (1955): Gene-tagged chromosome translocations in eleven stocks of mice. *J. Genet.*, *53*, 154.

Cattanach, B. M., Pollard, C. E. and Isaacson, J. H. (1968): Ethyl methanesulfonate induced chromosome breakage in the mouse *Mutation Res.*, *6*, 297.

CHANDLEY, A. C. (1971): Personal communication.
COURT BROWN, W. M. and SMITH, P. G. (1969): Human population cytogenetics. *Brit. med. Bull.*, *25*, 74.
EDWARDS, R. G. and SEARLE, A. G. (1963): Genetic radiosensitivity of specific post-dictyate stages in mouse oocytes. *Genet. Res.*, *4*, 389.
EVANS, E. P., BRECKON, G. and FORD, C. E. (1964): An air-drying method for meiotic preparations from mammalian testes. *Cytogenetics*, *3*, 289.
EVANS, E. P., FORD, C. E., SEARLE, A. G. and WEST, B. J. (1970): Studies on the induction of translocations in mouse spermatogonia. III. Effects of X-irradiation. *Mutation Res.*, *9*, 501.
EVANS, E. P., LYON, M. F. and DAGLISH, M. (1967): A mouse translocation giving a metacentric marker chromosome. *Cytogenetics*, *6*, 105.
FORD, C. E. and CLEGG, H. M. (1969): Reciprocal translocations. *Brit. med. Bull.*, *25*, 110.
FORD, C. E., SEARLE, A. G., EVANS, E. P. and WEST, B. J. (1969): Differential transmission of translocations induced in spermatogonia of mice by irradiation. *Cytogenetics*, *8*, 447.
GROPP, A., TETTENBORN, U. and VON LEHMANN, E. (1970): Chromozomenvariation vom Robertsonschen Typus bei der Tabakmaus, *M. poschiavinus* und ihren Hybriden mit der Laboratoriumsmaus. *Cytogenetics*, *9*, 9.
HECHT, F. and KIMBERLING, W. J. (1971): Patterns of D chromosome involvement in human (DqDq) and (DqGq) Robertsonian rearrangements. *Amer. J. hum. Genet.*, *23*, 361.
JACOBS, P. A., AITKEN, H., FRACKIEWICZ, A., LAW, P., NEWTON, M. S. and SMITH, P. G. (1970): The inheritance of translocations in man: data from families ascertained through a balanced heterozygote. *Ann. hum. Genet.*, *34*, 119.
LEJEUNE, J., DUTRILLAUX, B. and DE GROUCHY, J. (1970): Reciprocal translocations in human populations, a preliminary analysis. In: *Human Population Cytogenetics*, pp. 82–87. Editors: P. A. Jacobs, W. H. Price and P. Law. University Press, Edinburgh.
LÉONARD, A. and DEKNUDT, G. (1967): Relation between the X-ray dose and the rate of chromosome rearrangements in spermatogonia of mice. *Radiat. Res.*, *32*, 35.
LÉONARD, A. and DEKNUDT, G. (1968*a*): Chromosome rearrangements after low X-ray doses given to spermatogonia of mice. *Canad. J. Genet. Cytol.*, *10*, 119.
LÉONARD, A. and DEKNUDT, G. (1968*b*): The sensitivity of various germ-cell stages of the male mouse to radiation induced translocations. *Canad. J. Genet. Cytol.*, *10*, 495.
LÉONARD, A. and DEKNUDT, G. (1969): Dose response relationship for translocations induced by X-irradiation in spermatogonia of mice. *Radiat. Res.*, *40*, 276.
LÜNING, K. G. and SEARLE, A. G. (1971): Estimates of the genetic risks from ionizing irradiation. *Mutation Res.*, *12*, 291.
LYON, M. F. and MEREDITH, R. (1966): Autosomal translocations causing male sterility and viable aneuploidy in the mouse. *Cytogenetics*, *5*, 335.
LYON, M. F. and MORRIS, T. (1969): Gene and chromosome mutation after large fractionated or unfractionated radiation doses to mouse spermatogonia. *Mutation Res.*, *8*, 191.
LYON, M. F., MORRIS, T., GLENISTER, P. and O'GRADY, S. E. (1970*a*): Induction of translocations in mouse spermatogonia by X-ray doses divided into many small fractions. *Mutation Res.*, *9*, 219.
LYON, M. F., PHILLIPS, R. J. S. and GLENISTER, P. (1970*b*): Dose-response curve for the yield of translocations in mouse spermatogonia after repeated small radiation doses. *Mutation Res.*, *10*, 497.
LYON, M. F., PHILLIPS, R. J. S. and SEARLE, A. G. (1964): The overall rates of dominant and recessive lethal and visible mutation induced by spermatogonial X-irradiation of mice. *Genet. Res.*, *5*, 448.
LYON, M. F. and SMITH, B. D. (1971): Species comparisons concerning radiation-induced dominant lethals and chromosome aberrations. *Mutation Res.*, *11*, 45.
MEREDITH, R. (1969): A simple method for preparing meiotic chromosomes from mammalian testis. *Chromosoma (Berl.)*, *26*, 254.
MURAMATSU, S., NAKAMURA, W. and ETO, H. (1971): Radiation-induced translocations in mouse spermatogonia. *Jap. J. Genet.*, *45/5*, *46*, 281.
OFTEDAL, P. (1968): A theoretical study of mutant yield and cell killing after treatment of heterogeneous cell populations. *Hereditas (Lund)*, *60*, 171.
PHILLIPS, R. J. S. and SEARLE, A. G. (1964): The effect of dose-rate on the yield of translocations and dominant lethals following spermatogonial irradiation of mice. *Genet. Res.*, *5*, 468.
RUSSELL, L. B. and MONTGOMERY, C. S. (1969): Comparative studies on X-autosome translocations in the mouse. I. Origin, viability, fertility, and weight of five *T* (X; 1)'s. *Genetics*, *63*, 103.
RUSSELL, L. B. and RUSSELL, W. L. (1956): The sensitivity of different stages in oogenesis to the

radiation induction of dominant lethals and other changes in the mouse. In: *Progress in Radiobiology*, pp. 187–192. Editors: J. S. Mitchell, B. E. Holmes and C. L. Smith. Oliver and Boyd, Edinburgh.

Russell, L. B. and Wickham, I. (1957): The incidence of disturbed fertility among male mice conceived at various intervals after irradiation of the mother. *Genetics*, *42*, 392.

Russell, W. L. (1967): Repair mechanisms in radiation mutation induction in the mouse. *Brookhaven Symp. Biol.*, *20*, 179.

Savković, N. V. and Lyon, M. F. (1970): Dose-response curve for X-ray-induced translocations in mouse spermatogonia. I. Single doses. *Mutation Res.*, *9*, 407.

Searle, A. G. (1971): Symposium on mammalian radiation genetics. July 7–9th, 1970. Summary and synthesis. *Mutation Res.*, *11*, 133.

Searle, A. G. and Beechey, C. V. (1971): Unpublished information.

Searle, A. G., Beechey, C. V., Corp, M. J. and Papworth, D. G. (1972): A dose-rate effect on translocation induction by X-irradiation of mouse spermatogonia. *Mutation Res.*, *15*, 89.

Searle, A. G., Beechey, C. V., Evans, E. P., Ford, C. E. and Papworth, D. G. (1971): Studies on the induction of translocations in mouse spermatogonia. IV. Effects of acute gamma irradiation. *Mutation Res.*, *12*, 411.

Searle, A. G., Evans, E. P., Ford, C. E., West, B. J. with appendix by Papworth, D. G. (1968): Studies on the induction of translocations in mouse spermatogonia. I. The effect of dose-rate. *Mutation Res.*, *6*, 427.

Searle, A. G., Evans, E. P. and West, B. J. (1969): Studies on the induction of translocations in mouse spermatogonia. II. Effects of fast neutron irradiation. *Mutation Res.*, *7*, 235.

Searle, A. G., Ford, C. E. and Beechey, C. V. (1971): Meiotic disjunction in mouse translocations and the determination of centromere position. *Genet. Res.*, *18*, 215.

A. Léonard

Laboratoire de Génétique, Département de Radiobiologie CEN/SCK, Mol, Belgique

Données récentes sur les taux de mutations radio-induites chez les mammifères

Jusqu'aux environs de 1950, l'estimation des effets génétiques d'une exposition aux radiations ionisantes était basée essentiellement sur les résultats des recherches expérimentales poursuivies sur la Drosophile. Depuis lors cependant, on a accumulé de plus en plus de données sur un organisme bien plus proche de l'homme, puisqu'il s'agit d'un mammifère, la souris. C'est donc avant tout des recherches effectuées sur les effets génétiques des radiations ionisantes chez les petits mammifères et de l'extrapolation possible à l'homme des résultats obtenus que je vous entretiendrai ici.

Au fur et à mesure que progressaient ces travaux sur les mammifères certains chercheurs ont été amenés à penser que les 'vraies' mutations, entendez par là les simples changements de bases dans l'acide désoxyribonucléique, étaient rarement induites par les radiations ionisantes chez ces organismes. Une bonne partie des arguments en faveur de cette théorie provient de la comparaison de l'induction dans les spermatogonies, d'une part des mutations de locus spécifiques qui jusqu'il y a peu étaient considérées comme des mutations géniques typiques, et d'autre part des translocations (Léonard, 1971) qui constituent assurément un des exemples les plus caractéristiques de remaniement chromosomique. Il est en effet particulièrement intéressant de comparer la réponse de ces deux types d'effets génétiques aux critères utilisés en radiogénétique pour distinguer les mutations géniques des mutations dites chromosomiques. Ces critères sont (Wolff, 1967) la forme de la relation dose d'irradiation-taux de mutations, l'efficacité biologique relative (RBE) des différents types de rayonnement, l'effet du débit et du fractionnement. Si on considère en effet la théorie des cibles il apparaît qu'un seul point d'impact est suffisant pour induire une mutation ponctuelle ou génique ou une délétion terminale tandis que des remaniements plus complexes du type translocation ou délétion interstitielle requièrent deux points d'impact. On peut en déduire que les changements génétiques du premier type ont un taux variant linéairement avec la dose d'irradiation et ne sont pas influencés par le débit, le fractionnement ou l'énergie du rayonnement. Les changements génétiques du second type par contre sont influencés par le débit, le fractionnement et l'énergie du rayonnement; de plus leur taux varie comme le carré de la dose reçue. Ces critères ont en fait une valeur beaucoup plus théorique que pratique étant donné que la réaction primaire à l'irradiation peut être perturbée par différents facteurs tels que l'élimination sélective des cellules les plus lésées (Russell, 1965*a*, *b*; Oftedal, 1968), le manque d'homogénéité des populations cellulaires irradiées (Gerber et Léonard, 1971) et les phénomènes de restauration (Kimball, 1966; Sobels, 1969*a*; Russell, 1967*b*). C'est en se basant sur l'existence de ces différents facteurs que l'on a expliqué et admis jusqu'à ces derniers temps que les mutations de locus spécifiques et les translocations, que l'on considérait cependant comme fondamentalement différentes, pouvaient l'une et l'autre varier linéairement avec la dose d'irradiation et être in-

fluencées par le débit, le fractionnement et l'énergie du rayonnement. D'autres arguments expérimentaux ont été émis également en faveur de la théorie que dans les organismes supérieurs les mutations induites sont rarement de type génique. Ce sont les recherches de Lifschitz et Falk (1968) chez la Drosophile, l'analyse des mutants de la tomate (Khush et Rick, 1968) et les travaux de Brewbaker et Natarajan (1960), Lewis (1951) et Pandey (1965) sur le locus *S* d'auto-incompatibilité. Si l'on tient compte cependant des résultats expérimentaux récents enregistrés par L.B. Russell (1971) qui a analysé de façon très détaillée la région *d-se* du chromosome II de la souris et qui sont en faveur de la nature génique des mutations de locus spécifiques, on se rend compte que la controverse est loin d'être terminée.

C'est pour cette raison, que dans cet exposé je traiterai des changements génétiques induits par les radiations sans tenir compte de leur nature exacte.

Je n'envisagerai cependant que très brièvement l'induction de la sémistérilité. Il s'agit là en effet d'un problème qui est actuellement étudié principalement au moyen de techniques relevant de la cytogénétique et dont le Dr A.G. Searle vient de vous entretenir.

RÉSULTATS DES RECHERCHES EXPÉRIMENTALES

1. MUTATIONS DE LOCUS SPÉCIFIQUES

Ces mutations ont, depuis 1951, fait l'objet des travaux remarquables poursuivis tant à Harwell qu'à Oak Ridge. La technique utilisée, je vous le rappelle, consiste à croiser des souris traitées ou non traitées de type sauvage à des animaux homozygotes pour certains gènes récessifs. Une mutation pour un de ces locus existant à l'état hétérozygote chez les hybrides pourra être détectée dans les descendants pour autant évidemment que la mutation ait un effet phénotypique visible. Cette technique a d'ailleurs été utilisée chez la Drosophile par Valencia et Muller (1949) et Bonnier et Lüning (1949). Quoique requérant des nombres impressionnants d'animaux, elle présente des avantages certains puisque les mutations sont détectées en F_1 alors que les mutations autosomales récessives de locus non spécifiques nécessitent deux générations pour être mises en évidence. De plus les mutations de locus spécifiques sont discernables par un simple examen et autorisent des comparaisons avec ce qui a été obtenu chez la Drosophile. Utilisant un groupe de 7 locus spécifiques Russell et ses collaborateurs se sont avant tout attachés à l'étude des mutations de locus spécifiques induites dans les spermatogonies et dans les oöcytes.

a. Mutations de locus spécifiques produites par irradiation des spermatogonies

Dans les spermatogonies le taux de mutations de locus spécifiques augmente avec la dose d'irradiation administrée. Il diminue brusquement pour des doses d'irradiation supérieures à 600 R, ce qui est attribué à une élimination sélective des cellules les plus radiosensibles à la fois à la mort cellulaire et aux mutations induites (Russell, 1965*a*, 1968). Alors que chez la souris mâle le taux moyen de mutation spontanée des 7 locus utilisés varie entre $0{,}46 \times 10^{-5}$ et $1{,}0 \times 10^{-5}$ par locus et par génération, Russell (1951, 1956, 1965*a*) a obtenu après irradiation par rayons X un taux moyen de mutations de $2{,}2 \times 10^{-7}$ par locus, par gamète et par R.

Utilisant les 6 locus HT (dont un seul était commun aux groupes PT et HT) (Tableau 1), Lyon et ses collaborateurs (Lyon et Morris, 1966, 1969) ont obtenu un taux de mutations de $0{,}78 \times 10^{-7}$ par locus, par gamète et par R significativement inférieur à celui trouvé par Russell mais cependant 2 à 3 fois supérieur à ce que l'on observe chez la Drosophile. Il convient d'ajouter qu'un test de viabilité des homozygotes a montré que sur 5 mutations testées sur les 7 obtenues par Lyon et Morris (1966, 1969) une seule était létale à l'état homozygote tandis que chez Russell 75 % des mutations pour les 7 locus spécifiques étaient létales à l'état homozygote.

Les travaux de Russell (1963*a*, *b*, 1965*b*, 1968) ont mis en évidence l'influence du débit sur

TABLEAU 1 *Génotype des animaux utilisés pour l'étude des mutations de locus spécifique*

Laboratoire	Symbole	Mutation	Groupe de 'linkage'	Référence
Oak Ridge (locus PT)	a	non-agouti	V	Russell (1951)
	b	brun	VIII	
	c^{ch}	chinchilla	I	
	d	dilution	II	
	p	dilution de la couleur rose de l'oeil	I	
	s	taches de couleur	III	
	se	oreilles courtes	II	
Harwell (locus HT)	a	non-agouti	V	Lyon et Morris (1966)
	bp	membres courts	V	
	fz	poils bouclés	XIII	
	ln	couleur de plomb	XIII	
	pa	couleur pâle	V	
	pe	couleur perle	XIV	

TABLEAU 2 *Effets du débit sur le taux de mutations de locus spécifiques induites par une irradiation des spermatogonies avec des doses de 300 à 600 R*

Nature du rayonnement	Débit par minute	Taux de mutations par locus, par gamète et par roentgen $\times 10^{-7}$
RX	1000	3,0
	90	2,2–2,9
	60–70	2,5
	9	1,4
γ	0,8	0,85
	0,009	0,80
	0,001	0,98–1,40

(D'après Russell, 1965*a*, 1968.)

le taux de mutations radio-induites dans les spermatogonies. Les résultats de ces travaux repris dans le Tableau 2 montrent que le taux de mutations induites par des doses de 300 à 600 R diminue lorsque le débit varie de 90 R/min à 0,8 R/min. Une diminution du débit jusqu'à 0,001 R/min ne modifie plus le taux de mutations. Cet effet du débit est interprété en termes de restauration. Ainsi que l'on pourrait s'y attendre étant donné l'efficacité biologique élevée de ce type de rayonnement Batchelor et coll. (1964, 1966, 1967) ont observé qu'une dose aiguë de neutrons est moins efficace qu'un dose chronique: 188 rads (+18 rads de contamination gamma) ont donné un taux moyen de mutations pour les locus PT de $0{,}15 \times 10^{-6}$ mutations par locus, par gamète et par R tandis que 62 rads (+42 rads de contamination gamma) répartis sur une période de 12 semaines ont donné $1{,}33 \times 10^{-6}$ mutations par locus, par gamète et par R.

Après des doses aiguës supérieures à 600 R de rayons X, le taux de mutations baisse fortement par suite d'une élimination sélective des cellules les plus radiosensibles (Russell, 1963*b*, 1965*a*, *b*). Si une dose élevée est administrée en deux fractions séparées par un intervalle de temps de 24 heures, on constate une augmentation de taux de mutations quelque soit le groupe de locus spécifiques utilisés (Russell, 1962; Lyon et Morris, 1969). Cet effet semble s'expliquer

à la fois par une synchronisation provoquée par la première fraction de la dose et par une élimination réduite des cellules les plus radiosensibles.

b. Mutations de locus spécifiques produites par une irradiation des oöcytes

On constate en général que l'irradiation des oöcytes mûrs avec des doses élevées de rayons X produit plus de mutations que l'irradiation des spermatogonies. Dès 1959, Russell et coll. ont clairement démontré que l'effet du débit était beaucoup plus prononcé chez les oöcytes que chez les spermatogonies. Aux très faibles débits d'ailleurs (0,009 R/min) on ne constate aucune augmentation de la fréquence des mutations spontanées après des doses de 258 à 600 R (Russell et Kelly, 1965). Selon Russell (1963*b*, 1965*b*, *c*) il est possible qu'une irradiation chronique produise moins de dommages au système de réparation qu'une irradiation aiguë ou encore que le système de réparation puisse seulement réparer une certaine quantité de dommages en un temps donné de sorte qu'une irradiation à faible débit permette une réparation optimum. Ces hypothèses ont été en partie confirmées par la découverte (Russell et Kelly, 1965) qu'une dose de 50 R donnée à un débit de 90 R/min à des souris femelles produit beaucoup moins de mutations (11 mutants sur 169.325 descendants) que le nombre attendu (28) sur la base d'une extrapolation linéaire des résultats obtenus avec 400 R (Russell, 1967*b*) et d'autre part par la mise en évidence de l'effet marqué du fractionnement. C'est ainsi qu'une dose de 400 R administrée à un débit de 90 R/min en 8 fractions de 50 R séparées l'une de l'autre par un intervalle de temps de 75 minutes a produit 13 mutants sur 23.387 descendants, ce qui est significativement en dessous des 34 attendus sur la base des résultats obtenus avec une dose aiguë de 400 R laquelle induit un taux moyen de $4{,}8 \times 10^{-7}$ mutations par locus, par gamète et par R.

La découverte la plus importante en ce qui concerne l'induction des mutations de locus spécifiques chez les femelles est cependant celle de la dépendance étroite existant entre la fréquence des mutations et l'intervalle de temps s'écoulant entre l'irradiation et la conception. Cet effet, d'abord mis en évidence pour une irradiation avec neutrons (Russell, 1965*c*) a récemment été confirmé pour une irradiation avec rayons X (Russell, 1967*b*). Après une exposition de souris femelles à une dose de 50 R de rayons X, 11 mutations ont été observées parmi les 155.882 descendants conçus durant les 7 premières semaines et aucun parmi les 71.324 descendants conçus plus tard. Il est difficile de dire avec certitude à l'heure actuelle si ces différences sont dues à des différences de radiosensibilité, à des mécanismes de restauration plus actifs ou à une sélection germinale. L'observation par Oakberg (1968) de l'incorporation plus intense de l'uridine tritiée dans les oöcytes jeunes pourrait indiquer que les mécanismes de réparation y sont de fait beaucoup plus effectifs mais nous ne devons pas perdre de vue, ainsi que le fait remarquer Searle (1966), que la plus grande sensibilité des oöcytes jeunes à la mort cellulaire radio-induite est en faveur de l'hypothèse d'une élimination sélective des cellules lésées.

2. MUTATIONS VISIBLES DOMINANTES ET RÉCESSIVES

Le taux de mutations visibles récessives spontanées de locus non spécifiques a été estimé à $7{,}04 \times 10^{-3}$ par génération mais avec de très larges variations (Lyon et coll., 1964). Après une irradiation des spermatogonies ces mêmes auteurs observent un taux moyen de $1{,}8 \times 10^{-5}$ mutants récessifs visibles par gamète et par R, ce qui semble nettement plus élevé que les valeurs que l'on peut déduire des expériences sur les mutations de locus spécifiques. Cette différence s'expliquerait peut-être en partie par le fait qu'un certain nombre des mutants pour un locus spécifique sont létaux à l'état homozygote. Ce taux de mutants récessifs visibles est quelques 40 fois plus élevé que le taux de mutants dominants visibles. Le taux de dominants visibles spontanés est en effet seulement de 81×10^{-7} par génération et le taux de mutations radio-induites $4{,}96 \times 10^{-7}$ par gamète et par R (Lüning et Searle, 1971, pour revue). Diverses hypothèses ont été émises (Report U.N., 1966) pour expliquer ces différences, telle l'existence dans

le génome de deux groupes de locus l'un, le plus abondant, pouvant donner des mutations récessives, l'autre moins nombreux comprenant des locus pouvant donner des mutations dominantes. Dans cette hypothèse le taux de mutations serait identique dans les deux groupes de locus. On pourrait également admettre que quelques rares locus puissent muter, mais à des taux différents, en une mutation dominante ou récessive. On peut encore ajouter que, tout comme cela se vérifie pour les mutations de locus spécifiques, le taux de mutants visibles dominants augmente aux doses élevées, que l'irradiation chronique avec des neutrons produit plus de mutations que l'irradiation aiguë et qu'elle est plus efficace que l'irradiation chronique avec des rayons gamma (Batchelor et coll., 1964, 1966, 1967).

3. MUTATIONS LÉTALES DOMINANTES ET RÉCESSIVES

a. Létalité dominante

Par létalité dominante il faut entendre les changements génétiques qui provoquent la mort des hétérozygotes avant la naissance ou peu de temps après, à l'exclusion de tous les changements qui affectent les gamètes eux-mêmes et les rendent incapables d'une fertilisation normale. Les létaux dominants résultant d'une irradiation des stades préméiotiques sont surtout la conséquence des translocations induites à ces stades alors que ce sont des délétions qui expliquent en grande partie la létalité provoquée par une irradiation des stades postméiotiques.

Irradiation des cellules reproductrices mâles Pour la létalité dominante comme pour les autres mutations on observe une différence nette de radiosensibilité entre les différents types cellulaires. L'irradiation des spermatogonies donne chez la souris un taux de 0,4 à $1,3 \times 10^{-4}$ létaux dominants par gamète et par R (Lyon et coll., 1964; Sheridan, 1965; Pomerantzeva et Ramaiya, 1969; Schröder, 1969), et chez le rat un taux voisin de 0,9 à $1,2 \times 10^{-4}$ par gamète et par R (Taylor, 1968; Taylor et Chapman, 1969*a*, *b*). Il existe cependant d'une espèce à l'autre (Lyon et Smith, 1971) certaines différences en ce qui concerne le moment exact où la mort intervient avant la naissance. Ici également le fractionnement d'une dose faible réduit le taux de mutations radio-induites (Sheridan, 1968) tandis que l'inverse s'observe pour le fractionnement d'une dose élevée (Lyon et Morris, 1969). Ces effets contradictoires s'expliquent sans doute par des phénomènes de restauration dans le premier cas et d'élimination sélective dans le second. Ajoutons encore que le taux de létaux dominants induits par une irradiation des spermatogonies est influencé par le débit (Philips et Searle, 1964; Lyon et coll., 1964) et que les létaux dominants induits par irradiation des spermatogonies continuent à être transmis aussi longtemps que dure la vie de l'animal (Sheridan, 1965).

Le taux de létaux dominants induit par une irradiation des spermatozoïdes est voisin de $1,8 \times 10^{-3}$ par gamète et par R (Léonard, 1966; Léonard et Deknudt, 1967). Entre les espèces existent certaines différences (Lyon, 1970; Lyon et Smith, 1971; Gilliavod et Léonard, 1971). C'est ainsi que si la souris et le rat ont une radiosensibilité voisine, le lapin et le cobaye sont moins radiosensibles.

Irradiation des cellules reproductrices femelles Ce sont les oöcytes irradiés durant la métaphase de la première division méiotique qui sont les plus radiosensibles. L'anaphase I et la métaphase II sont moins radiosensibles mais le sont cependant encore beaucoup plus que le stade des pronucléus. L'irradiation des oöcytes mûrs produit presque autant de létaux dominants que l'irradiation des spermatozoïdes (Edwards et Searle, 1963).

b. Létalité récessive

Il convient de distinguer ici les létaux récessifs autosomiques des létaux récessifs liés au sexe. Les létaux récessifs autosomiques ont été étudiés chez la souris par Lyon et coll. (1964) et par Lüning (1964). Ces auteurs quoique utilisant des techniques différentes sont arrivés après irradiation des spermatogonies à des estimations très voisines l'une de l'autre ($2,46 \times 10^{-4}$

par gamète et par R et 0,8–2,0 $\times 10^{-4}$ par gamète et par R). Ces valeurs sont nettement inférieures à ce que l'on pourrait s'attendre en se basant sur les travaux de Russell avec les locus spécifiques mais concordent assez bien avec les estimations basées sur les résultats de Lyon et Morris (1966). Chez le rat (Taylor et Chapman, 1969*a*, *b*) le taux de létaux récessifs est de $1,6 \times 10^{-4}$ par gamète et par R.

Différentes estimations des taux de létalité récessive liée au sexe ont été proposées tant pour le rat que pour la souris. Chez cette dernière espèce le taux est de $1,7 \times 10^{-4}$ par gamète et par R (Searle, 1964) et chez le rat de $1,6 \times 10^{-4}$ par gamète et par R. Tout porte à croire cependant que les vrais létaux récessifs liés au sexe contribuent en fait fort peu à ces taux ainsi que le montrent les résultats récents enregistrés par Schröder (communication personnelle).

EXTRAPOLATION À L'HOMME DES RÉSULTATS DES RECHERCHES EXPÉRIMENTALES

L'extrapolation à l'homme des résultats des recherches expérimentales effectuées sur les rongeurs demande d'une part que les cellules reproductrices de l'homme aient une radiosensibilité voisine de celle des rongeurs et que d'autre part les conditions d'irradiation soient à peu près semblables.

1. RADIOSENSIBILITÉ DES CELLULES REPRODUCTRICES MÂLES

Les résultats des recherches expérimentales montrent bien (Tableau 3) que quelque soit le type de mutation envisagé la radiosensibilité génétique des stades postméiotiques est beaucoup plus élevée que celle des stades préméiotiques. S'il existe évidemment des différences énormes

TABLEAU 3 *Radiosensibilité génétique comparée des différents types de cellules mâles*

Type cellulaire	Mutation		
	Létalité dominante par R $\times 10^{-4}$	Translocation par R $\times 10^{-5}$	X ^{M}O par R $\times 10^{-5}$
Spermatozoïde	18,0	25,4	2,0
Spermatide	38,0	72,5	4,3
Spermatocyte mûr	28,0	7,4	1,0
Spermatocyte jeune	24,0	20,8	2,9–3,3
Spermatogonie	1,1	0–3,6	0,07

(D'après Léonard, 1965; Léonard et Deknudt, 1968; Russell et Saylors, 1963.)

quant aux taux de mutations radio-induites, ce sont toujours les spermatides qui sont les plus radiosensibles. Ces considérations se sont vérifiées pour toutes les espèces étudiées jusqu'à présent (Lyon, 1970; Lyon et Smith, 1971) et on peut raisonnablement penser qu'elles sont également d'application pour l'homme. Etant donné que la radiosensibilité des spermatogonies de l'homme paraît 2 à 3 fois plus élevée que la radiosensibilité des spermatogonies de la souris (Oakberg et Heller, 1966) il est possible cependant que le taux de mutations produit par une irradiation des spermatogonies humaines soit inférieur par suite d'une sélection germinale plus intense.

2. RADIOSENSIBILITÉ DES CELLULES REPRODUCTRICES FEMELLES

Il paraît beaucoup plus délicat d'extrapoler à l'homme les données quantitatives obtenues

par irradiation des souris femelles. En effet, la morphologie nucléaire du stade diplotène auquel les oöcytes sont bloqués jusqu'au moment de l'ovulation varie largement d'une espèce à l'autre. Si l'on trouve un stade diplotène caractéristique chez l'homme, le singe Rhesus, la chèvre et le chien (Baker, 1966, 1971; Baker et Beaumont, 1967; Baker et Neal, 1969), chez la souris, le rat et les quelques espèces apparentées étudiées jusqu'à présent, le stade diplotène ressemble dans une certaine mesure à une interphase. Parallèlement à ces différences et aux activités métabolitiques qui y sont liées, on constate que les oöcytes du singe sont beaucoup plus radiorésistants à la mort cellulaire que ceux de la souris. C'est ainsi que 5000 R donnés aux oöcytes jeunes du singe ont le même effet que 15 R seulement administrés aux souris femelles. D'irradiations données *in vitro* ces mêmes auteurs ont pu conclure que les oöcytes humains sont aussi radiorésistants que les oöcytes du singe et qu'il n'y pas de différence entre les effets d'irradiations données *in vivo* et *in vitro*.

Il est raisonable de penser que les dommages produits aux chromosomes par une exposition aux radiations ionisantes sont responsables en partie de la mort des cellules et on a d'ailleurs pu montrer qu'il existait de fait une relation entre la sensibilité génétique et la sensibilité somatique d'une cellule (Oftedal, 1968). On pourrait dès lors en conclure que le danger de produire des mutations par irradiation des oöcytes humains est moindre que chez la souris. De plus si l'on se base sur les travaux de Russell (1965*c*) qui, nous l'avons vu, ont montré que les oöcytes de souris ovulés endéans les 7 premières semaines qui suivent une irradiation sont plus sensibles aux mutations radio-induites, on peut penser que si une femme irradiée s'abstient d'avoir des enfants les 12 à 18 premiers mois qui suivent une irradiation, le risque de mutations s'en trouvera fortement réduit. Il conviendrait cependant de remarquer que l'absence d'élimination sélective des cellules les plus lésées génétiquement pourrait être un facteur agissant en sens inverse. En conclusion il paraît hasardeux dans l'état actuel de nos connaissances d'estimer quantitativement les dangers génétiques d'une exposition des oöcytes humains à l'irradiation.

3. ESTIMATION DE LA DOSE REQUISE POUR DOUBLER LE TAUX DE MUTATIONS SPONTANÉES

Dans l'estimation pour la race humaine, des dangers génétiques d'une exposition aux radiations ionisantes, il est d'usage d'utiliser le concept de 'doubling dose'. Par ce terme on entend la dose d'irradiation requise pour doubler le taux de mutations spontanées ou encore la quantité d'irradiation nécessaire pour doubler la part prise par les effets génétiques dans les malformations congénitales ou les morts précoces.

Lüning et Searle (1971) se basant sur les résultats expérimentaux disponibles dans la littérature ont récemment calculé quelles étaient pour les spermatogonies les doses aiguës de rayons X à administrer pour doubler ce taux de mutations spontanées. Ces doses aiguës (Tableau 4) sont de 32 R pour les mutations de locus spécifiques (locus du groupe PT), 16 R pour les mutations visibles dominantes, 26 R pour les mutations visibles dominantes du squelette, 51 R

TABLEAU 4 *Doses d'irradiation nécessaires pour doubler le taux de mutations spontanées*

Mutations	Dose d'irradiation		
	Minimum	Moyenne	Maximum
Locus spécifiques	18 R	32 R	55 R
Dominants visibles	4 R	16 R	104 R
Dominants visibles du squelette	3 R	26 R	445 R
Sémistériles	8 R	31 R	138 R
Létaux récessifs	16 R	51 R	170 R

(D'après Lüning et Searle, 1971.)

pour la létalité autosomique récessive et 31 R pour la sémistérilité. Comme vous le voyez il s'agit là de doses d'irradiation très faibles. En ce qui concerne les létaux dominants induits par irradiation des spermatogonies, ces mêmes auteurs sont arrivés à la conclusion que si l'on admet que toutes les pertes prénatales sont dues chez les témoins comme chez les irradiés à la létalité dominante, la dose nécessaire pour doubler ce taux de mutations est de plusieurs milliers de roentgen. Ils en ont conclu qu'on ne pourrait utiliser ce critère pour estimer les dangers génétiques d'une exposition aux radiations ionisantes.

Sans vouloir diminuer l'intérêt de ces données, il convient cependant d'ajouter qu'elles ont une valeur plus théorique que pratique. En effet:

1. Elles sont basées uniquement sur les recherches expérimentales effectuées sur les spermatogonies et nous ne possédons que des données fort incomplètes pour les cellules reproductrices femelles. Dans ce dernier cas nous avons vu qu'il est très difficile d'extrapoler à l'homme les résultats d'expériences effectuées sur le rat ou sur la souris.

2. Hormis le cas d'une exposition accidentelle ou pour des raisons médicales, il est rare qu' une personne soit exposée à une dose aiguë de radiation d'un type bien déterminé. Dans la pratique les gens sont exposés à des doses fractionnées de rayonnements de nature diverse et d'efficacité biologique relative très différente. Nous avons vu pour chaque type de mutation l'influence que pourrait avoir le fractionnement d'une dose donnée et le débit auquel elle est administrée sur le taux de mutations radio-induites. Le Tableau 5 donne un aperçu de l'efficacité biologique relative des différents types de rayonnements pour les mutations de locus spécifiques, les dominants visibles et les dominants létaux. Si l'on tient compte de ces différents facteurs il est certain que la dose de radiation nécessaire pour doubler, par irradiation des spermatogonies, le taux de mutations spontanées est beaucoup plus élevée que ce que l'on a observé dans des conditions expérimentales strictes.

TABLEAU 5 *Efficacité biologique relative des différents types de rayonnement*

	Efficacité biologique relative	Référence
Spermatogonies		
Mutations de locus spécifiques		
Dose aiguë de rayons X/dose chronique de rayons gamma	3	Russell (1963*a*)
Neutrons/rayons X	5,8	Russell (1965*a*)
		Russell et Kelly (1964)
	5	Batchelor et coll. (1964, 1966, 1967)
Neutrons faible débit/gamma faible débit	18,1	Russell (1965*a*)
	23	Batchelor et coll. (1964, 1966, 1967)
		Searle et Philipps (1964, 1966, 1967)
Dominants visibles		
Neutrons chroniques/gamma chroniques	20	Batchelor et coll. (1966)
		Searle et Philipps (1967)
Spermatozoïdes		
Dominants létaux		
Dose aiguë de neutrons/dose aiguë de rayons X	8	Russell et coll. (1953, 1954)
	6	Sheppard et coll. (1957)

3. Enfin, difficulté supplémentaire et non des moindres, nous ne possédons pas encore à

l'heure actuelle, ainsi que cela ressort de la réunion de l'UNSCEAR tenue à New York en juin dernier, d'une base valable pour estimer chez l'homme le nombre de locus susceptibles de pouvoir muter.

RÉSUMÉ

Dans cette revue ont été envisagés tous les changements génétiques radio-induits chez les petits mammifères, quelqu'en soit la nature exacte.

Selon le groupe de locus utilisé (PT ou HT) le taux de mutants de locus spécifiques observés après irradiation des spermatogonies avec des rayons X varie de $2{,}2 \times 10^{-7}$ à $0{,}78 \times 10^{-7}$ par locus, par gamète et par R. Ce taux de mutations est influencé par le débit de la dose et son fractionnement. Après des doses supérieures à 600 R le taux de mutations diminue fortement par suite d'une élimination sélective des cellules les plus lésées. L'irradiation des oöcytes mûrs produit beaucoup plus de mutations que l'irradiation des oöcytes jeunes.

Le taux de mutants récessifs radio-induits chez les spermatogonies ($1{,}8 \times 10^{-5}$ par gamète et par R) est nettement plus élevé que le taux de mutants dominants visibles ($4{,}96 \times 10^{-7}$ par gamète et par R).

Le taux de létalité dominante induite par irradiation des spermatogonies varie de 0,4 à $1{,}3 \times 10^{-4}$ par gamète et par R, tandis que le taux produit par irradiation des spermatozoïdes est voisin de $1{,}8 \times 10^{-3}$ par gamète et par R. Le taux de létalité récessive induite par irradiation des spermatogonies varie quant à lui et selon les auteurs de 0,8 à $2{,}46 \times 10^{-4}$ par gamète et par R.

Si l'on peut raisonnablement extrapoler, dans une certaine mesure, à l'homme une partie des résultats expérimentaux obtenus par irradiation des cellules reproductrices mâles, il est pratiquement impossible dans l'état actuel de nos connaissances d'en faire autant en ce qui concerne les cellules reproductrices femelles.

REMERCIEMENTS

Je tiens à exprimer tous mes remerciements au Dr A. G. Searle et au Dr A. D. Tates qui ont bien voulu revoir le manuscrit de ce travail et me faire part de leurs suggestions. Ce travail a été réalisé avec l'aide du Contrat de Recherche Euratom/C.E.N. N° 059–65–7–BIOB et du 'Fonds de la Recherche Scientifique Fondamentale Collective'.

BIBLIOGRAPHIE

Baker, T. G. (1966): The sensitivity of oocytes in the post-natal rhesus monkeys to X-irradiation. *J. Reprod. Fertil.*, *12*, 183.

Baker, T. G. (1971): Comparative aspects of the effects of radiation during oogenesis. *Mutation Res.*, *11*, 9.

Baker, T. G. et Beaumont, H. M. (1967): Radiosensitivity of oogonia and oocytes in the foetal and neonatal monkey. *Nature (Lond.)*, *214*, 981.

Baker, T. G. et Neal, P. (1969): The effects of X-irradiation on mammalian oocytes in organ cultures. *Biophysik*, *6*, 39.

Batchelor, A. L., Philips, R. J. S. et Searle, A. G. (1964): High effectiveness of chronic neutron exposures for the induction of specific locus mutations in mice. *Nature (Lond.)*, *201*, 207.

Batchelor, A. L., Philips, R. J. S. et Searle, A. G. (1966): A comparison of the mutagenic effectiveness of chronic neutron- and α-irradiation of mouse spermatogonia. *Mutation Res.*, *3*, 218.

Batchelor, A. L., Philips, R. J. S. et Searle, A. G. (1967): The reversed dose-rate effect with fast neutron-irradiation of mouse spermatogonia. *Mutation Res.*, *4*, 229.

BONNIER, G. et LÜNING, K. G. (1949): Studies on X-ray mutations in the white and forked loci of *Drosophila melanogaster*. I. A statistical analysis of mutation frequencies. *Hereditas (Lund)*, *35*, 163.

BREWBAKER, J. L. et NATARAJAN, T. (1960): Centric fragments and pollen-part mutations of incompatibility alleles in *Petunia*. *Genetics*, *45*, 699.

CARTER, T. C., LYON, M. F. et PHILIPS, R. J. S. (1958): Genetic hazard of ionizing radiations. *Nature (Lond.)*, *182*, 409.

EDWARDS, R. G. et SEARLE, A. G. (1963): Genetic radiosensitivity of specific post-dictyate stages in mouse oocytes. *Genet. Res.*, *4*, 389.

EHLING, U. (1965): Dominant mutations affecting the skeleton in offspring of X-irradiated mice. *Genetics*, *54*, 1381.

FORD, C. E., SEARLE, A. G., EVANS, E. P. et WEST, B. J. (1969): Differential transmissions of translocations induced in spermatogonia of mice by irradiation. *Cytogenetics*, *8*, 447.

GERBER, G. B. et LEONARD, A. (1971): Influence of selection, non-uniform cell population and repair on dose effect curves of genetic effects. *Mutation Res.*, *12*, 175.

GILLIAVOD, N. et LÉONARD, A. (1971): Incidence of dominant lethal mutations after X-irradiation of spermatozoa of mice and rats. *Strahlentherapie*, *141*, 351.

KHUSH, G. S. et RICK, C. M. (1968): Cytogenetic analysis of the tomato genome by means of induced deficiencies. *Chromosoma (Berl.)*, *23*, 452.

KIMBALL, R. F. (1966): Repair of premutational damage. Dans: *Advances in Radiation Biology*, *Vol. II*, pp. 135–166. Rédacteurs: L. G. Augenstein, R. Mason et M. Zelle. Academic Press, New York, N.Y.

LÉONARD, A. (1965): Differential radiosensitivity of germ-cells of the male mouse. *Canad. J. Genet. Cytol.*, *7*, 400.

LÉONARD, A. (1966): Relation between the X-ray dose and the rate of dominant lethals induced by irradiation of mouse spermatozoa. *Mutation Res.*, *3*, 73.

LÉONARD, A. (1971): Radiation-induced translocations in spermatogonia in mice. *Mutation Res.*, *11*, 71.

LÉONARD, A. et DEKNUDT, GH. (1967): The rate of dominant lethals after low X-ray doses given to mouse spermatozoa. *Mutation Res.*, *4*, 234.

LÉONARD, A. et DEKNUDT, GH. (1968): The sensitivity of various germ-cell stages of the male mouse to radiation-induced translocations. *Canad. J. Genet. Cytol.*, *10*, 495.

LEWIS, D. (1951): Structure of the incompatibility gene. III. Types of spontaneous and induced mutation. *Heredity*, *5*, 399.

LIFSCHITZ, E. et FALK, R. (1968): Fine structure analysis of a chromosome segment in *Drosophila melanogaster*. Analysis of X-ray induced lethals. *Mutation Res.*, *6*, 235.

LÜNING, K. G. (1964): Studies of irradiated mouse populations. III. Accumulation of recessive lethals. *Mutation Res.*, *1*, 86.

LÜNING, K. G. et SEARLE, A. G. (1971): Estimates of the genetic risks from ionizing irradiation. *Mutation Res.*, *12*, 291.

LYON, M. F. (1970): X-ray induced dominant lethal mutations in male guinea-pigs, hamsters and rabbits. *Mutation Res.*, *10*, 133.

LYON, M. F. et MORRIS, T. M. (1966): Mutation rates at a new set of specific loci in the mouse. *Genet. Res.*, *7*, 12.

LYON, M. F. et MORRIS, T. M. (1969): Gene and chromosome mutation after large fractionated or unfractionated radiation doses to mouse spermatogonia. *Mutation Res.*, *9*, 191.

LYON, M. F., PHILIPS, R. J. S. et SEARLE, A. G. (1964): The overall rates of dominant and recessive lethal and visible mutation induced by spermatogonial X-irradiation of mice. *Genet. Res.*, *5*, 448.

LYON, M. F. et SMITH, B. D. (1971): Species comparisons concerning radiation-induced dominant lethals and chromosome aberrations. *Mutation Res.*, *11*, 45.

NEEL, J. V. (1970): Evaluation of the effects of chemical mutagens on man: the long road ahead. *Proc. nat. Acad. Sci. USA*, *67*, 908.

OAKBERG, E. F. (1968): Relationship between stage of follicular development and RNA synthesis in mouse oocyte. *Mutation Res.*, *6*, 155.

OAKBERG, E. F. et HELLER, C. G. (1966): Radiation response of spermatogonia in man. *Third International Congress of Radiation Research, Cortina d'Ampezzo*, Abstract 166.

OFTEDAL, P. (1968): A theoretical study of mutant yield and cell killing after treatment of heterogeneous cell populations. *Hereditas (Lund)*, *60*, 177.

PANDEY, K. K. (1965): Centric chromosome fragments and pollen-part mutation of the incompatibility gene in *Nicotiana alata*. *Nature (Lond.)*, *206*, 792.

PHILLIPS, R. J. S. et SEARLE, A. G. (1964): The effect of dose-rate on the yield of translocations and dominant lethals following spermatogonial irradiation in mice. *Genet. Res.*, *5*, 468.

POMERANTZEVA, M. D. et RAMAIYA, L. K. (1963): The genetic after effects of the action of fast neutrons on the sexual cells of male mice. *Dokl. Akad. Nauk SSSR*, *151*, 203.

POMERANTZEVA, M. D. et RAMAIYA, L. K. (1969): The mutagenic effect of different types of irradiation on the germ cells of male mice. I. Comparative genetic radiosensitivity of spermatogonia and of other stages of spermatogenesis. *Genetika (Leningrad)*, *5*, 103.

REPORT OF THE UNITED NATIONS SCIENTIFIC COMMITTEE ON THE EFFECTS OF ATOMIC RADIATION (1966): Supplement No. 14 (A/6314), 153 p.

REPORT OF THE UNITED NATIONS SCIENTIFIC COMMITTEE ON THE EFFECTS OF ATOMIC RADIATION (1970): A/AC.82/R.256, 89 p.

RUSSELL, L. B. (1971): Definition of functional units in a small chromosomal segment of the mouse and its use in interpreting the nature of radiation-induced mutations. *Mutation Res.*, *11*, 107.

RUSSELL, L. B. et SAYLORS, C. L. (1963): The relative sensitivity of various germ cell stages of the mouse to radiation-induced non-disjunction, chromosome losses and deficiencies. Dans: *Repair from Genetic Radiation Damage*, pp. 313–340. Rédacteur: F. H. Sobels. Pergamon Press, Oxford.

RUSSELL, W. L. (1951): X-ray induced mutations in mice. *Cold Spr. Harb. Symp.*, *16*, 327.

RUSSELL, W. L. (1956): Lack of linearity between mutation rate and dose for X-ray induced mutations in mice. *Genetics*, *41*, 658.

RUSSELL, W. L. (1962): An augmenting effect of dose-fractionation on radiation-induced mutation rate in mice. *Proc. nat. Acad. Sci. USA*, *48*, 1724.

RUSSELL, W. L. (1963*a*): The effect of radiation dose rate and fractionation on mutation in mice. Dans: *Repair from Genetic Radiation Damage*, pp. 205–217. Rédacteur: F. H. Sobels. Pergamon Press, New York, N.Y.

RUSSELL, W. L. (1963*b*): Evidence from mice concerning the nature of the mutation process. Dans: *Genetics Today*, *Vol. II*, pp. 257–264. Rédacteur: S. J. Geerts. Pergamon Press, New York, N.Y.

RUSSELL, W. L. (1965*a*): Studies in mammalian radiation genetics. *Nucleonics*, *23*, 53.

RUSSELL, W. L. (1965*b*): The nature of the dose-rate effect of radiation on mutation in mice. *Jap. J. Genet.*, *40*, 128.

RUSSELL, W. L. (1965*c*): Effect of the interval between irradiation and conception on mutation frequency in female mice. *Proc. nat. Acad. Sci. USA*, *54*, 1552.

RUSSELL, W. L. (1967*a*): Factors that affect the radiation-induction of mutations in the mouse. *An. Acad. brasil. Cienc.*, *39*, 66.

RUSSELL, W. L. (1967*b*): Repair mechanisms in radiation mutation induction in the mouse. Dans: *Recovery and Repair Mechanisms in Radiobiology*. *Brookhaven Symp. Biol.*, *20*, 179.

RUSSELL, W. L. (1967*c*): Recent studies on the genetic effects of radiation in mice. Dans: *Proceedings, I International Symposium on the Biological Interpretation of Dose from Accelerator-produced Radiation*, pp. 81–87. Rédacteur: R. Wallace. U.S. Atomic Energy Commission, Division of Technical Information, Washington, D.C.

RUSSELL, W. L. (1968): Recent studies on the genetic effects of radiation in mice. *Pediatrics*, *41*, 223.

RUSSELL, W. L. et KELLY, E. M. (1964): Relative biological effectiveness of neutron to X-ray induction of gene mutations in mouse postspermatogonial stages. *U.S. AEC Report ORNL-3608*, p. 83.

RUSSELL, W. L. et KELLY, E. M. (1965): Mutation frequency in female mice exposed to a small X-ray dose at high dose rate. *Genetics*, *52*, 471.

RUSSELL, W. L., RUSSELL, L. B., GOWER, J. S. et SHEPPARD, C. W. (1953): Neutron-induced dominant lethals in mice. *Genetics*, *38*, 688.

RUSSELL, W. L., RUSSELL, L. B. et KIMBALL, A. W. (1954): The relative effectiveness of neutron from a nuclear detonation and from a cyclotron in inducing dominant lethals in the mouse. *Amer. Naturalist*, *88*, 269.

SANKARANARAYANAN, K. (1971): Recent advances in mammalian radiation genetics and their relevance to the problem of genetic risk estimates in man. *Int. J. environm. Stud.*, *1*, 187.

SCHRÖDER, J. H. (1969): Dominant lethal mutations after irradiation of mouse spermatogonia with 600 R of X-rays. *Int. J. Radiat. Biol.*, *16*, 377.

SEARLE, A. G. (1964): Genetic effects of spermatogonial X-irradiation on productivity of F1 female mice. *Mutation Res.*, *1*, 99.

SEARLE, A. G. (1966): Progress in mammalian radiation genetics. Dans: *Radiation Research*, pp. 469–481. Rédacteur: G. Silini. North-Holland Publishing Co. Ltd., Amsterdam.

SEARLE, A. G. et PHILIPPS, R. J. S. (1964): Genetic effects of neutron-irradiation in mice. Dans: *Biological Effects of Neutron- and Proton Irradiations, Vol. I*, pp. 361–370. IAEA, Vienna.

SEARLE, A. G. et PHILIPPS, R. J. S. (1966): Genetic effects of fast-neutron exposures in mice. *Int. J. Radiat. Biol.*, *10*, 309.

SEARLE, A. G. et PHILIPPS, R. J. S. (1967): Genetic effects of high-LET radiation in mice. *Radiat. Res.*, *7*, *Suppl.*, 294.

SHEPPARD, C. W., SLATER, M., DARDEN, E. B., KIMBALL, A. W., ATTA, G. J., EDINGTON, C. W. et BAKER, W. K. (1957): Biological effects of fast neutrons from an internal target cyclotron: physical methods and dominant lethals in Drosophila. *Radiat. Res.*, *6*, 173.

SHERIDAN, W. (1965): The induction by X-irradiation of dominant lethal mutations in spermatogonia of mice. *Mutation Res.*, *2*, 67.

SHERIDAN, W. (1968): The effects of acute single or fractionated X-ray treatment on mouse spermatogonia. *Mutation Res.*, *5*, 163.

SNELL, G. D. et AEBERSOLD, D. C. (1937): The production of sterility in male mice by irradiation with neutrons. *Proc. nat. Acad. Sci. USA*, *23*, 374.

SOBELS, F. H. (1961): Genetic variation and maximum permissible level of radiation. Dans: *Proceedings, II International Congress of Human Genetics, Rome, 1961*, pp. 1240–1250.

SOBELS, F. H. (1969*a*): Recent advances in radiation genetics with emphasis on repair phenomena. Dans: *Proceedings, XII International Congress of Genetics, Tokyo, Vol. III*, pp. 203–223.

SOBELS, F. H. (1969*b*): Estimation of the genetic risk resulting from the treatment of women with ^{131}iodine. *Strahlentherapie*, *138*, 172.

TAYLOR, B. A. (1968): The frequency of X-ray induced recessive visible mutations in the rat. *Genetics*, *60*, 559.

TAYLOR, B. A. et CHAPMAN, A. B. (1969*a*): Genetic effects of spermatogonial irradiation on growth and age at sexual maturity in rats. *Genetics*, *63*, 441.

TAYLOR, B. A. et CHAPMAN, A. B. (1969*b*): The frequency of X-ray induced dominant and recessive lethal mutations in the rat. *Genetics*, *63*, 455.

VALENCIA, J. I. et MULLER, H. J. (1949): The mutational potentialities of some individual loci in *Drosophila*. Dans: *Proceedings, VIII International Congress of Genetics*, pp. 681–683.

WOLFF, S. (1967): Radiation genetics. *Ann. Rev. Genet.*, *1*, 221.

Chapter III Population genetics

L. L. Cavalli-Sforza

Department of Genetics, Stanford University Medical School, Stanford, Calif., U.S.A.

Pygmies, an example of hunters-gatherers, and genetic consequences for man of domestication of plants and animals*

Definitions of the genus *Homo*, and more generally of man, vary, but according to a popular one, man's origin was at least two million years ago. During almost all this time, man obtained his food not only as a food gatherer – like his close relatives, the anthropoid apes – but also as a hunter. It has only been during the last ten thousand years – a mere 0.5% of this long history – that a different mode of food acquisition arose, that based on domestication of plants and animals. The areas where this first happened were several, but only a few are known in detail: one in the Near East (where some cereals, cattle, pigs, sheep, and goats were domesticated), one in S.E. Asia (mainly rice), and one in Central America (corn and various vegetables). All probably arose independently from one another. Several other centers – partly or fully independent from these – may have existed but they are less well documented.

The spread of agriculture from the centers of origin was slow, as will be discussed later, and even today some ethnic groups still exist which live mostly or entirely by hunting and gather-

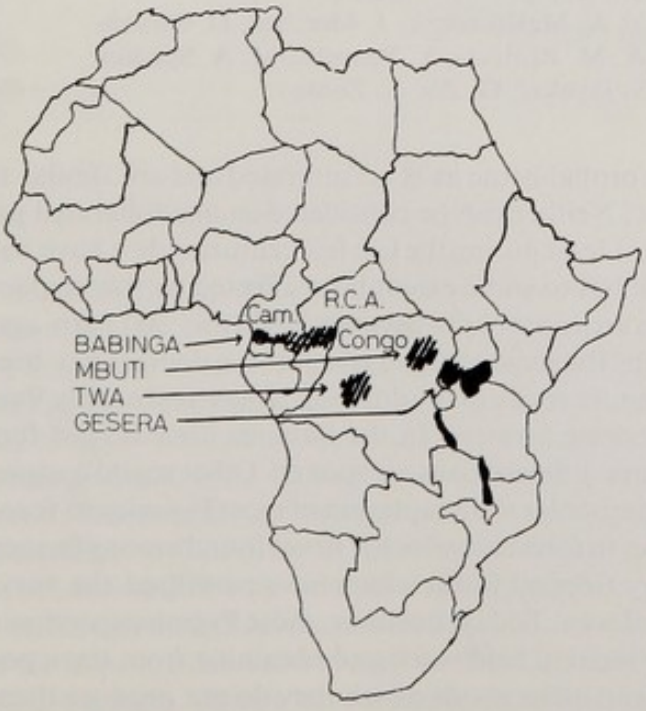


Fig. 1. Location of 4 main Pygmy groups.

* This research was supported in part by grants from the World Health Organization, the Wenner-Gren Foundation for Anthropological Research, and the Consiglio Nazionale delle Ricerche (International Biological Programme).

ing. Not all, but most of them are in Africa, and among these the largest group is that of Pygmies. This group is characterized, as is well known, by a small stature, but even more so by its socioeconomic structure. It is likely to have once been a much larger group, inhabiting perhaps most of central Africa between the twelfth parallel north and the sixteenth parallel south. Over 30,000 years ago, this area was occupied by people of the Sangoan culture, of whom Pygmies may be direct descendants. Today there still exist four major groups of Pygmies (Fig. 1) according to Murdock (1959): (1) Binga (or Babinga) numbering about 27,000 according to Murdock (which is not far from our estimates), living in the region between Cameroon, Congo Brazza, the Central African Republic, and Gabon; (2) the central Twa, numbering about 100,000 and being considerably acculturated and mixed in the central part of Congo Kinshasa; (3) the Gesera, about 9,000, living in Ruanda and Urundi, who have mostly adopted a sedentary life in the plains near Lake Kivu; and (4) the Mbutis, numbering about 32,000 (later estimates are somewhat higher), located in the Ituri forest in the north east of Congo Kinshasa, who reveal the least Negro influence in physique and culture. In addition, there are many splinter groups in central and south Africa.

Our work has concentrated mostly on the Babinga group, and has been concerned with a variety of aspects. Co-workers involved in it are listed in Table 1. Some data were obtained

TABLE 1 *Coworkers on project on African Pygmies, 1966–1971*

In the field:	F. Baschieri, G. Marin, F. Melgara, G. Roghi, M. Skolnick
Anthropology:	C. Turnbull
Blood groups:	L. E. Nijenhuis
Gm, Inv:	E. Van Loghem
Enzymes:	G. Modiano, A. S. Santachiara
Hemoglobin, G6PD:	L. Bernini, M. Siniscalco, W. W. W. De Jong
HLA:	W. and J. Bodmer, M. Nabholz
Other markers and measurements:	I. Barrai, P. Meera Khan, A. K. Ray, L. N. Went
Cytology:	F. Nuzzo De Carli
Parasitology and medicine:	S. Pampiglione, V. Pennetti
Endocrinology:	V. A. McKusick, T. J. Merimee, D. Rimoin
Nutrition:	A. M. Paolucci, V. Pennetti, M. A. Spadoni
Statistics:	S. Jayakar, G. Zei, L. Zonta

on the Mbutis as well. The two groups are probably the least acculturated and are similar to each other from a sociological point of view. Neither can be considered as unadulterated paleolithic or mesolithic hunters-gatherers, as at least during the last few centuries they have had contact with farmers, and their mode of life has to some extent been affected by this contact. It is known that farmers first settled in these areas probably between 2,000 and 300 years ago, though it is not known exactly when. Perhaps the most striking effect of the contact has been the loss of the original Pygmy language. Pygmies speak many different languages (mostly Bantu) which they have borrowed from neighboring farmers. In the Babinga area at least four different languages and in the Mbuti area three different ones are spoken. Other social customs have been more resistant to change, and in particular the adaptation of most Pygmies to forest life is still perfect. In fact, it is this adaptation to forest life which is never found among farmers and the persistence of large areas of primary tropical forest which have permitted the survival of Pygmies in a relatively unacculturated way. Today, however, most Pygmies spend part of their time near farmers' villages, helping them in field work and obtaining from them pottery, iron tools, palm wine, tobacco, cloth, and other goods which they do not produce themselves.

Our investigations were directed at several different problems which will be briefly summarized in what follows.

Demography Population density is of the order of 0.2 inhabitants per km^2. Birth and death rates are not known exactly, but there is some indication that infant mortality may be somewhat lower, and adult mortality higher, than among the neighboring farmers. Birth intervals are about four years on average. These data are similar to those obtained on the Yanomama and Bushmen, but perhaps not very different from those available for African farmers. The long birth interval is due to prolonged lactation and to a sex taboo lasting three years after the birth of a child. Pygmies are probably at an approximate demographic equilibrium, and it seems that social customs have been consciously or unconsciously adapted to stabilize population size. Stabilization has occurred at a fairly low level compared to that found for Bushmen. The biomass of Pygmies is only about twice as high as that of chimps living in a comparable area (3.6 kg/km^2 in the Ghana forest; Bourlière *et al.*, see Owen, 1966).

Demographic work is made difficult by the total lack of information on ages. Pygmies are physically sufficiently different from neighbors and other groups, especially in terms of anthropometric and growth patterns, that assessment of age by eye is unreliable. The distribution by age was studied in some camps by the following method. The birth order of all individuals of a group would be assessed by comparing birth order of the Pygmies with that of some neighboring farmers whose age was known at least approximately. It thus became possible to distinguish approximate 'age blocks' of the Pygmies. The outcome for one camp is given in Figure 2. The average expectation of life at birth is close to 21 years. The sex ratio seems normal.

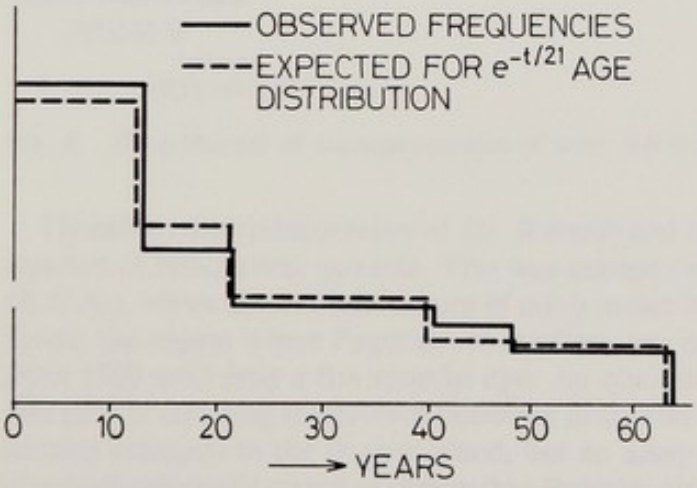


Fig. 2. Age distribution in a Pygmy camp.

Pygmies live in camps, which are moved fairly often – the building of huts requires less than a day. A camp is formed by 3 to 30 huts, with each hut corresponding to a nuclear family. Polygamy is rare. The location of a camp at the time when the Pygmies are living close to the farmers may vary from 50 yards to several miles from a village. Occasionally two or three (up to four) camps are close together. A fraction of the year, which varies greatly with Pygmy group and with area, is spent near the farmers and the rest of the time is spent hunting in the forest. Hunts may last days or months and may be at a considerable distance, even ten or more days' walk, from villages. In a few villages, especially at the fringe of the forest, Pygmies have become sedentary and show all degrees of acculturation to an agricultural economy.

Although these habits can be defined as nomadic, most Babinga Pygmies tend to gravitate to the same area and can usually be found, year after year, at the same villages, at least during the dry season. Camps have a highly changing composition, as some individuals may choose to spend a long period near the farmers' villages while others choose to go hunting. Families often split and then reunite at the end of a hunt. There are clans and totems, and marriage is usually exogamous (with some alleged preference for sister exchange). Cousin marriages are

forbidden, but information on genealogies is limited and respect for clan exogamy is not well documented.

The nomadic habits of Pygmies are expressed in the distribution of distances between birth places of husband and wife (Fig. 3) which shows a much greater dispersion than that of a group of Bantu-speaking neighboring farmers, the Issongos (Mbaiki area). Part of this dispersion is due to the fact that some births take place during long hunts, which may be several walking days away (a walking day is considered equivalent to 30 km). Probably a smaller fraction of the dispersion is due to marriages between Pygmies gravitating in different areas.

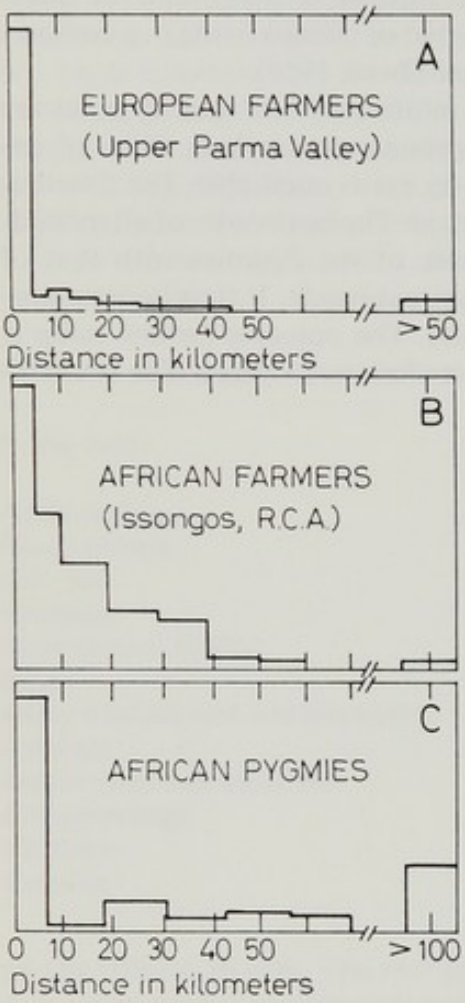


Fig. 3. Distributions of the distance between the birth places of husbands and wives in (A) one population of European farmers, (B) one population of African farmers, and (C) African Pygmies.

The migration matrix of Pygmies living in the Central African Republic was examined, and when tested by powering the geographic distribution was shown to be unstable (see Bodmer and Cavalli-Sforza, 1968), indicating that some areas would, if present migration patterns continued, become depopulated. This is, in fact, known to be the case. Toponomastic and historical observations indicate that Pygmies change their living areas in some discontinuous way. Probably a major factor in determining their migration is the retreat of the forest. Occasional splinter groups are found who live in the savannah far from the main forest but always close to some small forested area. These small groups are likely to disappear, at least culturally, if they finally become sedentary.

Mixed marriages occur only in the direction of Pygmy females marrying farmers. Pygmy females have a reputation for high fertility and are less expensive to acquire. The progeny is always absorbed into the farmers' culture. Such mixed marriages are limited to some areas and to some farmers' tribes, and are generally relatively rare. Under present conditions gene

flow into Pygmies must be extremely limited, whereas the reciprocal gene flow takes place with some regularity but is limited in area.

Physical characteristics The most typical peculiarity of Pygmies is their short stature. This actually varies with the Pygmy group from 144 cm for the Ituri (Congo Kinshasa) adult males to 159 cm in the Kribi region (Cameroon), the tallest Pygmies measured thus far. Figure 4 shows the distribution of the mean stature of over 300 African tribes. It is clear that Pygmies occupy a unique position at the lower end of the distribution.

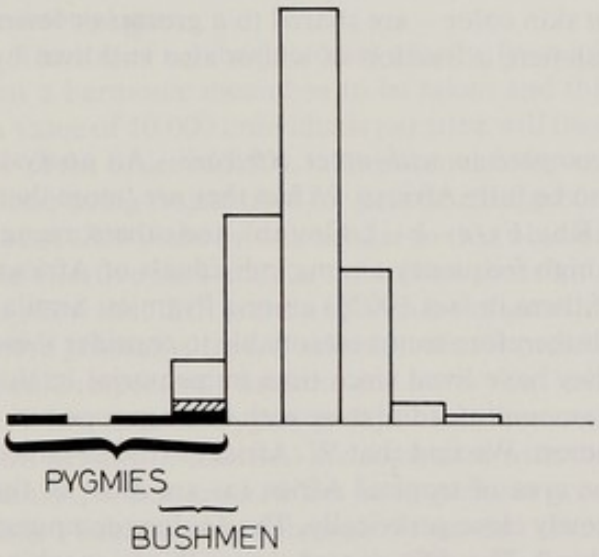


Fig. 4. Distribution of average statures of over 300 African tribes (data from Hiernaux, 1968).

Thanks to the collaboration of Dr. Rimoin and his associates, an endrocrinological investigation of stature was possible. This was carried out on Pygmies of the region of Bagandou (R.C.A.), where the average stature of adult males is 154 cm. It should be noted that the Ituri forest, the region where Pygmies are smallest, has been closed due to the Congolese civil war from 1960 until only a few months ago. An obvious approach to the endocrinological study was that of assaying the growth hormone produced by the pituitary. This was found to be in normal amounts in the Pygmy blood, but an assay of the sensitivity to the hormone by the standard metabolic tests has shown that Pygmies are remarkably insensitive even to very high doses. Local farmers showed a normal reactivity to the hormone, and the only two hybrids tested so far were normally sensitive.

The physiological aspects will be discussed in a paper by Dr. Rimoin *et al.* at this conference and therefore I will not expand on this most interesting topic (see also Rimoin *et al.*, 1969). Unfortunately, hybrids and backcrosses (occurring as explained only in one direction) are sufficiently rare, and the environmental conditions of Pygmies sufficiently different from those of the farmers, that a final word on the mode of inheritance of this trait cannot be made at present. A further difficulty is that people in whom crosses occur (*e.g.* the Issongos in the R.C.A. and the Babira in the Ituri) are smaller than the surrounding tribes, implying either the consequences of prolonged crossing, or of mixed marriages. By way of analogy it is interesting to note that a strain of mice of unusually small size, which happens to be called 'pygmy', shows a very similar endrocrinological condition (due to a recessive gene): normal production of the hormone, and resistance to its effects.

The possibility of direct environmental effect on stature should not be entirely eliminated, however. Pygmies live in a highly peculiar environment, the forest, that is in a dim green light which may have some effect on growth. The relative darkness, incidentally, is probably responsible, in part, for the lighter skin color of Pygmies with respect to the other Africans.

The selective mechanisms involved in the short stature are obscure. Better thermoregulation with a higher surface to weight ratio in a highly humid environment, and easier circulation of smaller bodies through the undergrowth may both be responsible. A smaller size is found also in many large forest mammals, *e.g.* the forest elephant is smaller than the savannah elephant and the same is true of buffaloes. Pygmy chimps and Pygmy hippos live in the dense forest. The smallest giraffid is the okapi, living in the Ituri forest.

Other physical characteristics of Pygmies are well known: a relatively large nose, a low limb to body ratio, kinky hair. In general, Pygmies are relatively easily distinguished from their neighbors, but not without error if physical traits alone are considered. Several of their characteristics – short stature, steatopygia, light skin color – are shared to a greater or lesser extent also by another protoafrican group, Bushmen, a fraction of whom also still lives by hunting and gathering in South Africa.

Genetic polymorphisms among Pygmies and comparison with other Africans An analysis of genetic markers among Pygmies shows them to be fully African. In fact they are 'more than African' if one considers that all alleles such as Rh_0, Fy(a−b−), Gm(ab), and others, recognized earlier as being African because of their high frequency among individuals of African origin, show an even higher frequency (some of them in fact 100%) among Pygmies. Similar considerations apply with respect to Bushmen. It therefore seems reasonable to consider these two groups as 'protoafricans', meaning that they have lived since time immemorial in this continent without any, or with only a moderate amount of, admixture with other groups.

The relationship with other Africans is of interest. We find that W. Africans (the so-called Forest Negro) and Bantu-speaking tribes in the area of tropical Africa (as are most of the farmers with whom we are concerned) are extremely close genetically. The *f* values computed on the basis of available markers were as in Table 2. The differences between Bantu-speaking

TABLE 2 *Measurements of the genetic divergence between Africans from West Africa and Bantu-speaking groups from West Africa*

f values given below express the divergence between the following groups:
A. Forest Negro (W. Africa)
B. Bantus of the R.C.A. (Central African Republic)
C. Bantus of Congo Kinshasa

	A	B	
B	0.0107		
C	0.0114	0.0143	Mean $f=0.0121$

These *f* values are computed averaging over 5–7 multiallelic polymorphic loci. If genetic *drift* alone were responsible for the difference, we expect

$$f=1-e^{-t/2N} \quad \left(f\simeq\frac{t}{2N} \text{ for small } f\right)$$

where t = separation time in generations.
N_e = effective size.
For $N_e=3000$, $t=2N_ef=72$ generations.
Separation time, with 25 years' generation time: 1800 years

or W. African groups seem very similar, and all are small, as expected on the basis of historical considerations. It is known, in fact, that Bantu-speaking tribes originated from a West African region about 2000 years ago. From the *f* values an independent estimate of this time can be obtained on the assumptions that only genetic drift is responsible for the observed genetic difference (expressed by the *f* value) and that the groups here considered have had no cross-migration since separation. The former hypothesis cannot be substantiated directly today. There is, however, one indirect test which is to make sure that the various loci employed do

not show a significant variation of the *f* values computed for each locus. This control (carried out using the Bartlett test for heterogeneity of variances) usually confirms the homogeneity of the *f* values computed for different loci, in agreement with the hypothesis of drift. This also confirms that averaging over various loci, as was done in Table 2, is appropriate. The second assumption (no cross-migration) is probably correct since groups far apart are being considered. The *f* value on these assumptions is related to the time *t* (in generations) since the groups separated by the formula given in Table 2. By this formula $-\log_e(1-f)$ (or, for low values of *f*, simply *f*) is equal to $t/2N_e$, where N_e is the effective population size. The relevant estimate of N_e seems to be that which can be derived using as 'population' the tribe, which is a linguistic and social unit.

The size of a tribe fluctuates greatly, from a few thousand to several hundreds of thousands, but a harmonic mean has to be taken and this will make the mean close to the lower limit. A value of 10,000 individuals per tribe will then be an acceptable rough estimate. N_e may then be of the order of 3,000, taking account of overlapping generations. On the assumption of drift alone being responsible for genetic divergence, separation time may then be calculated to be about 1800 years or very similar to that obtained from a consideration of historical facts. Were the effective sizes and the separation times known more precisely, this evidence might be used to show that drift is the major cause of genetic variation in the polymorphisms considered; or, more generally, that, if selection also is present, its disruptive and homeostatic effects, which would respectively increase and lower the *f* values, tend to balance each other.

When the two Pygmy groups most thoroughly examined (namely the Babingas or Western and Mbutis or Eastern) are compared with the 'standard' African (a pool of W. African and Bantus, which are a rather homogeneous group) as well as with Bushmen, the following results (Table 3) are obtained. From the 'standard' Africans we have excluded those tribes which are in closer contact with Pygmies and show, probably because of gene flow, a closer similarity to them. Table 3 shows that the three groups, E. Pygmies, Bushmen and 'standard'

TABLE 3 *f Values (averages of 4–14 loci) for some major African groups*

	W. Pygmies	E. Pygmies	Bushmen
'Standard' African	0.0329	0.0992	0.0900
W. Pygmies (R.C.A.)		0.0598	0.0819
E. Pygmies (Mbutis)			0.0805

Africans, all have approximately the same *f* values, of the order 0.09, corresponding to separation times of the order of 15,000 years. This is perhaps an appropriate order of magnitude for the separation time between the relevant cultures, namely the Stillbay culture for Bushmen, and the Sangoan for Pygmies. However, the identification of those extinct cultures with present inhabitants of the same region is still uncertain.

It may be noted that these *f* values are four or five times smaller than those between major human racial groups (Europeans, Africans, Orientals). Therefore, the creation of a separate major 'race' for Bushmen (Khoisanoid, Capoid) made by some anthropologists seems unwarranted.

Table 3 also shows that W. Pygmies are somewhat closer to 'standard' Africans than to the Mbuti Pygmies. The hypothesis that W. Pygmies are a mixture of 'standard' Africans and Pygmies (as represented more adequately by the smallest Pygmies, the Mbutis) has been considered (Cavalli-Sforza *et al.*, 1969). This question will be discussed also in what follows. It may be noted at this stage that the *f* values are not directly appropriate for an examination of admixture problems for which genetic distances (which are proportional to the square roots of *f* values) should be used instead (Cavalli-Sforza and Bodmer, 1971). On this basis W. Pyg-

mies are at a distance of 0.18 from 'standard' Africans and 0.24 from Mbutis. The sum of the two distances is 0.42, significantly larger than that between the two putative parents of the mixture ('standard' African and Mbutis) which is 0.31. This partially contradicts the hypothesis of admixture, which will deserve a more complete discussion later.

Microgeographic differentiation Most of the blood samples which we have collected (approximately 2,000) came from the region inhabited by W. Pygmies and it is on these that most of our investigations bear more directly. The external appearance, and more particularly the stature, of this group varies considerably.

As a higher stature may be *prima facie* evidence of greater admixture of Pygmies with other Africans, the most likely percentage of admixture (as determined by genetic markers) was computed by examining separately various areas. In Table 4 estimates of admixture and average statures are compared. Admixture was computed by the method outlined by Cavalli-Sforza *et al.* (1969). In no case was the admixture hypothesis completely substantiated by this method; that is, there was always a highly significant difference between the gene frequencies of the putatively mixed population and that of the artificial mixture between the two putative parents made by the populations which the method showed as most likely. Therefore, it would seem that either the parental Pygmy gene frequencies are badly represented in the present day Mbutis, or that if a mixture has occurred, much time has since elapsed. This corresponds with the fact that no gene flow into Pygmy populations is observed today. If any occurred, gene flow must have taken place some time ago.

TABLE 4 *Comparison of stature and estimates of per cent Bantu blood in subgroups of Babinga Pygmies*

Area	Longitude E (°)	Stature (ave.) Males	Stature (ave.) Females	Estimated % of Bantu blood
Kribi (Cameroon)	11	159.3	148.9	60.5
Abong Mbang (Cameroon)	14	154.1	146.0	59
Salo (R.C.A.)	16	154.2	144.5	52
Bambio (R.C.A.)	17	155.2	144.4	27
Bagandou (R.C.A.)	18	154.4	146.5	49.5
Mongoumba (R.C.A.)	19	152.5	148.8	65.5

As Table 4 indicates there is only a slight correlation between stature and per cent of calculated Bantu blood and then only when female stature data are used. The conclusions are not clearly in good agreement with the hypothesis of admixture. The following consideration

TABLE 5 *Polymorphisms and/or markers indigenous to Babinga Pygmies*

Mutants	Babinga Pygmies R.C.A.	Babinga Pygmies Cameroon
Hemoglobin delta		
Flatbush	2.1 ±0.3%	0.85±0.4%
Babinga	0.73±0.2%	0%(N=234)
Phosphoglucomutase		
PGM_2^6	6.3±0.8%	1.7±0.6%
Peptidase C		
C^2	1.4±0.4%	0% (N=162)
C^0	20.8±3.1%	7.9±7.9%

also confirms that if mixture occurred, much variation must have taken place since. In the analysis of W. Pygmies some new variants (Table 5) were detected which were not found anywhere else (at least not at polymorphic frequencies): (1) The delta hemoglobin chain mutant Flatbush. This had previously occurred in one Negro family of New York, but the gene frequency of this marker is almost 2% among Babingas, and the gene is regularly spread over the whole Babinga area, although it may be at a lower frequency in Cameroon. (2) The delta hemoglobin chain variant called Babinga (De Jong and Bernini, 1968), differing from the standard chain by one substitution at site number 136, was found first among these Pygmies. It has an overall gene frequency of 0.7% in the R.C.A. area. (3) The new allele at the locus 2 of phosphoglucomutase, PGM_2^6, occurs at a frequency of 0.5% and is evenly spread over the whole area (Santachiara Benerecetti *et al.*, 1969). (4) The new allele C^2, occurring at a frequency of 1.4%, and the silent allele C^0, occurring at a frequency of 21%, establish a new polymorphism at the peptidase C locus (Santachiara Benerecetti and Negri, 1970). In the winter of 1971 blood samples of 133 Mbuti Pygmies (266 genes) were analysed with a view that, if Babinga Pygmies are a mixture of approximately 50% Mbuti Pygmy and 50% Bantu blood, these variants, found only in the Babinga population, should be present at approximately twice the frequency among the Mbutis. Actually none of them was found, with the exception of one homozygous C^0. Although the sample examined is admittedly small, the difference between the cumulative expectation of these markers and that actually observed is significant. This further proves that Babinga Pygmies have been separated from the Mbutis for a long time, and have undergone in the meanwhile independent evolution, developing their own polymorphisms. The relative intermediacy of Babingas between Mbuti Pygmies (who live at their East) and 'standard' Africans, who lived originally at their West, may perhaps be the outcome of an early admixture at the time of first contact. Or, perhaps more likely, the overall picture is one of a general gradient that was established gradually over the millennia that preceded the rapid conquest of Central Africa by the Bantu speakers, by a limited amount of migration, insufficient to check the origin of local polymorphisms.

One further point of interest in the analysis of microgeographic variation is that of local genetic differentiation within the W. Pygmy group. Here, 22 villages or groups (in some cases a few of the smaller villages were combined) were examined in the R.C.A. and 7 in the Cameroon. Each group corresponds to at least one Pygmy camp, but usually to many. The analysis in the R.C.A. is much more complete and covers at least 1/3 of all Pygmy camps of the area. The analysis in Cameroon was limited to only two areas (Kribi and Abong Mbang, 3 and 4 villages, respectively), and the geographic coverage is less complete.

The variance of gene frequencies between villages was computed by various methods, and

TABLE 6 *f values (σ^2/pq) between villages computed as the mean of the f values of seven codominant loci*

Method	Babinga Pygmies of the R.C.A. (7 loci)
1. Matessi's	0.01032
2. Matessi's, corrected for bias	0.01055
3. Jayakar's	0.01507
4. Robertson's χ^2 (1951)	0.01667
5. Robertson's, maximum likelihood (1951)	0.01540
6. Cavalli-Sforza's χ^2	0.02387

Methods for computing variances by Matessi and Jayakar are unpublished. For Robertson's and Cavalli-Sforza's methods, see Cavalli-Sforza and Bodmer, 1971.

the results are given in Table 6. The various methods give different results. A simulation by Jayakar and Matessi (who have developed several of these methods) has not yet been completed, and we still do not know which of these methods should be preferable in general. It will be noted that the method previously used by the present author on the Parma data (Cavalli-Sforza, 1969) gives the highest estimates, and this probably accounts for much of the excess of the *f* value obtained from genetic markers in the Parma study. In the present analysis, only codominant markers were used, as dominance introduces further statistical difficulties, which are partly unsolved. The *f* values computed refer only to the R.C.A. data, as the work on the migration matrix with which a comparison has to be drawn was limited to the above. Exchange between the two Cameroon groups and the group living in the R.C.A. is practically zero at our level of investigation of migration, which was carried out by asking birth places of the individuals sampled and of their parents. Rare long distance migrants are occasionally observed but are too few for measuring the migration between areas far apart such as the R.C.A. area and the two Cameroon areas studied which are separated by several hundred km. At this point we prefer to take, as a precautionary measure, Robertson's maximum likelihood estimate, which gives values intermediate between those obtained with all methods. The estimate is 0.0154 ± 0.0038, the standard error being computed on the basis of the values obtained from the seven individual codominant loci used: Hb δ chain, PGM1, PGM2, MN, Tf, AP, 6PGD. This estimate can now be compared with the expected *f*, which was computed by the method of Bodmer and Cavalli-Sforza on the basis of the migration matrix. The latter is shown in Figure 5 (see also Malcolm *et al.*, 1969). The equilibrium value of *f* expected on the

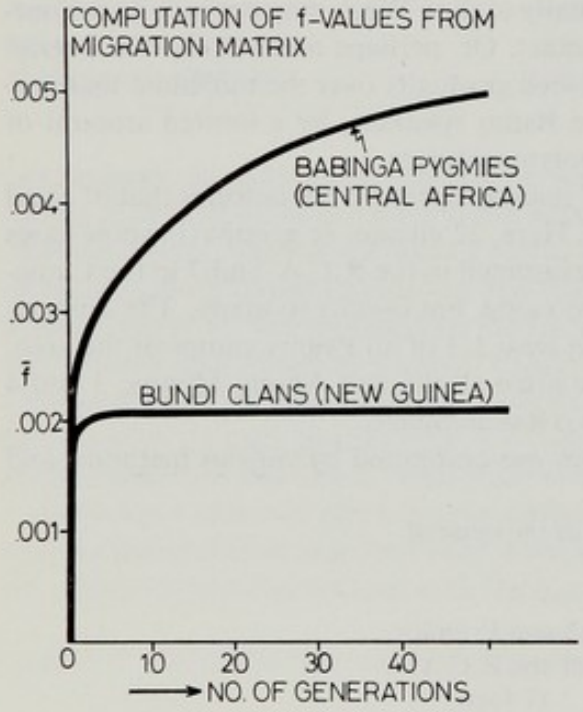


Fig. 5. The coefficient of kinship calculated from the migration matrix (see also Malcolm *et al.*, 1969).

basis of the migration matrix is somewhat above 0.005 and is reached after at least 50 generations. The time to get near to equilibrium is not very long and one might expect it to have been reached in the time available. Taken at face value, the expected *f* seems significantly below the observed one, but it is not easy to assess the standard error of the expected *f* value, which depends on sampling errors of the migration matrix. A Montecarlo method of estimating fluctuations in the migration matrix shows that its limits are fairly wide. Thus, the difference between observed and expected *f* is presumably not significant. At present a conservative

conclusion is that the *f* value indicating the heterogeneity of Babinga Pygmies in the Central African Republic is of the order 0.01.

Disease A medical and parasitological examination was carried out on a fraction of the individuals examined. Infestation with a great variety of parasites is extremely common, and is probably responsible for the very high values of gamma globulin concentrations in blood, as well as for spleen enlargement. The picture is, however, not markedly different from that found for the surrounding farmers (Pampiglione, unpublished data). The main differences observed are with respect to malaria, where farmers have shown a higher percentage of positive bloods (Table 7). It is of interest that among R.C.A. Pygmies, Hb^s has a somewhat lower

TABLE 7 *Malaria and sickle cell anemia among Pygmies and Bantus of Bagandou region of R.C.A. (January 1969)*

	Pygmies	Bantus	
Malaria +	148 (41.8%)	195 (60.7%)	343
parasites in blood −	206	126	332 $\chi^2=23.4$
	354	321	675

Hb^s: Pygmies 9.4%±0.6%, Bantus 13.7%±1.4%

frequency than among farmers (Table 7). The difference is significant. It should be noted that our figures for Hb^s among Pygmies are higher than earlier published ones (3.4% for Babinga Pygmies in the R.C.A.; see Hiernaux, 1968). In Cameroon the frequency of Hb^s among Pygmies is lower and drops to zero near the seaside. On the contrary with Mbuti Pygmies the gene frequency of Hb^s is very high (18%), confirming the earlier observations by Motulsky and others. If there is an increased frequency of malaria due to the agricultural mode of life, as has been claimed (Wiesenfeld, 1967), the actual difference is perhaps not so striking. It is true, however, that Pygmies have very low frequencies of other markers conferring resistance to malaria, such as G6PD, and also that there are probably other factors involved. For instance, Pygmies have a higher incidence of spleen enlargement than Bantus, in spite of the apparently lower malarial infestation. A more detailed malariological study of Pygmies and farmers occupying the same area would certainly be desirable.

One disease which is much more prevalent among Pygmies than among farmers is yaws, a treponematosis whose reservoir is suspected to be in wild monkeys. If this is correct, yaws might be a professional disease for Pygmies, who are hunters. The agent of yaws, *Treponema pertenue*, is very similar to that of syphilis and difficult to distinguish from it serologically. In view of earlier claims that syphilis is associated with ABO, an association between ABO and yaws was looked for and found (Table 8).

In spite of the very heavy infestation with parasites, general health conditions of Pygmies are,

TABLE 8 *Association between yaws and ABO blood groups*

	ABO blood groups	
	0	not 0
Clinical yaws absent	500	564
present	19 (3.7%)	7 (1.2%)
	519	571

$\chi^2_c=5.92$, $P<5\%$.

on average, good. Nutrition, as judged from a superficial analysis, seems excellent. The thickness of skin folds is high. We had to limit our examination of skin folds to the triceps and the subscapular region because steatopygia, observed mostly in women, but also in men, made the measurement of skin folds in the gluteal region difficult, and probably not very relevant from the point of view of nutrition. Several cases of kwashiorkor were, however, noticed among Pygmy infants. Further hints that the nutritional status is not perfect were observed by examination of the pattern of plasma amino acids. Paolucci *et al.* (1969) found that the amino acids in plasma had concentration profiles fairly different from those found in Caucasians. The profile was in the direction of that expected under protein deficiency. The most remarkable variation was in the increased level of phenylalanine, the level of tyrosine being more than two times higher than Caucasians (or black Americans). Phenotypically these Pygmies had phenylalanine:tyrosine ratios comparable to that of heterozygotes for phenylketonuria. Africans of non-Pygmy origin from the same region showed either the Caucasian pattern or an intermediate pattern, or, in a small minority of cases, the Pygmy pattern. In order to test whether the difference observed could have had an environmental origin, we subjected a group of twelve Pygmies to a diet with increased protein content. The results were clear-cut. In ten days of a diet with rice and sardines (which had been chosen by the Pygmies themselves as a desirable one) enriched with vitamins, the ratio of phenylalanine to tyrosine went progressively to normal (Paolucci and Pennetti, unpublished data). Although this proves an effect of the environment, it may still leave room for genetic predisposition. The most likely explanation seems that Pygmies are used, either because of a genetic predisposition or because of physiological adaptation, to a diet high in proteins. Pygmies on a strictly hunting and gathering diet are believed, as an approximate estimate communicated to me by Dr. Colin Turnbull, to eat about 30% of their food intake as meat. When they were examined by us, they were, however, living in the environment of the farmers and mostly sharing the farmers' diet, which is very high in carbohydrates and exceedingly low in protein. Further examination during the hunting season would be necessary to clarify the issue. It is worth recalling that Hiernaux observed a very high incidence of kwashiorkor in a Pygmy village that had just been acculturated. It is possible that populations that have not undergone, or only partially undergone, the transition to agriculture are not genetically or physiologically adapted to a low protein diet, and therefore suffer much more from an exposure to it than do people who are genetically or physiologically adapted to it. The problem of deciding if the adaptation to a low protein diet in farmers is physiological or genetic is not an easy one.

THE TRANSITION FROM HUNTING AND GATHERING TO AGRICULTURE AND ITS GENETIC CONSEQUENCES

I would like to mention at this stage some recent work done by Ammerman and Cavalli-Sforza (1971*b*) which bears on the transition from hunting and gathering to agriculture in Europe and Africa, and the genetic consequences of this transition.

There probably have been several independent areas in which agriculture arose and from which it spread (as previously mentioned). One of these areas on which much knowledge has accumulated today is the 'nuclear area', an origin of domestication in the Near East. By using radio carbon dates of the earliest 'neolithic' (or more precisely, 'early farming') settlements in the various regions of Europe, it is possible to map the advance of early farming (or, for short, the neolithic) in Europe, starting in the Near East. A remarkable regularity is observed (Fig. 6). There are, it is true, regions where the advance has been more rapid and others where it was slower, but there is considerable regularity. The average rate of advance of the neolithic wave front has been measured to be almost exactly one kilometer per year. The beginning of the expansion can be dated at approximately 9,000 years ago, but the onset of agricultural techniques was, as could be expected, earlier, and has developed probably over several mil-

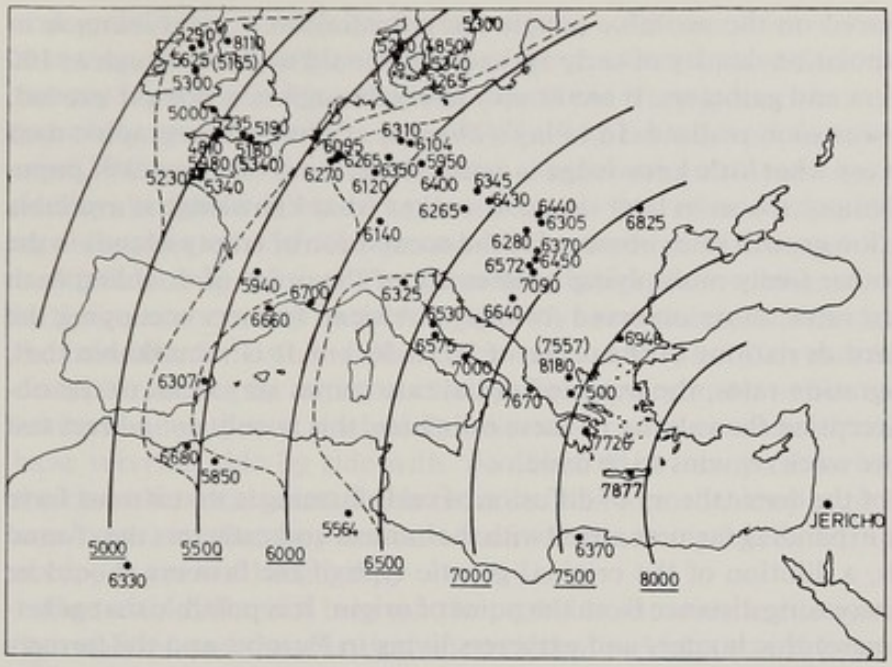


Fig. 6. Radio carbon dates of earliest farming settlements in Europe. All dates given are B.P. (before present). Solid lines are points equidistant from an arbitrary center in the 'nuclear area' of development of early farming which was located in this figure at Jericho. They are computed on the basis of rate of advance per year of 1 km. Dotted lines indicate some local variations in the rate of spread. (From Ammerman and Cavalli-Sforza, 1971*a*.)

lennia in the nuclear area before it took the shape characteristic of the phase of expansion.

At the time of expansion, several plants had been domesticated, essentially wheat and barley, and several animals (cattle, sheep, and goats). This complex of domesticates is found subsequently with very few exceptions over all the area to which the neolithic spread, starting from the Near East. There would be no reason why the expansion should happen only in one direction, namely towards Europe, but this is the only area where data are sufficiently dense that the actual expansion can be plotted with some precision. There are, however, several hints that the expansion also occurred in the other directions, namely towards Asia and Africa. In fact, what little data are available for Africa and Asia would point out that the rate of the spread of the neolithic was comparable to that observed in Europe.

The main genetic reason for interest in this problem arises from the fact that a culture as complex as the neolithic can hardly expand by cultural diffusion alone, but is more likely to spread with the people who originated it and carried it with them. We have called this process of diffusion *demic* as contrasted with a purely *cultural* diffusion where the trait or complex of traits is spread culturally unaccompanied by the displacement of people. The hypothesis of demic diffusion may be tested (admittedly indirectly) by checking if the observed rate of advance is compatible with the expected value obtained using this hypothesis. A mathematical theory was put forward some time ago for genetics (the wave of advance of advantageous genes) by R.A. Fisher (1937). Essentially the same theory can be made for the spread of an epidemic (Kendall, 1948) or in ecology (Skellam, 1951). Here we phrase the theory: A population which is (1) increasing in size and (2) has short range migration, random in direction (such as farmers with shifting agriculture are bound to have), *will expand with a constant radial rate*. The radial rate of advance is expected to be twice the geometric average of the growth rate and of the migration rate, measured in suitable units. One can therefore compare the observed rate of advance with that computed on the basis of the theoretical one for demic diffusion. Unfortunately there are not yet any direct measurements of the growth rate or the migration of neolithic people, but it is clear that the possession of agriculture, by increasing the carrying

capacity of the land, conferred on the neolithic people the potential for a great increase in population density. The population density of early agriculturals could even be as high as 100 to 1000 times that of hunters and gatherers. It seems very likely, though it is not yet proved, that the potential increase was soon realized. In today's absence of direct demographic data on early neolithics, one can use what little knowledge is available on the growth rates of populations that have occupied empty spaces in later times, as well as what knowledge is available on migration rates. Population growth rates observed in the occupation of empty islands in the Atlantic and Pacific or in other freely multiplying isolates are of the order of doubling each generation. As to migration rates, those observed in today's African farmers occupying the tropical forests give standard deviations of the order of 20 to 30 km. It is remarkable that, using these growth and migration rates, the expected radial rate comes very close to the observed rate. Clearly even accepting the validity of these estimates, this is only an indirect test of the theory and much more work remains to be done.

An important corollary of the demic theory of diffusion of early farming is that it must have genetic consequences. If the expanding farmers mixed with the hunters and gatherers they found when occupying new lands, a dilution of the original genetic type of the farmers should be observed, increasing with increasing distance from the point of origin. It is possible that genetic differences between the mesolithic hunters and gatherers living in Europe, and the farmers who gradually invaded it, coming from the Near East were not sufficiently important to allow a regular cline of genetic differences to be observed. But it should be noted that the fact that Basques show important genetic differences from the rest of Europeans (Mourant, 1954) is in agreement with the idea that they developed before the neolithic wave arrived in the area (around 3,000 B.C.) and their culture, language, and biological identity partially survived the impact with the newcomers. The situation is more clearcut in Africa. There the genetic differences between arriving neolithics and local mesolithics were probably more substantial and therefore a genetic cline could be established by the neolithic migration. A preliminary analysis of the distribution of genetic markers in Africa in the light of this theory suggests that a progressive diffusion of Caucasian genes through Suez – and perhaps the Red Sea – led to a progressive dilution of the original Caucasian genes. A satisfactory representation of the overall picture of African Rh types is thus obtained.

It is easy to understand how the neolithic wave in Africa may have stopped at the boundary formed by the forest at about 12° N latitude where none of the crops or animals which the Near Eastern farmers had originally developed would be able to survive. Secondary, or perhaps even independent, centers of domestication formed in those regions which were more compatible in their ecology. These centers permitted further expansions of the locally developed farming cultures. Of these, the Bantu spread to Central and South Africa is probably the most important example. It was notably facilitated by the use of iron. Thus the W. African type extended to many parts of Central and South Africa. The relative homogeneity of West, Central and South African Negroes must be the result of a rapid spread of a people endowed with a new technology that permitted rapid increase of population and rapid migration.

As mentioned before, we do not know exactly when Pygmies first came into contact with the invading farmers, but it is likely that this happened at least several centuries ago. Perhaps Babingas further to the west may have experienced the contact at an earlier time and in the beginning partial admixture with the invading people may have taken place. In any case, there must have been a long period in which Pygmies have been in contact with the agriculture which was introduced into West Central Africa by the Bantus and in some other, eastern, areas by other ethnic groups. A remarkable symbiosis between farmers and Pygmies has developed, but it is clear that Pygmies have, themselves, refused so far to take part in the agricultural revolution and remain one of the few people in the world who have not yet effected this transition, or have effected it only to a very minor extent.

It is interesting to mention by contrast what seems to have happened in Europe at the time of the agricultural expansion. Radio carbon dating of the latest mesolithic (the latest hunters

and gatherers) cultures in the same areas where neolithic cultures have been found shows that there is practically no overlap between mesolithics and neolithics. In other words, when the neolithic entered an area, the mesolithic disappeared. It is true that many of the data, especially on the mesolithic, are still too fragmentary and need further study with this idea in mind. When more data accumulate, some overlap between mesolithics and neolithics may perhaps be found. If we take present data at face value we should say that the mesolithic culture disappeared with the arrival of the neolithic in Europe. The reasons may have been multiple. It may be that European mesolithics were subjugated or essentially exterminated by the invaders; it may be that they quickly absorbed the new mode of life and freely mixed with the invaders. Still other explanations are possible, but they should take account of the rapid disappearance as far as known today. This is very much unlike the situation in Africa where hunters and gatherers, such as Pygmies who can be considered 'mesolithics' in many respects, have survived side by side with 'neolithics'* for many centuries, and are still maintaining their numbers with remarkable tenacity. It is true that they must have covered a much larger area once and that they are now confined to some pockets, but the extent of the region they occupy is not at all negligible.

One main difference between the probable situation in Europe at the time of the spread of the neolithic and the situation we see today in Africa comes from a consideration of climatic differences. The climate in Europe must have shown considerable seasonal variation with re-

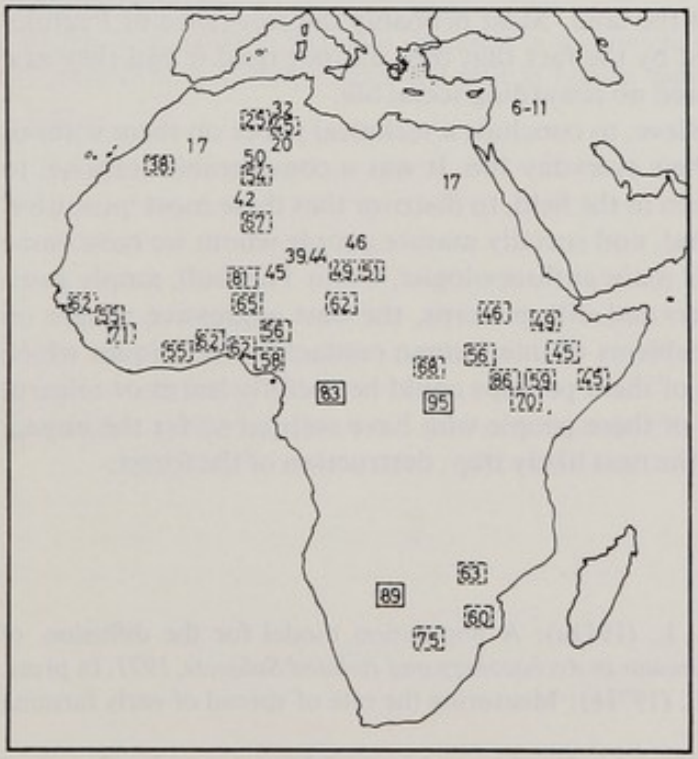


Fig. 7. Frequencies in the per cent of the Rh_0 allele, showing agreement with the hypothesis of spread of early farming from the Near East. This allele has a frequency of 3% in Europe and is slightly higher in the nuclear area. Unboxed numbers refer to populations considered 'white', and those in a dotted box to groups with dark skin usually, but not always, considered Negro. Numbers in solid boxes refer to protoafricans, namely Pygmies or Bushmen. Apart from irregularities due to historically known migrations such as those of Berber tribes in North Africa or of the Bantu invasion of Central and South Africa, the figures show a general cline which agrees which the idea of a migration by a group low in Rh_0 gene frequency from the nuclear area which has progressively mixed with a group of almost 100% Rh_0 allele gene frequency. (From Ammerman and Cavalli-Sforza, 1971*b*.)

* The word as applied here to Bantu invaders is, of course, inappropriate, as Bantus had iron.

latively hard winters, though it is believed that at the time of neolithic expansion the climate may have been milder. During those winters, the life of the hunter and gatherer must have been a more difficult one. Agriculture, however, permitted the abundant production of food easily stored for use in the winter. The advantage of agriculture must have been very evident, and the transition to it, if the hunters and gatherers were to survive, must have been a relatively simple proposition.

The same is not true of the African forests. Here agriculture has been and still is a very difficult adventure. Today, especially with the introduction of manioc and bananas, food production is easy, but by no means satisfactory. The crops thus obtained do not provide great variety, nor a nutritionally valuable food. The two named crops are newcomers, especially manioc, and we do not even know exactly what was in use before their introduction. Earlier crops must, however, have been definitely inferior to the presently used ones, considering the ease with which the latter have spread to a very large area in a short time (especially in the case of manioc). Agriculture in the tropical regions is still a difficult technical problem. Even sophisticated western agriculture does not have much to offer to substitute for the crops presently raised by the African farmers. It has only added a few cash crops which do not answer the problem of daily food.

By contrast, Pygmies have continued to live on the products of the forest, to which they have learned to adapt so well. They have no problems with the limited seasonal variation. They learned to establish and maintain fairly friendly relations with the invaders, without losing the effective ownership of most of the land. Most probably the resistance of Pygmies to the agricultural revolution is explained by the fact that they did not need it and they saw no justification in an economy which offered no rewarding social life.

It would be unfair to the Pygmies, I believe, to conclude a technical paper on them without mentioning some intriguing aspects of their everyday life. It was a considerable surprise, to me and to all the co-workers who have been in the field, to discover that these most 'primitive' people are among the most charming, kind, and socially mature people whom we have come across. We found the enthusiasm of their main anthropologist, Colin Turnbull, amply justified. Pygmies are extremely brave hunters and still, perhaps, the least aggressive people on earth. They have learnt to solve their problems of interhuman contact by techniques which we seem to have largely forgotten. Some of them perhaps could be usefully learnt or relearnt if there is time enough for further study of these people who have resisted so far the impact of civilization but who cannot withstand the next likely step: destruction of the forest.

REFERENCES

Ammerman, A. J. and Cavalli-Sforza, L. L. (1971*a*): A population model for the diffusion of early farming in Europe. *Proc., Research Seminar in Archaeology and Related Subjects, 1971*. In press.

Ammerman, A. J. and Cavalli-Sforza, L. L. (1971*b*): Measuring the rate of spread of early farming in Europe. *Man, 6*, 674.

Bodmer, W. and Cavalli-Sforza, L. L. (1968): A migration matrix model for the study of random genetic drift. *Genetics, 59*, 565.

Cavalli-Sforza, L. L. (1969): Human diversity. In: *Proceedings, XII International Congress of Genetics, Tokyo, Japan, 1968, Vol. 3*, pp. 405–416. Scientific Council of Japan.

Cavalli-Sforza, L. L. and Bodmer, W. (1971): *The Genetics of Human Populations*. W. H. Freeman and Co., San Francisco, Calif.

Cavalli-Sforza, L. L., Zonta, L. A., Nuzzo, F., Bernini, L., De Jong, W. W. W., Meera Khan, P., Ray, A. K., Went, L. N., Siniscalco, M., Nijenhuis, L. E., Van Loghem, E. and Modiano, G. (1969): Studies on African Pygmies. I. A pilot investigation of Babinga Pygmies in the Central African Republic (with an analysis of genetic distances). *Amer. J. hum. Genet. 21/3*, 252.

De Jong, W. W. W. and Bernini, L. (1968): Hemoglobin Babinga (δ chain 136 gly→Asp): a new delta chain variant. *Nature (Lond.), 219*, 1360.

Fisher, R. A. (1937): The wave of advance of advantageous genes. *Ann. Eugen. (Lond.), 7*, 355.

HIERNAUX, J. (1968): *La Diversité Humaine en Afrique Subsaharienne*. Editions de l'Institut de Sociologie de l'Université Libre de Bruxelles.

KENDALL, D. G. (1948): *Proc. Cambridge Phil. Soc.*, *44*, 591.

MALCOLM, L. A., BOOTH, P. B. and CAVALLI-SFORZA, L. L. (1969): Intermarriage patterns and blood group frequencies in the Bundi People of the New Guinea Highlands. In press.

MOURANT, A. E. (1954): *The Distribution of the Human Blood Groups*. Blackwell Scientific Publications, Oxford.

MURDOCK, G. P. (1959): *Africa: Its Peoples and their Culture History*. McGraw-Hill, New York.

OWEN, D. F. (1966): *Animal Ecology in Tropical Africa*. W. H. Freeman and Co., San Francisco, Calif.

PAOLUCCI, A. M., SPADONI, M. A., PENNETTI, V. and CAVALLI-SFORZA, L. L. (1969): Serum free amino acid pattern in a Babinga Pygmy adult population. *Amer. J. clin. Nutr.*, *22/12*, 1652.

RIMOIN, D. L., MERIMEE, T. J., RABINOWITZ, D., CAVALLI-SFORZA, L. L. and MCKUSICK, V. (1969): Peripheral subresponsiveness to human growth hormone in the African Pygmies. *New Engl. J. Med.*, *281*, 1383.

ROBERTSON, A. (1951): The analysis of heterogeneity in the binomial distribution. *Ann. Eugen. (Lond.)*, *16*, 1.

SANTACHIARA BENERECETTI, S. A., MODIANO, G. and NEGRI, M. (1969): Studies on African Pygmies. II. Red cell phosphoglucomutase studies in Babinga Pygmies: a common PGM_2 variant allele. *Amer. J. hum. Genet.*, *21*, 315.

SANTACHIARA BENERECETTI, S. A. and NEGRI, M. (1970): Studies on African Pygmies. III. Peptidase C polymorphism in Babinga Pygmies: a frequent erythrocytic enzyme deficiency. *Amer. J. hum. Genet.*, *22*, 228.

SKELLAM, J. G. (1951): Random dispersal in theoretical populations. *Biometrika*, *38*, 196.

WIESENFELD, S. L. (1967): Sickle-cell trait in human biological and cultural evolution. *Science*, *157*, 1134.

J. Neel, T. Arends, C. Brewer, N. Chagnon, H. Gershowitz, M. Layrisse, Z. Layrisse, J. MacCluer, E. Migliazza, W. Oliver, F. Salzano, R. Spielman, R. Ward and L. Weitkamp

Department of Human Genetics, University of Michigan School of Medicine, Ann Arbor, Mich., U.S.A.

Studies on the Yanomama Indians*

This presentation will assume that by now an adequate case has been made for the insights into population genetics which can *only* be gained by studies in depth of relatively unacculturated, tribal-type populations, and proceed directly to a consideration of one such effort. Some years ago, as the urgent need for such studies began to emerge – urgent not only for scientific reasons but because they will not be possible for much longer – our group elected to direct its attention towards the American Indian who, for reasons outlined elsewhere (Neel and Salzano, 1964), offered a number of unusual opportunities. A program was developed whose principal objectives could be subsumed under three headings (Neel and Salzano, 1964):

1. To develop a knowledge of population structure in groups of this type.
2. To apply the newer techniques of distance analysis based upon defined genetic characteristics to the complex task of unravelling the relationships of Indian tribes and estimating rates of genetic divergence.
3. To attempt to identify and even quantify the manner in which 'civilization', the definition of which grows more difficult each day, introduces the need for genetic adjustments.

At the outset there was some ambiguity in our minds as to the relative merits of a broad program covering many tribes and a program which studied in depth a relatively few tribes. Our pilot studies among the Xavante of the Brazilian Mato Grosso led to a series of hypotheses-generalizations concerning the above-mentioned objectives (Neel and Salzano, 1967). It was clear these were best pursued in a larger and even less disrupted group than the Xavante. After consideration of many possibilities, we turned attention to the Yanomama of Southern Venezuela and Northern Brazil. It quickly became apparent that this group was characterized by striking genetic microdifferentiation (Arends *et al.*, 1967). At the same time, a preliminary effort to develop a genetic network of the Central and South American Indian tribes for which adequate data were available raised many questions, the answers to which clearly were in part dependent upon an appreciation of the robustness of these networks (Fitch and Neel, 1969). The Yanomama were seen as a group in which not only could we study population divergence at the first, the village level, but in which, by a judicious use of ethnologic, anthropometric, and linguistic evidence, it would be possible to test the accuracy with which a genetic network portrayed the events of the recent and remote past. As one of the largest un-

* The financial support of the U.S. Atomic Energy Commission and the National Science Foundation is gratefully acknowledged. This program constitutes one of the Integrated Research Projects of the U.S. contribution to the International Biological Program.

acculturated and relatively undisturbed peoples to be found in South America, they were likewise felt to present unusual opportunities for demographic and biomedical studies. Accordingly, in effect we made the decision to look no further, but to concentrate on this single group. It is this preoccupation with one tribe which now distinguishes our program from the others to be described in the course of this Symposium today. You might say we are attempting to study evolution at the 'grass roots' level. However, although the Yanomama have received the bulk of the attention, it has been clear that some aspects of our findings could only be properly appreciated if data were available for the surrounding tribes, and so a limited effort has been directed towards the Piaroa, Macushi, and, especially, the Makiritare, since

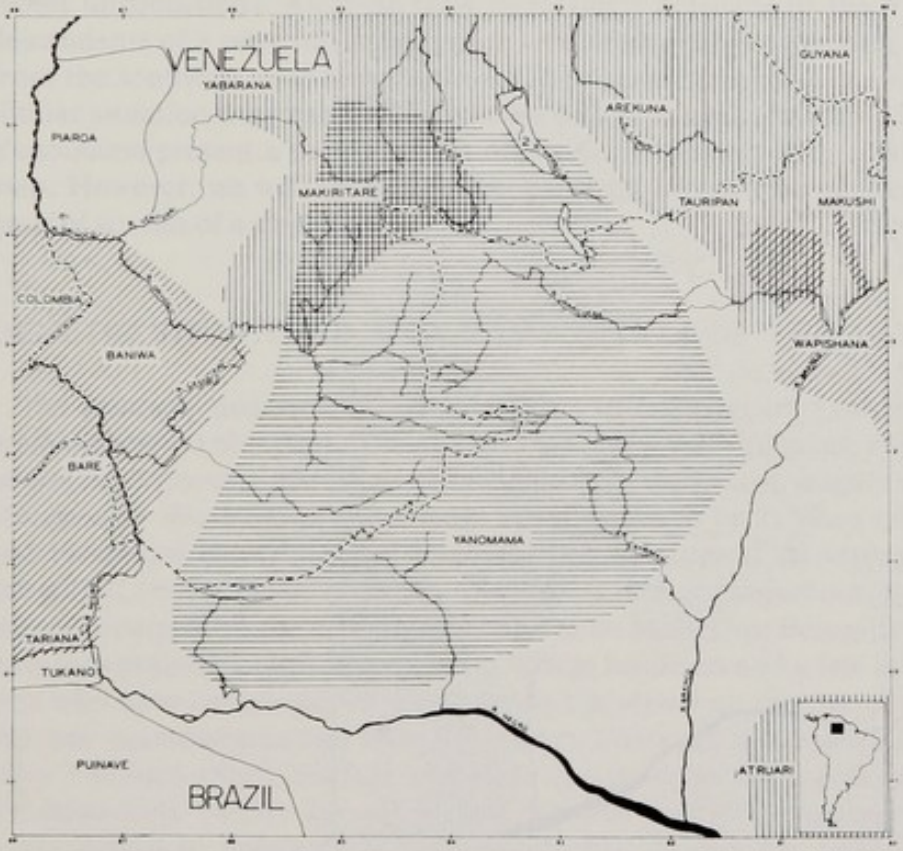


Fig. 1. Distribution of Yanomama and surrounding tribes in 1970. The distribution of language families is indicated as in Figure 2. Note the disappearance of the tribes numbered 3, 4, and 5 in Figure 2, as well as four Arawak groups (Kuriobana, Barauana, Manduaka, Manau) formerly contiguous to the Yanomama on the southwest, and the three Carib groups (Purukoto, Sapara, Paushiana) to the northeast. The area occupied by the Yanomama has more than doubled in the past century.

the latter are now in contact with the Yanomama along a broad and until recently, mutually hostile front, thus providing unusual opportunities for the study of gene flow across tribal boundaries.

The work has progressed slowly, more slowly than anticipated. The Yanomama would not be as relatively unacculturated as they are if they were readily accessible. Primarily a forest people, only in the past 20 years have they located some of their villages on the Upper Orinoco and some of its larger tributaries, to establish contacts with small missions, a move clearly dictated more by a desire for material gain than spiritual enlightenment. As a matter of fact, the Yanomama have been, compared with most other Indian tribes, quite resistant to spiritual change. Because of our difficulties in projecting to our colleagues the nature of this type of field work – but more especially because of our concern to document the precise condition

of the Yanomama at the time of study – we have been engaged in the preparation of a number of film studies. One particular film (shown at this Congress) was prepared with an additional objective, to show students that contemporary genetics involve something more than the study of the double helix. We think you will find this film of interest.

Figure 1 illustrates the present distribution of the Yanomama and their neighbors. There are by our latest estimate something like 200 Yanomama villages and 15,000 Yanomama scattered throughout an area of approximately 100,000 square miles. It seems quite clear that within the past 100 years the tribe has expanded to the north and west, this expansion bringing them into hostile contacts with the Makiritare. This expansion is thought to be numerical

Fig. 2. Distribution of Yanomama and surrounding tribes about 1850. The distribution of tribes speaking Carib languages is indicated by vertical hatching, the distribution of speakers of Arawak languages by diagonal hatching, and of the Yanomama, by horizontal hatching. There are 7 tribes with as yet 'unaffiliated languages' (distributions unshaded), namely (1) Awake, (2) Sape (Kariana) (3) Marakana, (5) Máku, and the Piaroa, Tukano and Puinave. The small tribe indicated by '4', the Guinau, was an Arawak-speaking tribe, left unshaded for purposes of clarity.

as well as geographical. Figure 2 is an effort to reconstruct their distribution as of approximately 100 years ago, based on data of Schomburgk (1841), D'Almada (1861), Koch-Grünberg (1923), and Migliazza (1967*a*). We see them at that time as a relatively isolated group, their 'heartland' the Parima Mountain Range on the Brazilian-Venezuelan border, with a surrounding buffer zone occasionally traversed by war parties. The small tribes indicated as the Máku, Awake, Kuriobana, and Marakana have in the past 100 years been decimated and/or absorbed by the Yanomama and some of their neighbors. The material possessions of the Yanomama are few in number and simple in construction (Zerries, 1955, 1964; Becher, 1960; Migliazza, 1966, 1967*b*; Chagnon, 1968*a*, *b*; Lizot, 1970), decidedly less elaborate than those of the nearby Makiritare (De Barandiaran,1962, 1966; Layrisse and Wilbert, 1966), Pemon (Simpson, 1940; De Armellada, 1946; Layrisse and Wilbert, 1966), or Macushi (Farabee, 1924). Their

language does not appear to be closely related to any of the principal linguistic families of South America (Gê, Arawak, Chibcha, Carib, Tupi-Guarani). They are small, often lightly built, almost pygmoid people. Zerries (1959) found the mean stature of 37 males to be 1520 mm; in our series of 391 males, it was 1531 mm, with a standard deviation of 48 mm. Of the 30 different South American tribes represented in Comas' summary (1971), only one is shorter in average stature. Whereas the Diego-a and acid phosphatase-A genes are present in polymorphic proportions in the other three tribes shown on Figure 1 thus far studied (Piaroa, Makiritare, Macushi), these two genes are only rarely encountered in the Yanomama, and then principally at the periphery of the tribe. In some villages their presence is clearly due to recent admixture (Chagnon *et al.*, 1970), and we are inclined to view these genes as relatively recent introductions. All these facts increasingly persuade us the Yanomama represent the descendants of a relatively small group who centuries ago wandered into this area, probably from the southeast, and have remained in relative isolation ever since. To the extent that a similar situation may have obtained time and time again as man spread over the earth, the Yanomama present a most unusual opportunity to study the genetic structure of primitive man. However, we well recognize how cautious we must be of over-generalizations on the basis of studies of a single group.

GENETIC STRUCTURE AND COMPOSITION OF THE YANOMAMA

The principal demographic characteristics of a cluster of Yanomama villages have been briefly described elsewhere (Chagnon, 1968*a*; Neel and Chagnon, 1968), and will in due time be treated *in extenso*. The ultimate breeding unit, the village, usually contains between 40 and 250 persons. We estimate the average age of males to be 21 years and of females, 23. Infant and childhood mortalities are relatively low by tropical standards, our working estimate being 18% (excluding infanticide). Marriage is lineage exogamous, the preferred type involving cross-cousins of the first degree (FaSiDa or MoBrDa). Polygyny, in theory permitted all men, is in practice largely limited to the village headman and a few other senior citizens. Women who have completed the childbearing age report an average of 3.8 livebirths. However, physical examinations and tests for urinary chorionic gonadotropins suggest a pregnancy every 3–4 years (Neel, 1969). It is obvious that child-spacing is practised. The principal means are intercourse taboos during the first year the mother is nursing the child and abortion, accomplished by traumatic but effective means. In addition, infanticide is not uncommon, although it has thus far been impossible to obtain exact figures. This is traditionally committed immediately after the birth of the child. We believe that liveborn children so killed are not reported to the anthropologist by the mother, so that the figure given above for livebirths does not include cases of infanticide. It also of course does not include abortion. This may account in part for the discrepancy between the reproductive history and our above-mentioned data on pregnancy rates.

Contention for leadership within the village is brisk. A small village will have but one headman, but a larger village will have several. Tensions between these headmen and their followers lead to village fissions. The minor fragment from the fission may form a new village or it may join another village. A village which finds itself decimated by warfare and under heavy pressure may also join a larger, stronger village, either temporarily or for an extended period. In addition to these relatively major losses and acquisitions of people by a village, there is a continuing exchange of individuals, either peacefully or as a result of raids on nearby villages. Although we have not yet worked out the migration matrices for a set of interconnected Yanomama villages, this has been done for six villages of a neighboring tribe, the Makiritare. The backward stochastic migration matrix, as defined by Bodmer and Cavalli-Sforza (1968), is shown in Table 1 (Ward and Neel, 1970). For those interested in developing realistic models of populations, we point out how anisotropic that pattern of exchange is. Unfortunately

for genetic manipulations of that matrix, there has been extensive exchange between these six villages and areas not contacted. Attempts to extend the matrix are in progress.

With this necessary background, we turn to two matters which have recently been occupying our attention. The first is an effort to extend our insight into the dynamics of the tribe by virtue of simulation. As Dr. MacCluer will describe in more detail in another session, a stochastic computer simulation model has been developed which attempts to reproduce most of the aspects of population structure just enumerated (MacCluer, Neel, and Chagnon, 1971; MacCluer and Neel, unpublished data). The demographic base for the model consists of the actual inhabitants of four interrelated villages, with populations of 50, 71, 86, and 244, distributed among 11 lineages. Computer runs, incorporating birth, marriage, reproduction and death, simulate periods of 200 to 400 years. Three of the kinds of questions this model has enabled us to explore are the following:

1. As mentioned earlier, the ethnographic data suggest that the Yanomama have been numerically as well as geographically an expanding population in the past 100 years, an unusual situation among Central and South American Indian tribes. Given the complexity of population structure, it was not immediately apparent how consistent the various demographic parameters collected in the field were with the apparent rate of expansion of the Yanomama. In 15 computer runs which extended at least 200 years, each with an input of the same 451 individuals, and the same program, the population size at the end of 200 years varied between 293 and 1121 persons, with a mean of 576.2. The wide range bespeaks for the role stochastic events may play in such a small population. Be this as it may, the output corresponds with the impression of an expanding population.

2. What is the level of inbreeding within the population? Of 124 marriages represented in the real, input population, there were only 37 in which all four grandparents of both spouses were known, and none where all eight great-grandparents were known. Clearly, a direct estimate of the coefficient of inbreeding would be of little value. In a simulated population whose growth fell in the midrange of the values given above, the value of the pedigree F for the last 7 generations of a 400-year run was 0.032. The limited capacity of the computer did not permit extending the analysis back further than 7 generations. An unexpected finding was that the coefficient of inbreeding increased as much between generations 6 and 7 as 4 and 5. The potential contribution of remote links of inbreeding to the total coefficient in small populations of this nature has probably not been appreciated. Thus, the true coefficient of inbreeding may be even higher than this already high estimate of 0.032.

3. In large populations surveyed for the occurrence of consanguineous marriage, there is of course a sampling variance attached to the estimate. In small populations, where the entire

TABLE 1 *A backwards stochastic migration matrix for a set of 6 interrelated Makiritare villages*

Village	N	$m_{.j}$					
		BD	C	E	F	G	HI
BD	368	0.783	0.079				0.016
C	128	0.180	0.774				
E	166			0.663			0.018
F	138				0.544		0.029
G	188	0.011		0.005		0.601	0.011
HI	334				0.003	0.036	0.560

The left half of the matrix describes exchanges between six contacted villages, designated with letters; the right half, exchanges with villages as yet uncontacted, designated by numbers. Numbers 1 through 5 designate, respectively, villages on the headwaters of the Ventuari, Cuntinamo, Padamo, Cunucunuma, and Caura Rivers. Number 6 indicates origin unknown, and number 7, Yanomama

group is enumerated, this variance does not exist in the usual sense. However, the contribution of stochastic events (such as number of first and second cousins available for marriage) to temporal fluctuations in the coefficient of inbreeding appears relatively high. For example, in 15 simulation runs extending over a period of 200 years for which the per cent of first cousin marriages was computed for 10-year periods, the absolute value of the change in frequency between successive 10-year periods was 3.8 $\pm$ 2.8%. The maximum difference observed involved a drop in the frequency of first cousin marriages from 21.6% in one 10-year period to 6.3% in the next. Under these conditions, the results of a single point survey could be quite misleading.

It is clear that sophisticated interplay between field work and computer model can provide important additional insights into population structure. On the other hand, it is also clear our model needs considerable improvement before we can refine some of the early findings described above or proceed with further questions. Some may not find this initial output to be a major accomplishment for simulation, but in our opinion until we have checked the model out on the smaller points we are not yet ready for the larger. Our simulation is a closed breeding system; it allows a village to exchange persons only with the other three villages. A second unrealistic feature is failure to allow for fission when a village exceeds a certain size, or fusion when its numbers drop below 25. Finally, our input, based on the four villages for which we had satisfactory data when the simulation was undertaken, has a deficiency of young persons, quite possible because of epidemic disease some 10-15 years before our studies began, and this is probably influencing our output for the first 100 simulated years in ways only dimly perceived. Efforts are now in progress to alter these and other features of the model.

A second matter currently occupying our attention is reproductive compensation in the Yanomama, and its possible role in determining gene frequencies. Formal treatments of selection generally disregard this possibility. Recent studies on consanguinity effects in Japan reveal what a subtle and pervasive phenomenon this may be (Schull *et al.*, 1970). As long as we thought of primitive man as reproducing at near capacity in order to offset the kind of high infant death rates formerly seen in tropical agricultural communities, there was little need to consider reproductive compensation as a factor in the explanation of gene frequencies. Now we recognize adequate opportunities in primitive man. In the extreme case of a recessive trait resulting in early death, complete reproductive compensation (replacement by a phenotypically normal child) will slow the rate of elimination by selection from q^2 to $2/3q^2$ (Motulsky *et al.*, 1971). Although this is a minor source of perturbation in certain estimates, such as mutation rates, compared with, for instance, the results of assuming equilibrium, nevertheless it is clear the phenomenon must be considered as we attempt to improve our genetic formulations.

$\alpha_{.j}$						
1	2	3	4	5	6	7
0.060	0.019				0.038	0.005*
0.047						
0.319						
	0.145	0.080			0.044	0.159
0.043		0.075	0.133	0.080	0.032	0.011
0.129	0.090	0.105	0.045	0.027		0.006

origin. The symbols $m_{.j}$ and $\alpha_{.j}$ denote the probability that individuals born in colony i have parents derived from colony j. The asterisk denotes one Venezuelan 'campesino' who fathered two children in the village.
From Ward and Neel (1970).

The major objective which underlies most investigations in the field of population genetics today is to understand the significance of the great amount of variability present in all properly studied species. In the case of man, this variability arose and presumably persisted for long periods at the tribal and pre-tribal levels of social organization. Accordingly, we feel that in simulation programs designed to explore the maintenance of genetic variation in human populations, a major emphasis must be on incorporating the complexities of a tribal-type organization; for most exercises these programs should probably include high levels of inbreeding and reproductive compensation.

The question of the genetic relationship of the Yanomama to other Indian tribes, and the internal genetic organization of the Yanomama, has thus far been approached through studies of allele frequencies at 30 loci in a total of 2416 persons distributed among 37 villages. To begin with, mean Yanomama gene frequencies with respect to the commonly studied polymorphisms are unusual among Indian tribes in the relatively high frequency of CDE (R^z) and

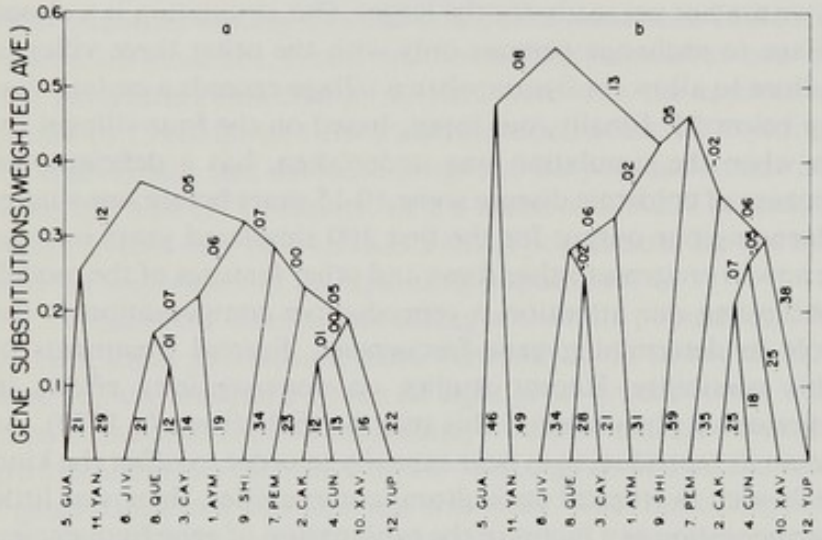


Fig. 3. A genetic network for 12 Indian tribes of Central and South America, after Fitch and Neel (1969). Network derived by the 'minimum-string method' of Edwards and Cavalli-Sforza (1963, 1964) and Cavalli-Sforza and Edwards (1967). Fig. 3a is based on exact distances derived by this method. Fig. 3b follows the same topology but bases distances on the minimum deviation method of Fitch and Margoliash (1967), which attempts to adjust the sum of the line segments between any two tribes to the best approximation of the genetic distance, as currently measured, between these two tribes. The tribes involved (left to right) are the Guaymi, Yanomama, Jivaro, Quechua, Cayapa, Aymara, Shipibo, Pemon, Cakchiquel, Cuna, Xavante, and Yupa.

CDe (R^1) and low frequency of cDE (R^2) of the Rh system, the high frequency of Hp^1 of the haptoglobin system, the low frequency of MS of the MNSs system, and, as indicated earlier, the virtual absence of the Di^a and AP^A genes (see Post *et al.*, 1968). Figure 3 presents a genetic network for 12 Indian tribes of Central and South America, derived by the 'minimum string method' of Edwards and Cavalli-Sforza (1963, 1964) and Cavalli-Sforza and Edwards (1967) (Fitch and Neel, 1969). Incidentally we found little difference between the network yielded by this method and by the cluster method of Edwards and Cavalli-Sforza (1965) and the minimum deviation method of Fitch and Margoliash (1967). The sole bases for selection of tribes for inclusion in this network were (1) determinations available on the ABO, MNSs, Rh, Kidd, Duffy, Diego and haptoglobin systems, (2) non-Indian admixture estimated at less than 5%, and (3) sample size greater than 200. The distinctive position of the Yanomama, together with the Guaymi, is obvious. These latter, incidentally, are a 'tribe' found in Panama, speaking the Talmancan division of the Chibchan language stock. They are apparently heterogeneous in origin, derived primarily from groups who retreated from the Caribbean shore of Panama

TABLE 2 *Range of gene frequencies for 9 genetic systems in 37 Yanomama villages*

System	Allele	Range
MNSs	MS	0.00–0.49
	Ms	0.20–0.79
Rh	R^2	0.00–0.26
	R^1	0.60–1.00
P	P^1	0.26–0.87
Duffy	Fy^a	0.35–1.00
Kidd	Jk^2	0.36–0.84
Le secretion	Le^2	0.28–0.74
Haptoglobin	Hp^1	0.51–0.99
Gc	Gc^1	0.66–0.97
PGM	$PGM^1{}_1$	0.82–1.00

southwards to the mountains of the interior (Johnson, 1948). The blood specimens on which their status is based, 240 in number, come from two collection sites but were pooled so that heterogeneity cannot be tested (Matson and Swanson, 1965). Here is an example of how the derivation of genetic networks from genetic distances creates unexpected bedfellows, who must be sorted out. At any rate, all of the foregoing findings strengthen the surmise that the Yanomama are the descendants of a rather small group and have been in relative isolation for some time.

There are two additional noteworthy genetic features of the Yanomama. One is the very marked genetic microdifferentiation. Examples are shown in Table 2. Thirty or more typings were available for each of the 37 villages on which the range is based. We now also have data on human histocompatibility antigen (HLA) types for 8 villages. This system, because of the number of alleles known, should be most helpful in studies of microdifferentiation, and also of the balance between drift and selection. As the Layrisses will report elsewhere in the Congress, the Yanomama possess at least 6 specificities at HLA locus 1, and another 6 at locus 2, with marked village differences. The microdifferentiation is conveniently measured by Wright's F_{ST} statistic, defined as $\sigma^2/\bar{p}\bar{q}$, where $\bar{p}$ is the population mean for a given gene, $\bar{q} = 1-\bar{p}$, and σ^2 is the variance of the values of p for the breeding units of the population (Wright, 1943). Table 3 presents estimates for F_{ST} on the basis of 8 codominant genetic sys-

TABLE 3 *F_{ST} (calculated as $\sigma^2/\bar{p}\bar{q}$) for 9 codominant alleles on the basis of determinations in 37 Yanomama villages and 7 Makiritare villages*

System	Yanomama	Makiritare
M–N	0.0681	0.0256
S–s	0.0663	0.0554
C–c	0.0954	0.0048
E–e	0.0663	0.0064
Fy^a–Fy^b	0.0561	0.0133
Hp^1–Hp^2	0.0611	0.0725
Gc^1–Gc^2	0.0648	0.0325
$PGM^1{}_1$–$PGM^2{}_1$	0.0329	0.0430
AP^A–AP^B	–	0.0142

tems studied in 37 Yanomama villages. Similar data are presented for 7 Makiritare villages (9 codominant systems). Comparable data are simply not yet available for other undisturbed tribal populations, but it seems quite likely that in general such populations will be found to have very high F_{ST} values. We will come to the possible significance of this for human evolution shortly.

A second noteworthy feature of the Yanomama is their low level of heterozygosity for both the established polymorphisms and the rare variants (Weitkamp and Neel, 1972). As Dr. Weitkamp will describe during this Congress, on the basis of electrophoretic studies of the phenotypic variation due to gene substitutions at 17 loci, polymorphic in one or more populations, we find that the average heterozygosity per locus is 3.5%. A composite European population – composite in the sense that all these same determinations have not been made on a single group – would manifest about 11.2% heterozygosity. 'Private' variants for these same systems in European populations have a frequency of about 1 per 1000 determinations; in the Yanomama, we have 10 private variants among 29,129 determinations, or about 1:3000. One interpretation of this low frequency of heterozygosity is that it is the result of the loss of genetic variation to be expected in a long isolated and inbred population. If this is so, then the relatively large numbers of alleles retained at the two linked HLA loci would seem to suggest strong stabilizing selection (or a high rate of origin of variants).

The various aspects of population structure we have touched upon so briefly must be viewed for both their 'short range' and their 'long range' implications. With respect to the former, we have argued that to the extent in human evolution that new tribes arose from villages breaking away from an existing tribe, there could be, because of the marked genetic microdifferentiation (high F_{ST}), a very large stochastic element in the gene frequencies of the new tribe (Neel and Ward, 1970). The long range implications of this structure are much less clear. It is possible that the various departures we find from the large, equilibrium, panmictic, replacement-type populations on which so many of our genetic formulations are based, are of relatively little import, and can for the most part be accommodated by adjustments for effective population size.

THE VALIDATION OF GENETIC NETWORKS

The genetic structure of a tribe may be described and analyzed by a number of approaches – distance functions and genetic networks, Wright's F-statistics or a Malécot-type formulation, for instance. We see these approaches as supplementing and overlapping, rather than competing. Each yields certain insights denied to the other – each makes certain assumptions which are probably not strictly correct. Thus far, our major effort has been directed towards genetic distances and networks, but other types of analysis are in progress. We have seen the Yanomama and Makiritare as an exceptional test grounds for the validation of genetic networks in small population groups. The independent approaches available to us for testing how well the networks do are five in number: ethnohistory, geographic relationships, physical anthropology, dermatoglyphics, and linguistics. Thus far, our contrasts have been restricted to the first three, but work on the other two approaches is under way. Time permits the presentation of only two examples of the results of such comparisons.

Let us consider first a contrast of genetic network with ethnohistory. By chance the fortunes of field work placed us in a position to explore this relationship among the Makiritare before we were ready to proceed with the Yanomama data. In the course of the expeditions of 1967, 1968 and 1969, we obtained blood samples from most of the inhabitants of seven different Makiritare villages. The location of the villages is given in Gershowitz *et al.* (1970). At each village an effort was made to collect information about the village history, with particular reference to village sites within this century. The composite history is shown in Figure 4. Each entry indicates a village site or name. Two points stand out: (1) five of the villages traced their

origins back to a closely related complex of villages located on the Upper Cunucunuma River, about 1910, and (2) two villages, designated as C and F, appeared to be clearly separate in origins. In recent years village C has exchanged numerous marital partners with a village (designated BD) of the major complex (details in Ward and Neel, 1970). Figure 5 is our best genetic network for these same 7 villages, based on gene frequencies at 11 loci (Rh, MNSs, Duffy, Kidd, Diego, P, Lewis, haptoglobin, Gc, acid phosphatase, PGM_1, and the secretor trait), and again derived by the approach of Edwards and Cavalli-Sforza (1963, 1964) and Cavalli-Sforza and Edwards (1967), with modifications as described in Ward and Neel (1970).

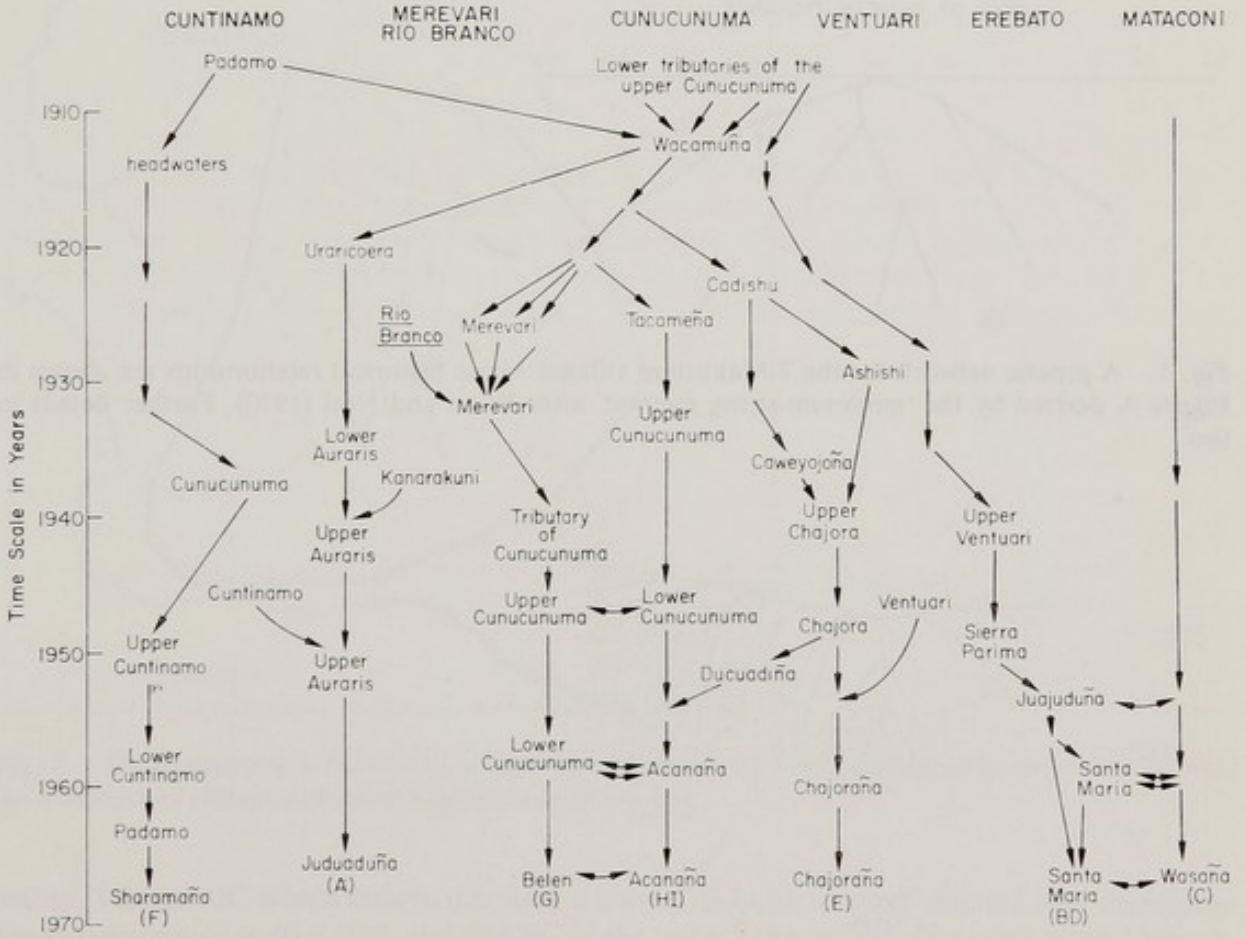


Fig. 4. A schema for the relationships over the past 60 years of 7 Makiritare villages. The labels at the top indicate major areas drained by the rivers whose names are given. The 7 names at the bottom (with letter designations) indicate the sites of the 7 villages studied. Each name in the schema itself indicates the site of a village occupied by the ancestors of these groups. Arrows indicate major additions or exchanges of persons.

The correspondence with ethnohistory is striking, with one exception: villages BD and C are indicated as having a common ancestry, whereas in fact we are confident that in origin BD is more closely related to the central cluster of villages. Here, then, is an example of how a high rate of exchange between two villages may influence a network. On the other hand, the intermediate position of BD between C and the remainder of the villages does reflect very well its hybrid origins, and illustrates why it is better to refer to these branching diagrams as genetic networks than as phylogenies.

We turn now to a contrast of a genetic network with geography, using as a test grounds the location of some 37 Yanomama villages. The genetic network, shown in Figure 6, was again

derived essentially by the 'minimum string method', utilizing the same 11 loci as previously. However, because of the number of villages involved, it was necessary to introduce several approximation procedures (Ward, 1972). Dr. Ward will present these procedures in greater detail elsewhere in this Congress. The positions of the 37 villages sampled, as best they can be determined in the absence of adequate mapping of the area, are given in Ward (1972). Although an effort has been made over the years to sample as widely as possible throughout the tribal distribution, logistic considerations such as the location of airstrips and navigable rivers have been important factors in limiting where we have penetrated. It has simply not been feasi-

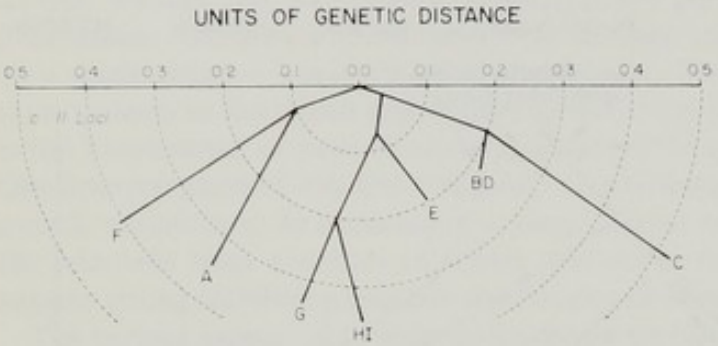


Fig. 5. A genetic network for the 7 Makiritare villages whose historical relationships are shown in Figure 4, derived by the 'minimum-string method' after Ward and Neel (1970). Further details in text.

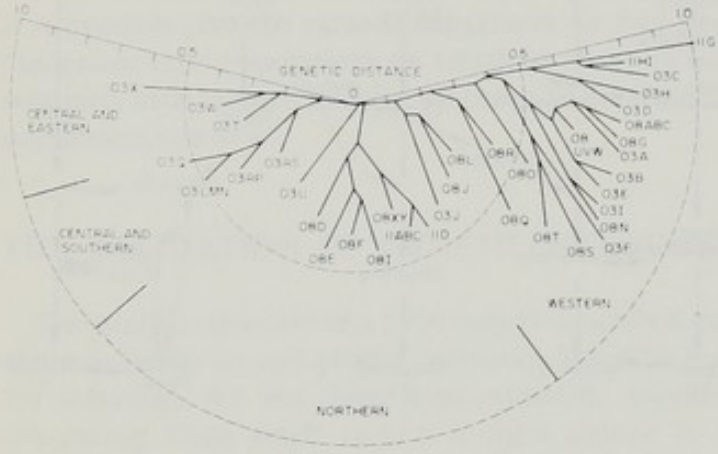


Fig. 6. A genetic network for 37 Yanomama villages, after Ward (1972). Details in text.

ble to lay out a sampling grid, especially in light of the fact that perhaps a third of Yanomama villages remain completely uncontacted by a non-Indian. Now, Yanomama villages are relocated approximately every 4 or 5 years, and under conditions of heavy pressure from warfare, may in the course of one of these relocations move 20 or 30 miles. An extreme example of movement under pressure has been described by Chagnon *et al.* (1970). For this reason, one might expect the correspondence between location and genetic affinities to be less than perfect.

For the purposes of this exercise, we will recognize 6 natural groupings of the villages shown in that network, as follows, reading from right to left: (1) 03X, 03W, 03T; (2) 03Q, 03LMN, 03KP, 03RS; (3) 03U, 08D, 08E, 08F, 08I, 08XY, 11ABC, 11D; (4) 03J, 08J, 08L, 08Q, 08R, 08O; (5) 08T, 08S, 03F, 08N, 03I, 03E, 03B, 08UVW, 03A, 08G, 08ABC; and (6) 03D, 03H, 03C, 11HI, and 11G. There is a measure of arbitrariness in these groupings and finer subdivisions are possible, but these are sufficient for the exercise. If, now,

the groupings of this network were more or less fortuitous, dictated by the noise in the system rather than genetic relationship, then the villages included in these groupings should be scattered more or less at random throughout the tribal distribution. In fact, however, as shown in Figure 7 it is possible to subdivide the Yanomama distribution into 6 non-overlapping areas within each of which, with no exceptions, one of the village clusters defined by the genetic network is found. Otherwise stated, none of the villages in cluster 1 of Figure 6 are found outside the area designated as 1 on the map, none in cluster 2 fall outside of area 2, etc. The exercise also provides another striking example of how the network reflects biological

Fig. 7. The geographic subdivisions of the area occupied by the Yanomama in which are located the 6 clusters of villages defined in Figure 6 and in the text.

reality. Village 03C which falls in the 'area 6 cluster' is in its 'formal' descent and traditional ties closely related to 03A, 03B, and 08ABC of the 'area 5 cluster' (*cf.* Chagnon, 1966, 1968*a*,*b*; Ward, 1972). However, it is on the border between area 5, which includes villages 03A, 03B, and 08ABC, and area 6, and our genealogical data reveal that of the 59 persons whom we sampled in 03C, 26 were either born in a village from area 6 or one of their parents was! The geographic pattern, despite the high mobility of the individual villages, suggests it would be of interest, with all due reservations, to compare our present findings with additional analyses based on the formulations of Malécot, and we are in the process of so doing.

Thirdly, we consider briefly a contrast between a network based on anthropometric distances (Mahalanobis' D^2) and one based on genetic markers, the latter again the gene frequencies at the same 11 loci mentioned earlier. The comparison has perforce been restricted to those 19 Yanomama villages for which both serological and anthropometric data are available. The correlation of entries in the two tables of pair-wise distances is very low: product moment, $r = 0.12$; Spearman's $r_s = 0.18$. The pair-wise distances are clearly not independent, however, which makes such correlations difficult to interpret. To evaluate the correspondence of the entire sets of genetic and anthropometric data, we have therefore devised a technique which compares the topology implied by one set with the data of the other (Spielman, 1971).

Underlying the technique is the observation that different topologies or networks applied to the same set of data, yield different total path lengths; the total amount of 'string' necessary to connect a set of points varies with the way in which the points are connected. Following Prim (1957) and Cavalli-Sforza and Edwards (1967), we assume that the smaller the total path length of a topology, the better it represents the relationships of the points to which it is applied. It follows that if we apply many topologies to a set of data, the one which is the best representative is that one which minimizes the total path length. For each set of data, therefore, we try to find the network which minimizes total path length; we thus obtain the topologies which best represent the genetic and anthropometric data respectively.

The original goal – to compare the relationships inferred from genetic data with those from anthropometric data – may now be rephrased. Of the many topologies which might be applied to the anthropometric data, we choose that one found to be best for the serological data and ask how well it represents the anthropometric data; *i.e.*, does it yield a relatively small total path length? To answer this question we need the distribution of total path lengths over all possible topologies (*cf.* Kidd and Sgaramella-Zonta, 1971). For 19 populations there are 6.33×10^{18} different networks. To examine the distribution, we have drawn a random sample of 1,000 networks and applied them to the anthropometric data, using an algorithm supplied by A. Edwards. The distribution is slightly skewed toward increasing path length, but not significantly different from the normal in kurtosis, when tested with standard tests of fit (ratios of functions of the 2nd, 3rd, and 4th central moments). The mean total path length is 39.14 (distance units) with standard deviation 2.01. The best topology found for the anthropometric data has total path length 25.87, more than 6 standard deviations below the mean of randomly chosen networks. The best topology found for the genetic data also represents the anthropometric data very well; it gives total path length 29.47, more than 4.5 standard deviations below the mean. To the extent that the distribution approximates the normal, we can say that the best topology found for the genetic data is in the best one-millionth (10^{-6}) part of the possible topologies for the anthropometric data.

It is also possible to base the comparison on the distribution of all possible topologies for the serological data. Looked at this way, the best topology found for the anthropometric data has total path length 4.25 standard deviations below the mean for randomly chosen nets applied to the serological data, and is therefore in the best hundred-thousandth (10^{-5}) part of the distribution of networks based on serology. Both approaches indicate a high degree of correspondence between the relationships implied by genetic and anthropometric data.

On the basis of these three experiences, it begins to appear that in small and subdivided populations of this type, genetic networks have a high validity but must be used with discrimination. However, there are at least two reasons why extreme caution is necessary in generalizing from this experience to all small, subdivided populations, or to larger populations. First, in those Indian tribes where we could study the process (Xavante, Makiritare, Yanomama), the sampling at the time of a village split is not in the least random, but along lineal lines. Successive repetitions of this process may result in greater genetic distances, and thus less ambiguous genetic networks, than when sampling is not so structured. Secondly, as noted, the Yanomama have apparently been expanding rapidly, in a more or less centrifugal fashion, during the past century, with less migration between all the villages of the complex than would be the case for a population with fixed boundaries. This might be expected to help preserve such differences as did arise between villages.

The third prong in this program, as mentioned at the outset, was the hope of gaining some insight into the selective pressures that had shaped our species, and how these pressures might be changing. Elsewhere we have recently summarized some of the kinds of data being collected (Neel, 1971). Time permits no discussion of this topic; let us note simply that we are slowly accumulating a body of data which in due time, in conjunction with the work of others, should help us appreciate just how great are the biomedical adjustments involved in man's transition from this cultural level to the present.

CONCLUDING REMARKS

The foregoing presentation has been intended to illustrate the range of studies possible on a tribal-type population. Until such populations are better understood, attempts to interpret the broad picture of genetic variation in man and the course and tempo of human evolution must remain highly speculative.

REFERENCES

Arends, T., Brewer, G., Chagnon, N. A., Gallango, M. L., Gershowitz, H., Layrisse, M., Neel, J. V., Shreffler, D., Tashian, R. and Weitkamp, L. (1967): Intratribal genetic differentiation among the Yanomama Indians of Southern Venezuela. *Proc. nat. Acad. Sci. USA, 57/5*, 1252.

Becher, H. (1960): Die Surára und Pakidái: Zwei Yanomámi-Stämme in Nordwestbrasilien. In: *Mitteilungen aus dem Museum für Völkerkunde in Hamburg, Vol. 26*, p. 138. Komissionsverlag Gram, De Gruyter and Co., Hamburg.

Bodmer, W. F. and Cavalli-Sforza, L. L. (1968): A migration model for the study of random genetic drift. *Genetics, 59/4*, 565.

Cavalli-Sforza, L. L. and Edwards, A. W. F. (1967): Phylogenetic analysis: Models and estimation procedures. *Amer. J. hum. Genet., 19/3*, 233.

Chagnon, N. A. (1966): *Yąnomamö Warfare, Social Organization and Marriage Alliances*. Thesis, University of Michigan.

Chagnon, N. A. (1968*a*): *Yąnomamö: The Fierce People*, pp. xiv and 142. Holt, Rinehart and Winston, New York, N.Y.

Chagnon, N. A. (1968*b*): Yąnomamö social organization and warfare. In: *War: The Anthropology of Armed Conflict and Aggression*, pp. 109–159. Editors: M. Fried, M. Harris and R. Murphy. Natural History Press, Garden City, New York, N. Y.

Chagnon, N. A., Neel, J. V., Weitkamp, L. R., Gershowitz, H. and Ayres, M. (1970): The influence of cultural factors on the demography and pattern of gene flow from the Makiritare to the Yanomama Indians. *Amer. J. phys. Anthrop., 32/3*, 339.

Comas, J. (1971): Anthropometric studies in Latin American Indian populations. In: *The Ongoing Evolution of Latin American Populations*, pp. 333–394. Editor: F. M. Salzano. Charles C. Thomas Co., Springfield, Ill.

D'Almada, M. G. L. (1861): Descrição relativa ao Rio Branco e seu território (1787). *Rev. trimestrial Inst. hist. geogr. etnogr. Brasil (Rio de Janeiro), 24*, 617.

De Armellada, C. (1946): *Como son los Pemones de la Gran Sabana*, pp. xx and 100. Elite, Caracas.

De Barandiaran, D. (1962): Actividades vitales de subsistencia de los Indios Yekuana o Makiritare. *Antropologica (Caracas), 11*, 1.

De Barandiaran, D. (1966): El habitado entre los Indios Yekuana. *Antropologica (Caracas), 16*, 1.

Edwards, A. W. F. and Cavalli-Sforza, L. L. (1963): The reconstruction of evolution. *Ann. hum. Genet., 27/1*, 105.

Edwards, A. W. F. and Cavalli-Sforza, L. L. (1964): Reconstruction of evolutionary trees. In: *Phenetic and Phylogenetic Classification, No. 6*, pp. 67–76. Systematics Association (British Museum, Natural History), London.

Edwards, A. W. F. and Cavalli-Sforza, L. L. (1965): A method for cluster analysis. *Biometrics, 21/2*, 362.

Farabee, W. C. (1924): *The Central Caribs, Vol. X*, pp. 299. Anthropological Publications of the University Museum, Philadelphia, Pa.

Fitch, W. M. and Margoliash, E. (1967): Construction of phylogenetic trees. *Science, 155/3760*, 279.

Fitch, W. M. and Neel, J. V. (1969): The phylogenetic relationships of some Indian tribes of Central and South America. *Amer. J. hum. Genet., 21/4*, 384.

Gershowitz, H., Layrisse, M., Layrisse, Z., Neel, J. V., Brewer, G., Chagnon, N. A. and Ayres, M. (1970): Gene frequencies and microdifferentiation among the Makiritare Indians. I. Eleven blood group systems and the ABH-Le secretor traits: a note on Rh gene frequency determinations. *Amer. J. hum. Genet., 22/5*, 515.

JOHNSON, F. (1948): Central American cultures: an introduction. In: *Handbook of South American Indians, Vol. 4*, pp. 43–68. Government Printing Office, Washington, D. C.

KIDD, K. K. and SGARAMELLA-ZONTA, L. A. (1971): Phylogenetic analysis: concepts and models. *Amer. J. hum. Genet., 23/3*, 235.

KOCH-GRÜNBERG, T. (1923): *Von Roroima zum Orinoco, Vol. 3*. Ethnographie, Stuttgart.

LAYRISSE, M. and WILBERT, J. (1966): *Indian Societies of Venezuela: Their Blood Group Types, Monograph 13*, p. 318. Fundación La Salle de Ciencias Naturales, Venezuela.

LIZOT, J. (1970): Aspects économiques et sociaux du changement culturel chez les Yanômami. *L'Homme, 11/1*, 32.

MACCLUER, J. W. and NEEL, J. V.: The simulation of inbreeding intensity among the Yanomama. Unpublished manuscript.

MACCLUER, J. W., NEEL, J. V. and CHAGNON, N. A. (1971): Demographic structure of a primitive population: a simulation. *Amer. J. phys. Anthrop., 35/2*, 193.

MATSON, G. A. and SWANSON, J. (1965): Distribution of hereditary blood antigens among Indians in Middle America. VIII. In Panama. *Amer. J. phys. Anthrop., 23/4*, 413.

MIGLIAZZA, E. (1966): Cultura materiale degli Scirianá. I. *Terra Ameriga, 7*, 3.

MIGLIAZZA, E. (1967*a*): Grupos lingüísticos do territorio Federal de Roraima. In: *Atas do Simpósio sôbre a Biota Amazônica, Belem, Brasil, 1966, Vol. 2 (Antropologia)*. Conselho Nacional de Pesquisas, Rio de Janeiro.

MIGLIAZZA, E. (1967*b*): Cultura materiale degli Scirianá. II. *Terra Ameriga, 8*, 9.

MOTULSKY, A. G., FRASER, G. R. and FELSENSTEIN, J. (1971): Public health and long-term genetic implications of intrauterine diagnosis and selective abortion. In: *Birth Defects, Original Article Series, Vol. 8/5*, p. 22. Editors: D. Bergsma, A. G. Motulsky, C. Jackson and J. Sitter. The National Science Foundation, Washington, D. C.

NEEL, J. V. (1969): Some aspects of differential fertility in two American Indian tribes. In: *Proceedings, VIII International Congress of Anthropological Sciences, Tokyo, Japan, 1968, Vol. I*, p. 356. Science Council of Japan, Tokyo.

NEEL, J. V. (1971): Genetic aspects of the ecology of disease in the American Indian. In: *The Ongoing Evolution of Latin American Populations*, pp. 561–590. Editor: F. M. Salzano. Charles C. Thomas Co., Springfield, Ill.

NEEL, J. V. and CHAGNON, N. A. (1968): The demography of two tribes of primitive relatively unacculturated American Indians. *Proc. nat. Acad. Sci. (Wash.), 59/3*, 680.

NEEL, J. V. and SALZANO, F. M. (1964): A prospectus for genetic studies of the American Indian. *Cold Spr. Harb. Symp. quant. Biol., 29*, 85.

NEEL, J. V. and SALZANO, F. M. (1967): Further studies on the Xavante Indians. X. Some hypotheses-generalizations resulting from these studies. *Amer. J. hum. Genet., 19/4*, 554.

NEEL, J. V. and WARD, R. H. (1970): Village and tribal genetic distances among American Indians, and the possible implications for human evolution. *Proc. nat. Acad. Sci. USA, 65/2*, 323.

POST, R. H., NEEL, J. V. and SCHULL, W. J. (1968): Tabulation of phenotype and gene frequencies for 11 different genetic systems studied in the American Indian. In: *Biomedical Challenges Presented by the American Indian, Vol. 85*, pp. 141–185. Pan American Health Organization, Washington, D. C.

PRIM, R. C. (1957): Shortest connection networks and some generalizations. *Bell Systems technol. J., 36*, 1389.

SCHOMBURGK, R. H. (1841): *Reisen in Guiana und am Orinoco während der Jahre 1835–1839*, p. 510. Leipzig.

SCHULL, W. J., FURUSHO, T., YAMAMOTO, M., NAGANO, H. and KOMATSU, I. (1970): The effect of parental consanguinity and inbreeding in Hirado, Japan. IV. Fertility and reproductive compensation. *Humangenetik, 9*, 294.

SIMPSON, G. G. (1940): Los Indios Kamarakotos. *Rev. Fomento, III/xxii, xxv*, 201.

SPIELMAN, R. (1971): Anthropometric and genetic differences among Yanomama villages. Ph.D. Thesis, University of Michigan.

WARD, R. H. (1972): The genetic structure of a tribal population, the Yanomama Indians. V. Comparison of a series of genetic networks. *Ann. hum. Genet.*, in press.

WARD, R. H. and NEEL, J. V. (1970): Gene frequencies and microdifferentiation among the Makiritare Indians. IV. A comparison of a genetic network with ethnohistory and migration matrices; a new index of genetic isolation. *Amer. J. hum. Genet., 22/5*, 538.

WEITKAMP, L. R. and NEEL, J. V. (1972): The genetic structure of a tribal population, the Yanomama

Indians. IV. Eleven erythrocyte enzymes and summary of protein variants. *Ann. hum. Genet.*, *35/4*, 433.

WRIGHT, S. (1943): Isolation by distance. *Genetics*, *28*, 114.

ZERRIES, O. (1955): Some aspects of Waika culture. In: *Proceedings, 31 International Congress of Americanists, São Paulo, Brasil, 1954*, pp. 73-88.

ZERRIES, O. (1959): Anthropologische Beobachtungen unter den Waika-Indianer des oberen Orinoco, Venezuela. *Dtsch. Gesellsch. Anthropol.*, *6*, 93.

ZERRIES, O. (1964): *Waika: Die Kulturgeschichtliche Stellung der Waika-Indianer des Oberen Orinoco im Rahmen der Völkerkunde Südamerikas, Band I.* Klaus Renner Verlag, Munich.

William J. Schull

Center for Demographic and Population Genetics, University of Texas at Houston, Houston, Tex., U.S.A.

Primitive populations – some contributions to the understanding of human population genetics

Some years ago in his provocative *The Challenge of Man's Future* Harrison Brown sketched movingly and eloquently the emergence of mankind from an existence little different from that of other animals, one dominated by the search for food, the avoidance of predators, and the need to reproduce, to the rise of urban culture. Though somewhat fanciful, perhaps, his description of the changing demographic relationships, the rise and fall of fertility and mortality with the progression from hunter-gatherer through primitive agriculturalist to contemporary city-dweller, provides limitless opportunities for speculation on the nature and rapidity of changes in the human gene pool which presumably accompanied this cultural and demographic evolution. Heretofore, these speculations would have remained just that – untestable conjectures. A variety of fairly recent developments, ranging from new theoretical formulations of population structure and measures of evolutionary divergence to innovations which facilitate the collection, transport, storage and analysis of specimens essential to genotyping, promise more. These developments, coupled with a sense of urgency stemming from the realization that the few remaining populations existing at a level of economic organization plausibly commensurate with that characteristic of much of man's past are under increasing threat of extinction or absorption, have stimulated renewed and broadened interest in primitive communities.

It should be clear at the outset of the remarks to follow the sense in which the term primitive is used. Presumably when man emerged from the company of the other great apes, he was a hunter and gatherer, moving in small bands or groups with little social organization. The structure which did exist was certainly not based upon the recognition of clans, lineages, and elaborate rules of social conduct. He was not only economically primitive but also culturally uncomplicated. Such groups do not now exist; those which are available for study have evolved further. They are primitive in the sense that their economies are based upon hunting and gathering, simple digging-stick and hoe or slash-and-burn agriculture, but complex in their social organization. These are the kinds of peoples under scrutiny in South America, Africa, and possibly Australia, and the gardeners of the Pacific Islands, Bougainville and New Guinea are not far removed. It is in this context then that 'primitive' is applied; it is not necessarily synonymous with high fertility, mortality, or inbreeding as some hold (Morton, 1968).

The purpose of this presentation is to attempt an enumeration of some of the contributions of studies of primitive societies to the understanding of hominid evolution and to the development of more cogent genetic models. However, an exhaustive and critical review, study-by-study, is not feasible in the allotted time. We shall perforce focus on just two aspects, namely, the demography of these societies and the evidence for microdifferentiation at the village and tribal level. This choice is not solely a matter of convenience. A major problem as yet unre-

solved in terms of classical population genetics is the ubiquity in man and numerous other organisms, for that matter, of genetic variation in polymorphic proportions. Classical single-locus theory has been unable to explain without some equivocation the cause of even one such polymorphism. Some ascribe this to an overly simple view of the action of selection (Lewontin, 1971), but insofar as man is concerned, a ponderable explanation is the use of discrete time models to represent the behavior of a sexually reproducing species with age-structured populations. Little is as yet known about the element of approximation involved in this practice, but it could be considerable. Charlesworth (1970) has recently shown, for example, that one bulwark of population genetics, Fisher's Fundamental Theorem, does not hold for age-structured populations if either selection is strong or population number fluctuates greatly. Surely the latter must have occurred frequently in man's past. The effective size of an age-structured population is substantially less (possibly 30–40%, see Nei, 1966) than the census number, and if the latter is small, as appears evident, clearly the former must be even smaller. But further quantitation which the study of primitive human groups provides rather than speculation seems needed if the importance of varying birth and death schedules is to be appraised, particularly in small populations.

The demography of primitive man Traditionally, demography is taken to mean the statistical study of populations with respect to births, deaths, marriages, etc., and obviously can embrace primitive communities for heuristic if not other reasons. Until quite recently, however, demographers have evinced little interest in non-industrial societies. They shied away from the inexactness of the limited data on these groups, and their methodologies were predicated on a quality and quantity of information which is simply not obtainable on most tribal societies. There is evidence of change. Techniques are being developed which permit the estimation of demographic rates in the absence of classical census and vital statistical data; without these rates it is difficult to project a population's size or composition, and hence to evaluate the impact of differing social structures at this early level of economic organization.

Most of the studies to be described briefly here were motivated by a desire to record and evaluate the circumstances surrounding and constraining the early evolution of man, and the observations which have accrued, though they may leave something to be desired in the eyes of a demographer, represent a significant step forward from the semi-quantitative and uncritical approaches of the past. But population analysis of so ecologically generalized a species as the human, one which can exist, expand, and make unique adjustments to environments as diverse as the altiplano of South America, the equatorial regions of Africa and Southeast Asia, or the circumpolar areas of Russia, Canada, or Greenland, if approached from particulars, becomes an almost endless exercise. It would seem clear, therefore, that if statements of general evolutionary import with respect to primitive man are to be made, they must be free of unique ecological adaptations. This appears possible only through the construction of a 'standard' or a series of 'standard' hypothetical, pre-agricultural populations which entails the estimation of range values for such dissimilar parameters as population size, fertility and mortality rates, birth spacing, length of life, sex ratio, and ages of menarche and menopause.

Only four sources of information are available from which this estimation can proceed; these involve the study of (1) non-human primate populations, (2) prehistoric and protohistoric human skeletal remains and artifacts, (3) historical demography, and finally (4) contemporary human populations. Systematic exploitation of all of these sources for the ends just cited has only begun; as a consequence, comprehensive inventories of the information available or potentially available through most of these avenues do not yet exist. Some effort (see Baker and Weiner, 1966) has been made to enumerate contemporary populations of possible interest in Africa, the Americas, Asia, Australia and the circumpolar areas, but even these are patently incomplete. We shall, therefore, limit our attention to the Americas partly for convenience and partly because more populations are represented, and even here to just five tribal groups, namely, the Juruna and Xavante of Brazil, the Macá of Paraguay, the Cashinahua of Peru, and the Yanomama of Venezuela. It should be noted that these five tribes differ substantially

TABLE 1 *Some demographic characteristics of five 'primitive' South American Indian tribes at varying levels of acculturation*

Tribe	Age distribution				Mean age*	Prevalence of polygamy	Infertile adult women	Mean number of livebirths	Mortality before age 15 (%)	Reference
	0–14	15–30	31–	Unk.						
Cashinahua										
♂	55.1	23.8	21.1**	–	17.8 ± 14.8	17 of 64	not reported	2.4*** ± 0.2	22†	Johnston *et al.*, 1969
♀	43.3	24.7	32.0	–	21.5 ± 15.5					
Juruna										
♂	50.0	36.7	13.3	–	19.1 ± 16.3	none now	0 of 13	3.2 ± 0.8	10	De Oliveira and Salzano, 1969
♀	53.6	25.0	21.4	–	16.2 ± 12.4					
Macá										
♂	28.5	17.3	32.5	21.7	27.5 ± 20.8	none now	2 of 53	3.6 ± 0.3	36	Salzano *et al.*, 1970
♀	28.1	23.1	31.3	17.5	26.3 ± 19.5					
Xavante										
♂	40.4	30.5	13.6	15.5	17.4 ± 13.1	16 of 37	0 of 35	4.7 ± 0.3	33	Neel and Chagnon, 1968
♀	37.6	34.3	17.6	10.2	18.3 ± 14.2					
Yanomama										
♂	34.3	44.8	20.9	–	20.8 ± 15.3	50 of 105	1 of 34	2.6 ± 0.3	16	Neel and Chagnon, 1968
♀	29.5	47.9	22.6	–	23.4 ± 16.4					

* The value which follows the mean is the standard deviation of the age distribution and *not* the standard error of the mean.
** These values have been estimated from age distributions reported in decades.
*** Some incomplete families are included; data are given on all married women older than 20 years of age.
† These are described as childhood deaths, and it is not clear what the upper age limit of childhood may be.

in size, and degree of acculturation, and only the Yanomama give evidence of being an expanding population.

Among the principal demographic findings to emerge thus far from studies of these groups are the following:

1. Within tribes, life is organized about villages which are usually endogamous and vary from 50 to 250 or so persons. Village size and endogamy encourage substantial inbreeding, an effect partially offset by certain culturally imposed restrictions on mate selection and the need for political alliances.

2. These are young populations; as can be seen from Table 1 the average age of males and females, even with allowance for errors of estimation, exceeds 25 years only in the Macá, the most acculturated of the five tribes.

3. Mortality and fertility appear lower than among sedentary agriculturalists. Birth intervals are longer than those commonly associated with reproduction unmodified by contraception or pregnancy wastage which would seem to imply some conscious family limitation. In the Yanomama, at least, the control of 'child spacing' is largely explicable in terms of abortion, infanticide, and lactational taboos which proscribe cohabitation. It is difficult to generalize these observations, however, for as Nag (1962) has amply demonstrated, cross-cultural comparisons of the factors affecting human fertility in non-industrial societies are fraught with the possibility of misinterpretation.

4. Virtually all, if not all females are married during some portion of their reproductive spans, and sterility is less frequent than is characteristic of present populations which on cultural and historical grounds may be assumed to be non-contracepting and free of venereal disease. For example, some 3.4% of ever-married Hutterite women of completed fertility are childless (Eaton and Mayer, 1954).

5. Polygyny, concurrent or serial, is common and with it the opportunity for a few males to make disproportionate contributions to reproduction. A consequence of this is a greater variance in number of surviving offspring among males than among females.

6. Migration tends to be of two kinds, namely, a small more-or-less steady, but presumably involuntary component which results from raiding and wife-stealing, and a larger episodic movement, generally of family groupings, arising from disharmonies within a village.

Some of these observations, especially those related to fertility, have been made by Carr-Saunders (1922) and Krzwicki (1934), earlier students of primitive societies. The data on which their conclusions rested, however, are very much less satisfactory than those here cited.

It is obvious that extrapolations from findings such as those just described on one contemporary primitive population to prehistoric and protohistoric man or to a 'standard' hypothetical population must be extremely guarded. If similar situations obtain in other primitive groups, as in this instance, possibly somewhat less caution need be exercised; however, it must be borne in mind that congruence may merely reflect the very limited, and atypical (largely tropical rain forest) portion of the earth's surface occupied by such societies. Some other confirmation is obviously needed, and this may be provided by historical and paleodemography. Clearly the latter is not likely to reveal all of the niceties of social stratification, marriage rules, etc. which characterized past populations, but paleodemography may provide needed evidence on the probable nature and size of such groups, their sex and age distributions, and certain disease experiences (see Brothwell, 1971). It may even be possible to measure fecundity at least crudely in skeletal populations from the state of the female pelvis, and these measures when correlated with other pathognomonic (or nearly so) skeletal changes could afford a rough basis for estimating relative fertilities (Angel, 1969).

Insofar as the paleodemographic record is concerned it is reassuring to note that estimates of the longevity of certain North American Indian skeletal populations accord reasonably well with the values in Table 1. Blakely (1971) has examined the mortality profiles of four prehistoric populations, two dating from the Archaic (2500-900 BC), one the Hopewellian (*c.* 200 AD), and one the Middle Mississippian (920-1120 AD) period. In all, the average age at

death was estimated to be 30 years of age or less, and in all, males appear to have lived from one to seven years longer than females, on the average. Mortality in the first decade of life varied from 28 to 48% with a small mode at 3–4 years of age which may reflect unsuccessful weaning. A striking similarity occurs in these profiles which reflect a temporal span of not less than 3500 years. Estimates of this nature are not without their errors, of course, but these do not appear large enough to obscure the basic similarities among the sets. One possible discrepancy between these data and the findings on contemporary populations previously described involves the estimate of pre-reproductive mortality. The skeletal material suggests a higher death rate than the living populations; it is difficult to ascribe this difference to biases for the obvious ones are likely to lead to an underrepresentation of the young and very young in the skeletal data. Further studies are clearly indicated.

Osteologic evidence also suggests that the pre-Columbian inhabitants of the Americas suffered less from contagious diseases than from arthritides, nutritional and metabolic disturbances, and nonspecific infections associated with traumata (St. Hoyme, 1969). While few diseases can be diagnosed from a study of bones alone, the skeletal findings imply a fairly healthy people, and this accords with observations on many contemporary groups, especially the Xavante and Yanomama who have been studied in some clinical depth. While the paleodemographic record is apt to remain fragmentary, particularly in those areas currently occupied by primitive groups, much of the information which is available or may become available involves populations which existed in the temperate zone, an area poorly represented among living primitive societies.

Historical demography can provide similar confirmatory evidence, particularly on population sizes and movements which can make contemporary observations intelligible or attest to their generality. Harvey (1967), for example, in a study of the aboriginal Cahuilla population of Southern California, based upon historical accounts has estimated the tribe to have consisted of 2500–3000 individuals in the middle of the nineteenth century distributed over some 22 settlements. He further estimated single lineage clans to have averaged 50–60 persons where the larger clans with several collateral lineages may have ranged upwards of 200 or 250 individuals. These are values very similar to those cited for contemporary Amerindian groups. Finally, he suggests that the decline of this population was neither cataclysmic nor its causes obvious, but may have been due to a small but steady drain of young women occasioned by the encroaching Western Europeans. In a society where the possible mate choices are few, the loss of a single woman through raiding or other means can reduce the few substantially.

Data of these general kinds are relevant to a number of general biologic problems of current moment. We cite only one. Ecologists are prone to believe that the numbers in a population are generally regulated by some internal adaptation so that the resources which sustain the population are not overexploited. And this notion has been frequently invoked with respect to primitive man (see, *e.g.*, Carr-Saunders, 1922). Of late, it has even been suggested that the apparent absence of environmental degradation associated with primitive economies is an outgrowth of a conscious effort at population regulation rather than the result of a technology too simple to threaten the environment greatly. Williams (1966) has pointed out in detail the logical difficulties inherent in any theory of self-regulation to an optimal number, but resolution of this issue is less likely to come from a philosophic examination of the fundaments of the arguments than evidence, if such can be adduced, that some populations do, indeed, optimize the relationship between their number and their resources.

Differentiation of local communities To understand evolution whether of man or any other organism is, in effect, to understand the forces which contribute to subdivision and differentiation of a species. Several theoretical approaches to a characterization of this process of local differentiation of gene frequencies are presently in vogue, each with a somewhat different objective. One of these has been termed population structure analysis and another the microtaxonomic approach. Both presuppose some degree of isolation of local groups or populations, and seek to describe and measure the rate and degree of evolutionary diver-

gence. Insofar as the first of these is concerned, the simplest model of structure, the so-called 'island model' of Sewall Wright (Wright, 1931), envisages a population divided into a series of panmictic groups which, on occasion, receive a small proportion of immigrants, the latter being representative of the population as a whole. While this may be true of some human communities, present as well as past, more commonly it is observed that individuals are distributed more or less discontinuously to form numerous colonies, and are exchanged mainly between adjacent or nearby groups. Isolation may thus be viewed as a function of the distance between colonies. Wright (1943, 1946) was the first to attempt to include this notion in a theory of population structure; he proposed a model which assumes a population to be uniformly distributed over a large territory, but that the parents of any given individual are drawn from a small surrounding region, a neighborhood – hence the common name of the model. Kimura (1953), and Kimura and Weiss (1964) have subsequently shown that both the 'island' and the 'neighborhood' models are limiting cases of one which Kimura (1953) has termed the 'stepping stone' model. The latter supposes a series of colonies, infinite in number, which exchange members so that the array can pass into a continuum with scattered clusters of high density, and ultimately a uniform continuum. Kimura and Weiss (1964) have shown that under these circumstances the correlation of the gene frequencies between two colonies will decay as a function of the number of intervening steps, *i.e.*, of the distance which intervenes.

A number of the consequences of this model were anticipated by the work of Malécot (1948, 1959, 1966) which has seen wider application to human populations and is concerned with the decay in the mean coefficient of kinship (defined as the probability that two homologous genes, one from individual I and the other from J, are descended from the same gene) under pressure of mutation, migration, and selection as distance increases. As generally applied to human populations, Malécot's model assumes that the distribution of the distance, say d, between the birthplaces of pairs of individuals is such that as d increases the mean coefficient of kinship is of the form

$$\phi(d) = ae^{-bd}d^{-c}$$

where the constant a is the mean coefficient of kinship in local populations; b varies with the systematic pressures due to mutation, migration, and selection and the standard deviation of the distance d in the past, and finally c is the dimensionality of migration. Derivation of this form assumes that d is large in comparison with its standard deviation, mating is random, there is no 'cline' in geographic selection, and migration whether normal, exponential, stepping stone or other is stable in time and isotropic if multidimensional.

As Yasuda and Morton (1967) have noted, application of this method of analysis requires 'a large sample of individuals of known phenotype and place of birth or residence, drawn from a sedentary population in a region with a diameter not enormously greater than the standard deviation of migration'. While the robustness of Malécot's arguments when applied to the real world has yet to be determined rigorously, advocates of this method of analysis argue that it is the only one which provides a precise description of population structure for comparison of different mating systems and migration patterns (Morton, 1968), and furthermore it can be readily applied to gene frequencies, phenotypes, or even anthropometric data. Though possibly a self-serving appraisal, this point of view is not entirely without justification for Malécot's model patently attempts to predict change directly in terms of mutation, selection and migration, the traditional parameters familiar to the geneticist.

A variety of industrialized societies, for example, Belgium (Dodinval, 1970), Brazil (Yasuda and Morton, 1967), Japan (Imaizumi and Morton, 1969), Sweden (Imaizumi and Morton, 1969), and Switzerland (Morton *et al.*, 1968), have been examined in this manner, but the appropriate data on primitive groups are more limited. Table 2 summarizes the scanty observations which exist. Though it is obviously premature to expect unambiguous conclusions, a few 'trends' are worthy of note. First, the mean coefficient of local kinship is much higher

TABLE 2 *Estimates of local kinship and systematic pressures based upon Malécot's model of the decline in mean coefficient of kinship with distance for four non-industrial societies*

Region	Local kinship a	Systematic pressures b	Migration dimensionality c	Reference
Central and South American Indians	0.0253 ± 0.0064	0.0032 ± 0.0016	linear	Roisenberg and Morton, 1970
Bougainville	0.0510 ± 0.0080	0.1050 ± 0.0230	linear	Friedlaender, 1971
Micronesia	0.0328 ± 0.0058	0.0014 ± 0.0005	linear	Imaizumi and Morton, 1970
New Guinea All of island	0.0126 ± 0.0025	0.0009 ± 0.0005	linear	Imaizumi and Morton, 1970
Four isolated areas	0.0296 ± 0.0064	0.0519 ± 0.0164	linear	Imaizumi and Morton, 1970

The model: $\varphi(d) = ae^{-bd}d^{-c}$
where $\varphi(d)$ is the coefficient of kinship at distance d, and the nature of the constants a, b, and c are indicated above.

and less variable than is characteristic of industrialized societies where values ranging from 0.00064 (Japan, density-adjusted estimate) to 0.00437 (Switzerland, Alpine area, density-adjusted estimate) have been reported (for a summary see Imaizumi and Morton, 1969). Possibly this is to be expected given the sizes of the local populations and their endogamous nature.

Second, the values of b are highly variable, differing by as much as two orders of magnitude. Since b is itself a function of a number of variables, namely, the pressures of mutation, long-range migration, selection, and the variability in the distance between the birthplaces of parents and offspring, a number of interpretations are possible. Thus, small values of b may imply migration rates which are high relative to the systematic pressures, but only if selection is assumed to be small and invariant with distance. Large values of b are commonly construed as evidence of high subdivision since they are presumed to arise because the variability between birthplaces of parent and offspring (or marital partners) is small. This general tendency to interpret b in terms of migration assumes that mutation and selection are small compared with the effects of migration. One finds, for example, assertions such as the following (Friedlaender, 1971): 'Within Bougainville, the high estimates of a and b both are attributable to extreme population subdivision. This would lead to both increased localized inbreeding ... and to small standard deviations in marriage distances, σ (resulting in high values of b)'. Of course these conjectures tacitly assume that migration is linear, for otherwise the estimates of localized inbreeding and the systematic pressures may be markedly different.

Finally, though this does not emerge solely from the data in Table 2, wherever the dimensionality of migration has been separately estimated it appears more nearly linear than of higher dimensions. An important exception is Micronesia where the dimensionality is intermediate between linear and two-dimensional dispersion (Imaizumi and Morton, 1970). If migration is indeed linear or nearly so, the assumption of isotropism which many investigators would be reluctant to make becomes unnecessary. But one may question whether an estimate of c derived from a model which presupposes homogeneous and isotropic migration is the best estimate of non-linear or non-uniform migration. This is an important issue for as Malécot (1955, 1959, see pp. 190–191) has stated the mean coefficient of kinship at distance zero, that is, the local mean, is sensitive to dimensionality and hence to the assumption of isotropic migration. This statement is borne out by Imaizumi and Morton's (1970) estimates for Micronesia which reveal a two-fold difference in the estimate of the local mean depending upon whether migration is assumed to be linear or not, and a ten-fold difference in the estimation of the systematic pressures although the latter are not supposed to be particularly sensitive to dimensionality (Malécot, 1955).

As previously stated, the microtaxonomic approach also presupposes an isolation of local groups and attempts to measure their divergence on a hierarchical basis. A surprising number of different indices have been presented for the measurement of distances between populations based on attribute data (*e.g.*, Sanghvi, 1953; Sokal and Sneath, 1963; Cavalli-Sforza and Edwards, 1967; Fitch and Margoliash, 1967; Steinberg *et al.*, 1967; Balakrishnan and Sanghvi, 1968; Hedrick, 1971). The merits of these alternatives have been reviewed at some length in the literature (see Balakrishnan and Sanghvi, *op. cit.*; Kurczynski, 1970; Goodman, 1972); suffice it here to say that there appears to be a positive correlation in their results (Fitch and Neel, 1969; Kidd and Sgaramella-Zonta, 1971; Barrai, personal communication). Adherents to the microtaxonomic approach insist upon a distinction between its use as a means to reconstruct and represent a sequence of evolutionary events, and as a measure of genetic similarities (or dissimilarities). In the former instance, if the various assumptions, *e.g.*, additive or minimum evolution, do not hold, the evolutionary tree which emerges is unlikely to be meaningful, but this would not invalidate the method as a tool to describe genetic similarities.

One or more of these measures of genetic distance have been used to explore the global divergence among some of the 'races' and ethnic groupings of man (Cavalli-Sforza and Edwards, 1964), the phylogenetic relationships of some Indian tribes of Central and South Amer-

ica (Fitch and Neel, 1969), and differentiation among local communities of Bougainvillians (Friedlaender *et al.*, 1971), New Guineans (Sinnett *et al.*, 1970), Australian aborigines (Sanghvi and Kirk, personal communication), Babinga pygmies (Cavalli-Sforza *et al.*, 1969), and the Makiritare and Yanomama Indians of Venezuela (Ward and Neel, 1970; Neel and Ward, 1970). The findings of these studies, especially those related to the differentiation of local groups with which we are primarily concerned, do not lend themselves readily to numerical summarization. One is obliged, therefore, to make qualitative appraisals which are not easily translated, save under quite restrictive assumptions, into measures of migration, mutation, selection, and the like, or even rates of gene substitution. Thus, insofar as the studies which have focussed on local groups are concerned, all find the 'evolutionary tree' which emerges to agree at least generally with the history (often legends) of the group. But estimates of genetic divergence can vary with the number and choice of genetic systems from which they are derived, and as yet there is no distributional form by which one can assert that a given result is to be expected or is unexpected based upon a probability statement. Thus, by what criterion could one reach a different conclusion than that just cited? More interesting and less subjective is the observation that the distance among local communities within a single Amerindian tribe can be almost as great (85–90%) as that associated with a dozen such tribes (Neel and Ward, 1970). This invites speculation and these authors suggest that this may be a contributing factor to the apparently more rapid rate of amino acid substitutions, and presumably evolution in man than other organisms.

Thus far, apparently only one set of observations, that on the Bougainvillians, has been subjected to both population structure analysis and the microtaxonomic approach. The results are difficult to compare. As previously noted, the former analysis reveals evidence of high local inbreeding, and little migration among the local communities. It is concluded from the phylogenetic analysis, on the other hand, that 'a striking amount of variability' exists which is 'related to geographic, linguistic, and migrational differences'. Unfortunately, whereas the expressions 'high' and 'little' can be given quantitative meaning as has been seen (Table 2), 'striking' and 'related to' cannot. Herein lies one of the principal advantages ascribed to population structure analysis over the phylogenetic approach in characterizing the differentiation of local communities; comparisons of findings can be analytic in the former instance, but only descriptive in the latter. It can be argued, of course, that the seemingly greater objectivity of the former is spurious if the assumptions, which some individuals will find too restrictive, are violated. Moreover, can one approach, in fact, be more objective than the other if they are functionally connected? Genetic distance, at least as computed in some fashions, can be shown to be approximately equal to the square root of the standardized variance of gene frequencies among the groups under scrutiny; whereas the coefficient of kinship is merely this standardized variance. Thus, to a close approximation, the coefficient of kinship, the correlation in gene frequencies, and genetic distance would appear to measure the same thing. But if this is so, to greater or lesser extent the advantages claimed for one must apply to all, and similarly the disadvantages. The choice must then reflect personal preferences, and not an inherently 'better' or 'best' approach.

Nei (1972) has proposed a measure of genetic distance which may resolve this dilemma of choice, or contribute importantly to its resolution. The measure of the number of accumulated gene differences per locus, the genetic distance, he proposes is the negative natural logarithm of the normalized identity of genes between two populations, say X and Y, with respect to all loci studied. The identity is defined as

$$I = J_{XY}/\sqrt{J_X J_Y}$$

where J_X, J_Y, and J_{XY} are the arithmetic means of the probabilities of identity of genes at the given loci in the average individual in X (the coefficient of inbreeding for the average individual in X), in Y, and at a pair of loci one associated with an individual in X and the other in Y (the coefficient of kinship). This metric has yet to be applied extensively to human data or to be

compared with the results of the techniques previously described. Hopefully, this brief account of some of the findings from studies of primitive societies will have convinced you that we have a considerable distance to travel to understand the forces which contribute to gene frequency changes in small human populations. Patently, more field and theoretical research are needed; the former to refine our observations and to provide some measure of the variation which may have existed in pre- and protohistoric times among human communities, and the latter to develop less restrictive models insofar as the nature of migration, etc. may be concerned. Further consideration of some of the philosophic implications of our research are to be encouraged. Thus, while all of us might agree that a necessary condition for human evolution is local differentiation, does it follow that local differentiation, as measured in a society of the kind we have described, constitutes evolution?

Though this presentation has emphasized studies on primitive populations, other groups such as the pastoralists of Africa and the agriculturalists of central America, possibly but a few millennia removed in the cultural scale, are being profoundly affected by events about them and should also be considered. What is obviously needed is a perspective on all levels from hunting and gathering to a contemporary urban existence. Indeed, it may be argued that the resident of Paris, London, New York, or Tokyo, for example, finds himself in a milieu for which past development has ill prepared him – the stress of numbers, environmental degradation, etc., which is the fare of many of us, pose new demands upon our genetic resources. We may be witnessing or about to witness changes in our genetic heritage as profound as those which are thought by some to have accompanied the introduction of agriculture.

REFERENCES

Angel, J. L. (1969): Paleodemography and evolution. *Amer. J. phys. Anthrop.*, *31*, 343.

Baker, P. T. and Weiner, J. S. (1966): *The Biology of Human Adaptability*. Clarendon Press, Oxford.

Balakrishnan, V. and Sanghvi, L. (1968): Distance between populations on the basis of attribute data. *Biometrics*, *24*, 859.

Blakely, R. L. (1971): Comparison of the mortality profiles of Archaic, Middle Woodland, and Middle Mississippian skeletons. *Amer. J. phys. Anthrop.*, *34*, 43.

Brothwell, D. R. (1971): Paleodemography. In: *Biological Aspects of Demography*, pp. 111–130. Editor: W. Brass. Taylor and Francis, Ltd., London.

Brown, H. (1954): *The Challenge of Man's Future*. The Viking Press, New York.

Carr-Saunders, A. M. (1922): *The Population Problem*. Clarendon Press, Oxford.

Cavalli-Sforza, L. L. and Edwards, A. W. F. (1964): Analysis of human evolution. In: *Proceedings, XI International Congress of Genetics, Vol. 3*, pp. 923–933. Editor: S. J. Geerts. Pergamon Press, New York, N. Y.

Cavalli-Sforza, L. L. and Edwards, A. W. F. (1967): Phylogenetic analysis: models and estimation procedures. *Amer. J. hum. Genet.*, *19*, 234.

Cavalli-Sforza, L. L., Zonta, A., Nuzzo, F., Bernini, L., De Jong, W. W. W., Meera Khan, P., Ray, A. K., Went, L. N., Siniscalco, M., Nijenhuis, L. E., Van Loghem, E. and Modiano, G. (1969): Studies on African pygmies. I. A pilot investigation of Babinga pygmies in the Central African Republic (with an analysis of genetic distance). *Amer. J. hum. Genet.*, *21*, 252.

Chagnon, N. A., Neel, J. V., Weitkamp, L. R., Gershowitz, H. and Ayres, M. (1970): The influences of cultural factors on the demography and pattern of gene flow from the Makiritare to the Yanomama Indians. *Amer. J. hum. Genet.*, *32*, 339.

Charlesworth, B. (1970): Selection in populations with overlapping generations. I. The use of Malthusian parameters in population genetics. *Theoret. Populat. Biol.*, *I*, 352.

De Oliveira, A. E. and Salzano, F. M. (1969): Genetic implications of the demography of Brasilian Juruna Indians. *Soc. Biol.*, *16*, 209.

Dodinval, P. A. (1970): Population structure of A, B, O, AB blood groups in Belgium. *Hum. Hered.*, *20*, 169.

Eaton, J. W. and Mayer, A. J. (1954): *Man's Capacity to Reproduce*. Glencoe Press, Glencoe, Ill.

Fitch, W. M. and Margoliash, E. (1967): Construction of phylogenetic trees. *Science*, *155*, 279.

FITCH, W. M. and NEEL, J. V. (1969): The phylogenetic relationships of some Indian tribes of Central and South America. *Amer. J. hum. Genet.*, *21*, 384.

FRIEDLAENDER, J. S. (1971): The population structure of South-Central Bougainville. *Amer. J. phys. Anthrop.*, *35*, 13.

FRIEDLAENDER, J. S., SGARAMELLA-ZONTA, L. A., KIDD, K. K., LAI, L. Y. C., CLARK, R. and WALSH, R. J. (1971): Biological divergence in South-Central Bougainville: an analysis of blood polymorphism gene frequencies and anthropometric measurements utilizing tree models, and a comparison of these variables with linguistic, geographic, and migration 'distances'. *Amer. J. hum. Genet.*, *23*, 253.

GOODMAN, M. M. (1972): Distance analysis in biology. *Evolution*, in press.

HARVEY, H. R. (1967): Population of the Cahuilla Indians: decline and its causes. *Eugen. Quart.*, *14*, 185.

HEDRICK, P. W. (1971): A new approach to measuring genetic similarity. *Evolution*, *25*, 276.

IMAIZUMI, Y. and MORTON, N. E. (1969): Isolation by distance in Japan and Sweden compared with other countries. *Hum. Hered.*, *19*, 433.

IMAIZUMI, Y. and MORTON, N. E. (1970): Isolation by distance in New Guinea and Micronesia. *Archaeol. phys. Anthrop. Oceania*, *5*, 218.

JOHNSTON, F. E., KENSINGER, K. M., JANTZ, R. L. and WALKER, G. F. (1969): The population structure of the Peruvian Cashinahua: demographic, genetic, and cultural relationships. *Hum. Biol.*, *41*, 29.

KIDD, K. K. and SGARAMELLA-ZONTA, L. A. (1971): Phylogenetic analysis: concepts and methods. *Amer. J. hum. Genet.*, *23*, 235.

KIMURA, M. (1953): 'Stepping stone' model of population. *A. R. Nat. Inst. Genet. Japan*, *3*, 62.

KIMURA, M. and WEISS, G. (1964): The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics*, *49*, 561.

KRZWICHI, L. (1934): *Primitive Society and Its Vital Statistics*. Macmillan and Co., London.

KURCZYNSKI, T. W. (1970): Generalized distance and discrete variables. *Biometrics*, *26*, 525.

LEWONTIN, R. C. (1971): Genes in populations – end of the beginning. *Quart. Rev. Biol.*, *46*, 66.

MALÉCOT, G. (1948): *Les Mathématiques de l'Hérédité*. Masson et Cie, Paris.

MALÉCOT, G. (1955): Comments on the paper of Motoo Kimura. *Cold Spr. Harb. Symp.*, *20*, 52.

MALÉCOT, G. (1959): Les modèles stochastiques en génétique de population. *Publ. Inst. Statist. Paris*, *8*, 173.

MALÉCOT, G. (1966): Identical loci and relationship. In: *Proceedings, V Berkeley Symposium on Probability and Statistics, Vol. 4*, p. 317.

MORTON, N. E. (1968): Problems and methods in the genetics of primitive groups. *Amer. J. phys. Anthrop.*, *28*, 191.

MORTON, N. E., YASUDA, N., MIKI, C. and YEE, S. (1968): Population structure of the ABO blood groups in Switzerland. *Amer. J. Hum. Genet.*, *20*, 420.

NAG, M. (1962): *Factors Affecting Human Fertility in Nonindustrial Societies: A Cross-cultural Study*. Yale University Publications in Anthropology, Number 66, New Haven, Conn.

NEEL, J. V. and CHAGNON, N. A. (1968): The demography of two tribes of primitive, relatively unacculturated American Indians. *Proc. nat. Acad. Sci. USA*, *59*, 680.

NEEL, J. V. and WARD, R. H. (1970): Village and tribal genetic distances among American Indians, and the possible implications for human evolution. *Proc. nat. Acad. Sci. USA*, *65*, 323.

NEI, M. (1966): Genetic structure of human populations. II. Differentiation of blood group gene frequencies among isolated populations. *Heredity*, *21*, 183.

NEI, M. (1972): Genetic distance between populations. *Theoret. Populat. Biol.*, in press.

ROISENBERG, I. and MORTON, N. E. (1970): Population structure of blood groups in Central and South American Indians. *Amer. J. phys. Anthrop.*, *32*, 373.

ST. HOYME, L. E. (1969): On the origins of New World paleopathology. *Amer. J. phys. Anthrop.*, *31*, 295.

SALZANO, F. M., MOREONO, R., PALATNIK, M. and GERSHOWITZ, H. (1970): Demography and H-Le^a salivary secretion of the Macá Indians of Paraguay. *Amer. J. phys. Anthrop.*, *33*, 383.

SANGHVI, L. D. (1953): Comparison of genetical and morphological methods for a study of biological differences. *Amer. J. phys. Anthrop.*, *11*, 385.

SINNETT, P., BLAKE, N. M., KIRK, R. L., LAI, L. Y. C. and WALSH, R. J. (1970): Blood, serum protein and enzyme groups among Enga-speaking people of the Western Highlands, New Guinea, with an estimate of genetic distance between clans. *Archaeol. phys. Anthrop. Oceania*, *5*, 236.

SOKAL, R. R. and SNEATH, P. H. A. (1963): *Principles of Numerical Taxonomy*. Freeman, San Francisco, Calif.

STEINBERG, A. G., BLEIBTREU, H. K., KURCZYNSKI, T. W., MARTIN, A. and KURCZYNSKI, E. M. (1967): Genetic studies of an inbred isolate. In: *Proceedings, III International Congress of Human Genetics*, pp. 267–289. Editors: J. F. Crow and J. V. Neel. The Johns Hopkins Press, Baltimore, Md.

WARD, R. H. and NEEL, J. V. (1970): Gene frequencies and microdifferentiation among the Makiritare Indians. IV. A comparison of a genetic network with ethnohistory and migration matrices; a new index of genetic migration. *Amer. J. hum. Genet.*, *22*, 538.

WILLIAMS, G. C. (1966): *Adaptation and Natural Selection*. Princeton University Press, Princeton, N.J.

WRIGHT, S. (1931): Evolution in Mendelian populations. *Genetics*, *16*, 97.

WRIGHT, S. (1943): Isolation by distance. *Genetics*, *28*, 114.

WRIGHT, S. (1946): Isolation by distance under diverse systems of mating. *Genetics*, *31*, 39.

YASUDA, N. and MORTON, N. E. (1967): Studies on human population structure. In: *Proceedings, III International Congress of Human Genetics*, pp. 249-265. Editors: J. F. Crow and J. V. Neel. The Johns Hopkins Press, Baltimore, Md.

Albert Jacquard

Institut National d'Etudes Démographiques, Paris, France

Utilisation des généalogies pour la mesure de l'évolution génétique d'une petite population

I. LES INDIVIDUS ET LES GÈNES

L'objet de la génétique des populations est l'étude des transformations d'un groupe humain dans le temps. Le généticien de population se meut donc dans un espace 'individus-temps' (Fig. 1).

Mais chaque *individu* est mortel; il ne se prolonge que grâce à une technique qui constitue un paradoxe pour un être par définition 'indivisible': il est capable de fabriquer une copie de la moitié de lui-même et, en accolant cette demie-copie à la demie-copie fournie par un autre individu de fabriquer un troisième individu qui à son tour.... C'est tout le processus, bien connu, de la reproduction sexuée.

Cette succession d'êtres 'indivisibles' qui se prolongent en se divisant est évidemment d'étude malaisée, car l'objet même de l'observation est évanescent, fugitif. Un individu ne peut être identifié qu'à lui-même, mais à aucun des individus qui l'ont précédé ou qui lui succèdent, ce qui exclut toutes comparaisons autres que les comparaisons globales établies par les démographes.

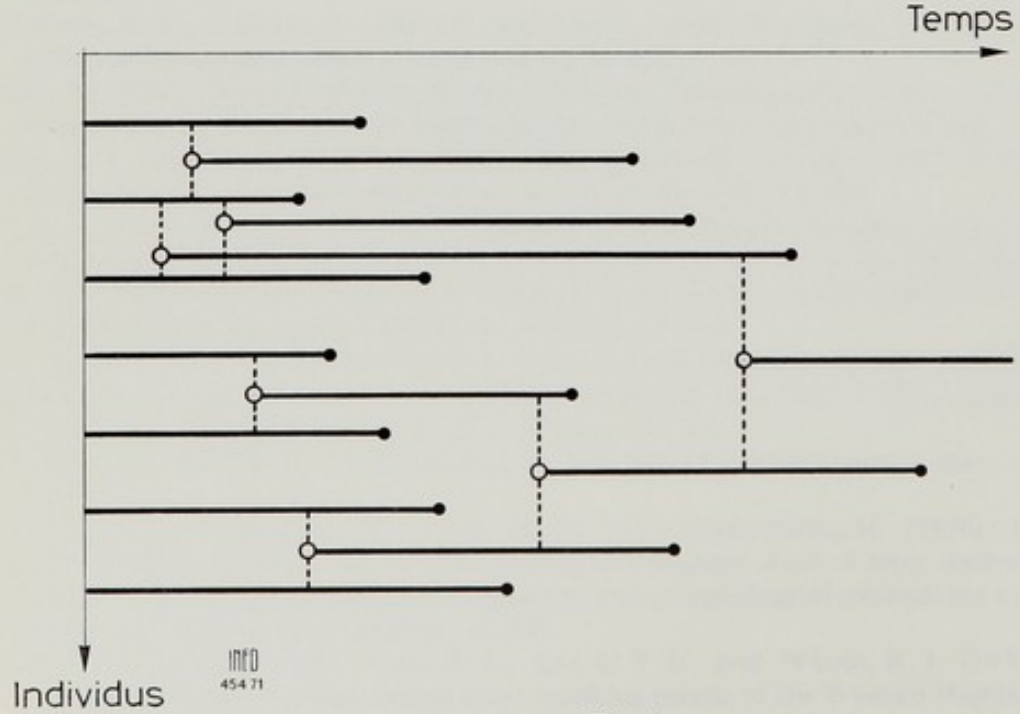


Fig. 1.

Enfin, l'ascendance d'un individu correspond à un ensemble extrêmement nombreux, dès que l'on considère plusieurs générations puisque chacun a 2 parents, 4 grands-parents, 2^n ancêtres à la génération n.

Le recours au concept de *gène* permet de parvenir à une vision infiniment plus simple de la réalité. On ne considère plus alors les individus mais les gènes dont ils sont porteurs. Certes ces gènes peuvent être considérés comme mortels puisqu'ils disparaissent avec l'individu qui les porte, mais ils se prolongent en réalisant d'eux-mêmes une copie intégrale et non plus une demie-copie. Un gène n'a qu'un ancêtre, aussi loin que l'on remonte dans le passé; à tout moment, il est donc possible d'identifier ce gène à son ancêtre, et aussi à tous les gènes qui ont le même ancêtre: il s'agit de la même réalité, on peut donc effectuer des comparaisons dans le temps, ce qui n'est pas possible si l'on considère les individus (Fig. 2).

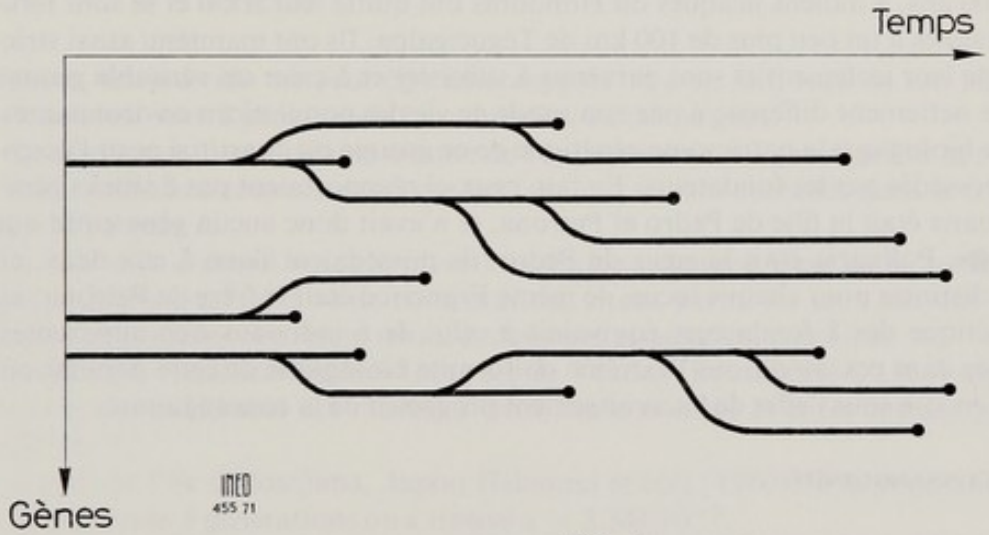


Fig. 2.

Mais, selon le nombre de duplications qu'il a subies, un gène peut avoir un nombre de descendants variables. Peu à peu la composition de la population de gènes évolue; autrement dit la population d'individus, considérés non plus comme des éléments équivalents d'un ensemble, mais comme des êtres doués de caractéristiques biologiques, se transforme.

Notre problème est de chercher à caractériser cette transformation, d'en donner si possible une 'mesure'.

Les données de base à partir desquelles nous pouvons réaliser cette mesure sont les généalogies. Nous supposons ici que nous sommes en mesure d'identifier pour chaque individu les deux individus dont il est issu, son père et sa mère, et ainsi de proche en proche sur un certain nombre de générations.

Cette information de base peut être donnée soit sous forme d'une liste de triplets de filiation (individu, père, mère) soit sous forme d'un graphique, mais tous ceux qui ont manipulé des généalogies savent combien ces graphiques deviennent vite inextricables: admettons donc que nous possédions les triplets de filiation pour l'ensemble de la population sur quelques générations.

Nous l'avons vu, si l'on veut progresser aisément, l'objet de l'étude doit être non l'individu mais les gènes qu'il porte. Or ceux-ci sont insaisissables, leur origine paternelle ou maternelle est inconnaissable. Supposons que nous prenions au hasard l'un des deux gènes de X (c'est l'opération que X réalisera lorsqu'il procréera), nous ne pouvons savoir si ce gène vient de son père ou s'il vient de sa mère; nous savons seulement que ces deux éventualités ont la même probabilité, 1/2.

Dès le départ, en raison même de la nature du mécanisme qui est à la base de la reproduction sexuée, nous devons donc avoir recours à une attitude probabiliste: dans cette optique la génétique des populations n'est pas une science où l'on peut décrire la réalité, mais une science

où il faut énumérer l'ensemble des possibles et s'efforcer d'affecter à chacun de ces possibles une probabilité.

Tel est donc le problème: à partir de données généalogiques, caractériser l'ensemble des gènes possédés par une population, au moyen de mesures probabilistes. Deux voies peuvent être suivies que nous allons rappeler en nous basant sur l'exemple d'une population réelle, illustrant de façon presque idéale les phénomènes qui peuvent survenir dans une petite population: une tribu de Jicaques du Honduras.

II. LES JICAQUES DE LA MONTAÑA DE LA FLOR

Il y a environ 100 ans, 8 indiens jicaques du Honduras ont quitté leur tribu et se sont réfugiés dans les montagnes à un peu plus de 100 km de Tégucigalpa. Ils ont maintenu aussi strictement que possible leur isolement et sont parvenus à subsister et à créer un véritable groupe humain autonome nettement différencié par son mode de vie des populations environnantes.

Du point de vue biologique le patrimoine génétique de ce groupe est constitué pour l'essentiel par les gènes possédés par les fondateurs. En fait, ceux-ci n'apportaient pas 8 stocks génétiques distincts: Juana était la fille de Pedro et Petrona, et n'avait donc aucun gène autre que ceux de ses parents, Polinaria était la sœur de Pedro, ils possédaient donc à eux deux, en moyenne, 3 gènes distincts pour chaque locus, de même Francisco était le frère de Petrona: au total le stock génétique des 8 fondateurs équivalait à celui de 6 individus non apparentés.

On peut imaginer dans ces conditions l'extrême uniformité biologique de cette population, uniformité accrue encore sous l'effet de l'accroissement progressif de la consanguinité.

ÉVOLUTION DE LA CONSANGUINITÉ

Dès la seconde génération force a été pour les Jicaques de ce groupe d'épouser un ou une apparenté. Au fil des générations les réseaux d'ascendance sont vite devenus inextricables et la plupart des couples ont été constitués d'individus ayant un grand nombre d'ancêtres communs.

Pour caractériser la liaison parentale entre deux individus on calcule généralement le 'coefficient de parenté ρ' des deux membres du couple (qui est aussi le 'coefficient de consanguinité f' de chacun de leurs enfants).

Ce coefficient est, par définition, la probabilité pour qu'un gène pris au hasard chez un membre du couple et un pris au hasard au même locus chez l'autre membre soient 'identiques', c'est-à-dire, soient la reproduction d'un même gène ancêtre. La méthode de calcul de ce coefficient est théoriquement fort simple; il suffit d'énumérer tous les ancêtres communs des deux individus A et B étudiés; de compter pour chaque ancêtre C_i le nombre n_i de générations qui séparent A de C_i et le nombre p_i de générations qui séparent B de C_i, enfin d'effectuer, pour tous les 'chemins' possibles de A à B par l'intermédiaire d'un ancêtre C_i la sommation:

$$\rho_{AB} = \Sigma_i \left(\tfrac{1}{2}\right)^{n_i + p_i + 1}$$

Dans le cas par exemple du couple de la 4ème génération constitué de Julio et Mencha, on constate que leur réseau d'ascendance correspond à un coefficient $\rho = 0{,}18359$ (Fig. 3).

Comme on l'a déjà indiqué, le coefficient de parenté d'un couple est aussi le coefficient de consanguinité de chacun de leurs enfants, ce coefficient étant défini comme la probabilité pour que les deux gènes reçus en un même locus par ces enfants soient 'identiques'. On peut ainsi à chaque génération, en pondérant les coefficients de parenté par les nombres d'enfants, calculer la moyenne des coefficients de consanguinité.

Cette moyenne, souvent désignée comme le 'coefficient de Bernstein: α', caractérise la possibilité pour les 2 gènes possédés en un même locus par un individu pris au hasard dans la génération, d'être la copie d'un même gène ancêtre. Si cette éventualité s'est effectivement

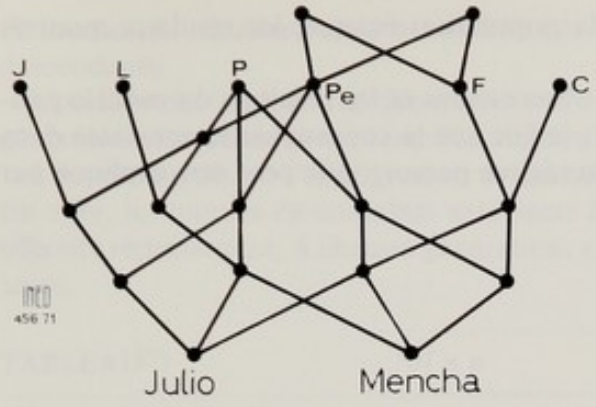


Fig. 3.

réalisée, cet individu ne peut être hétérozygote; il a en fait, reçu de son père et de sa mère, non pas vraiment 2 gènes, mais 2 exemplaires du même gène.

Le coefficient α est ainsi une mesure de l'appauvrissement génétique de la population.

Les coefficients moyens des 5 générations qui se sont succédés dans la tribu sont montrés dans le Tableau 1.

Il faut tout d'abord noter le niveau exceptionnellement élevé de la consanguinité atteinte dès la génération 3; à titre de comparaison, citons parmi les taux moyens de consanguinité les plus élevés jusqu'à présent mesurés dans une population humaine:

- celui de l'Andhra-Pradesh (côte est de l'Inde) (Dronamraju, 1964) où, en raison de la fréquence considérable des mariages entre oncle et nièce et entre cousins germains, α est de $2.280\,10^{-5}$;
- celui de l'île d'Hosojima, Japon (Ishinuki et coll., 1960) où la population est très isolée; en considérant 5 générations on a trouvé $\alpha = 3.341\,10^{-5}$.

Les taux de consanguinité des Jicaques sont ainsi trois fois plus élevés que ceux considérés jusqu'à présent comme des maximums.

TABLEAU 1

Génération	Effectif	$a_g \times 10^5$
1	7	0
2	34	0
3	176	6.872
4	247	9.218
5	101	6.560

D'autre part, les taux observés peuvent être comparés à ceux qui résulteraient d'un régime 'panmictique', c'est-à-dire d'une absence totale de choix du conjoint. Malécot a montré que, dans ce régime panmictique, les coefficients α_g des générations successives sont liés par la relation de récurrence:

$$\alpha_g = \frac{1}{2N_{g-2}} + \left(1 - \frac{1}{N_{g-2}}\right)\alpha_{g-1} + \frac{1}{2N_{g-2}}\alpha_{g-2}$$

où N_{g-2} est le double de la moyenne harmonique des effectifs N_1 d'hommes et N_2 de femmes dans la génération g-2, soit:

$$N = 4\,\frac{N_1 \times N_2}{N_1 + N_2}$$

L'application de cette relation donne, pour la population jicaque, les résultats montrés dans le Tableau 2.

Pour les générations 3 et 4, l'accord entre les observations et les résultats du modèle panmictique peut être considéré comme satisfaisant; le fait que la consanguinité constatée dans la génération 3 soit inférieure à celle résultant du régime panmictique peut être expliqué par la prohibition des mariages frère-sœur.

TABLEAU 2

Génération	N_1	N_2	N	$\alpha \times 10^5$
1	4	3	6,86	0
2	19	15	33,52	0
3	96	80	174,54	7.289
4	–	–	–	8.563
5	–	–	–	8.820

Pour la génération 5, le coefficient α subit, contrairement à ce que laisse prévoir la théorie, une sensible diminution. Mais cette fois l'explication est évidente: l'isolat n'est pas resté totalement fermé; certains garçons ont épousé des indiennes provenant d'autres communautés et même des 'Ladinos'; malgré la faible importance numérique de cette immigration, elle a suffi pour provoquer un recul de la consanguinité moyenne.

TRANSFORMATION DU PATRIMOINE GÉNÉTIQUE

Le coefficient de consanguinité moyen α des générations successives ne constitue qu'un bien pauvre résumé des informations contenues dans les généalogies. Certes, il peut être interprété d'un point de vue biologique comme significatif de l'érosion génétique subie par le groupe: nous savons, par exemple, grâce au calcul de α, que la patrimoine génétique s'est trouvé appauvri à la génération 4 d'environ 9%, c'est-à-dire que la probabilité, pour un indien pris au hasard dans cette génération, d'être hétérozygote pour un locus donné est inférieure de 9% à ce qu'elle était chez les fondateurs. Mais bien d'autres modifications sont intervenues dans ce patrimoine; en particulier, la plus grande fécondité de certains couples a entraîné une plus large répartition des gènes dont ils étaient porteurs; indépendamment de l'apparentement plus ou moins intense des couples qui se sont constitués, une évolution de la structure génétique de la population s'est produite. C'est cette évolution que nous allons tenter de caractériser.

Pour le faire, il suffit de s'interroger, en face de chaque individu, sur la provenance de ses gènes: ceux-ci proviennent avec la probabilité 1/2 de son père, 1/2 de sa mère; les gènes de son père à leur tour provenaient ...; on peut ainsi calculer pour chaque individu la probabilité pour qu'un de ses gènes provienne de chacun des fondateurs ou de chacun des immigrants. En faisant la moyenne de ces probabilités pour les diverses générations on obtient les résultats montrés dans le Tableau 3.

Ce tableau montre tout d'abord, combien la variance de la fécondité des couples et de la mortalité des enfants a provoqué une très rapide modification de la structure génétique: ainsi Leon et Petrona par exemple, dont les parts étaient égales à l'origine, ont eu rapidement des importances très différentes: dès la génération 2, les gènes de Petrona sont 2,26 fois plus nombreux dans la population que ceux de Leon; par la suite l'écart s'est constamment amplifié; ce rapport atteint 5,4 à la génération 5.

Autrement dit, à côté de l'"effet de fondateur' souvent mentionné, résultant de l'hétérogénéité des fondateurs par rapport à l'ensemble de la population dont ils sont issus, apparaît un 'effet

de fondation' résultant de la variabilité des fécondités de ces fondateurs et de leurs premiers descendants.

Un autre trait caractéristique de cette évolution est l'invasion brutale, à partir de la 4ème génération, des gènes provenant d'immigrants métis et indiens.

L'étude directe de l'immigration ne permet pas d'avoir une idée claire de ce phénomène. En effet, les entrées de conjoints extérieurs à la communauté n'ont porté que sur de faibles effectifs représentant, à chaque génération, environ 4% de l'ensemble des individus procréateurs.

TABLEAU 3

Génération	Effectif	Fondateurs								Immigrants	
		Leon	Francisco	Caciana	Juan	Polinaria	Pedro	Petrona	Total	Indiens	Métis
1	7	7	143	143	143	143	143	143	1000	–	–
2	34	34	88	74	191	176	199	199	1000	–	–
3	176	176	66	78	167	151	208	208	957	43	–
4	247	247	54	68	162	146	195	195	888	81	31
5	101	101	24	31	132	126	129	129	602	186	212
Moyenne pondérée		55	66	66	160	146	187	187	867	82	51

Une immigration portant sur un effectif aussi limité est ressentie comme faible à la fois par la communauté concernée et par les ethnologues ou les démographes qui l'étudient; elle a cependant été suffisante pour modifier très profondément la structure génétique de la population. Chez les enfants de la 5ème génération 40% des gènes proviennent des immigrants. Ce résultat n'est paradoxal qu'en apparence, il a pour cause la fécondité supérieure des couples comportant un conjoint immigré.

Le cas des Jicaques met ainsi en évidence le peu de signification, du point de vue génétique, des mesures de l'immigration basées uniquement sur le nombre des individus entrés dans le groupe. Ce qui importe à long terme, c'est le nombre de gènes incorporés par les immigrants dans le patrimoine de la communauté; la mesure de leur influence doit donc tenir compte du nombre de leurs descendants. Or, sans être une règle, la fécondité des couples comportant un immigré est très souvent constatée dans les faits.

MESURE DE L'ÉVOLUTION GÉNÉTIQUE

A chaque génération g, la structure génétique est représentée par un ensemble de n probabilités $x(1, g)$, $x(2, g)\ldots$ $x(n, g)$, n étant le nombre de fondateurs et d'immigrants. Cet ensemble constitue un vecteur caractéristique de la génération.

Pour évaluer la rapidité avec laquelle cette structure génétique se déforme de génération en génération, il convient de définir ce qu'est la 'distance' entre deux vecteurs: pour bien correspondre à notre intuition de l'écart qui sépare deux structures génétiques, une telle distance doit respecter les propriétés ci-après*:

- la distance entre deux générations ne dépend que des probabilités les concernant et de la probabilité moyenne de chacun des divers fondateurs;
- la distance entre deux générations caractérisées par les mêmes probabilités est nulle;
- si deux fondateurs ont constamment des probabilités proportionnelles (cas de Francisco et Caciana par exemple), on ne change pas les distances entre générations si l'on remplace ces

* *Cf*: J. P. Benzecri: *Analyse des Correspondances*. I.S.U.P., Paris.

deux probabilités par leur somme, ce qui revient à remplacer les deux individus par le couple qu'ils ont constitué.

On peut constater que la 'distance euclidienne' classique, obtenue en faisant la somme de carrés des écarts entre deux générations:

$$d^2(g, g') = \Sigma_i (x_{ig} - x_{ig'})^2$$

a bien les deux premières propriétés, mais pas la troisième.

Par contre, en calculant une distance conforme à une 'métrique de χ^2', définie par:

$$d^2(g, g') = \Sigma_i \frac{(x_{ig} - x_{ig'})^2}{x_{i.}}$$

où $x_{i.}$ est la moyenne des probabilités du fondateur i pour l'ensemble des générations pondérée par les effectifs de ces générations, on est en possession d'un critère d'écart entre les générations qui jouit des trois propriétés énoncées. Dans le cas de la structure génétique des Jicaques, on obtient:

$d(1, 2) = 15{,}6$ $\quad d(2, 3) = 6, 3$ $\quad d(3, 4) = 6, 7$ $\quad d(4, 5) = 29{,}7$

mesures qui traduisent bien le ralentissement de l'évolution entre les générations 2 et 4, en raison de l'accroissement de l'effectif de la population, pour l'accélération provoquée, lors de la constitution de la génération 5, par la forte fécondité des couples immigrés.

III. RÉSUMÉ

L'évolution du patrimoine génétique d'un groupe peut être mesurée soit, comme il est classique, d'après la valeur du coefficient de consanguinité des générations successives, soit en évaluant les contributions des divers fondateurs dans ces générations.

L'exemple de la tribu des indiens jicaques du Honduras étudiée ici montre l'intérêt de cette dernière méthode, qui permet en outre le calcul d'une 'distance génétique' entre génération et par conséquent une mesure de la rapidité de l'évolution génétique.

BIBLIOGRAPHIE

DRONAMRAJU, K. (1964): Andhra-Pradesh. *Population*, *19*, 291.
ISHINUKI, N. et coll. (1960): Hosojima. *Amer. J. hum. Genet.*, *12*, 67.

N. E. Morton

University of Hawaii, Hawaii

Population genetics

Population genetics is the application of mathematics to genetics. One approach is to consider separately populations under different demographic conditions, such as pre-neolithic, primitive, peasant, or industrial. However, only a few poorly understood properties of gene pools reflect such cultural differences, and so it seems more powerful to examine specific genetic processes over a spectrum of environments. In this symposium we consider the processes that lead to polymorphism, the effects of mutation, and the determinants of population structure. Time prevents us from exploring other processes, such as those producing familial aggregations of disease and those resulting from altered reproductive patterns, which are currently the subject of active research.

Our speakers reflect a profound change in population genetics, which a generation ago might almost have been equated to evolutionary genetics. Today the main thrust is no longer to feel comfortable with the biological record of evolution, but rather to understand the factors responsible for diversity in contemporary populations. Less appeal is made to the consensus of knowledgeable men, and more to empirical tests of hypotheses. Differential equations, the characteristic tools of evolutionary theory, have been largely replaced by probability and statistics.

Most of the genes identified in man are rare, with frequencies less than 1%. Such genes have been called *idiomorphs*. Some have severe effects on health, and most of these owe their frequencies to the opposing forces of mutation and adverse selection, while a smaller proportion represent balanced selection. Many of the properties of rare recessive, detrimental genes can be determined by study of the genetic load revealed through inbreeding. However, by far the majority of rare genes are not known to cause impairment. Traditionally it was supposed that imperceptible selection kept these genes rare, but the discovery of many electrophoretically variant proteins with apparently normal activity and stability has raised the possibility of quasi-neutrality: that is, selection may be of about the same order as the mutation rate, and therefore not distinguishable. A substantial fraction of protein evolution may be due to slow fixation of quasi-neutral mutations, although the evidence is still inconclusive.

Largely through electrophoresis, it has become apparent that in human populations a large fraction of loci have two or more common alleles, which are called *polymorphs*, and the locus is said to be a *polymorphism*. From the rate of amino acid substitution in specific proteins, it appears that quasi-neutral alleles are involved, but this is consistent with more intense selection in some generations. Thus we do not yet know the role of balanced selection, especially heterozygote advantage, in the origin and maintenance of polymorphism.

One of the facts not explained on the hypothesis of quasi-neutrality is that estimates of kinship from gene frequency variance among ethnic groups show a pronounced peak as the mean gene frequency approaches $\frac{1}{2}$, implying weaker and perhaps variable systematic pres-

sure on the most common genes. If mutation were the main systematic pressure, estimates of kinship should be uniform instead of parabolic. Another anomaly is that mutation rates are known to be nearly proportional to numbers of generations, being much less per unit of absolute time in mammals than bacteria. Unlike mutation, protein evolution shows little retardation in higher organisms. Therefore, at the present time the evidence points to very low systematic pressures on protein evolution, but does not clearly support fixation of neutral mutations.

Another fact has sometimes been thought to argue against neutral polymorphism: namely, the same alleles are often polymorphic in different regions, as if maintained by balanced selection. Kimura has shown that this is also to be expected for neutral alleles if there is so much migration that the whole species behaves as a breeding unit with regard to fixation; that is, the pressure toward fixation in any locality is less than the normalizing effect of migration. Thus *population structure*, defined as the variation of gene frequencies among subpopulations, partially determines polymorphism and the fate of mutations. It was the genius of Wright and Malécot to see population structure as the resultant of random drift in small local populations, opposed by a systematic pressure due largely to migration, but also to mutation and normalizing selection. Wright's concept of neighbourhood size, defined in terms of the variance of migration, does not correspond to local populations, whereas Malécot's recognition of kinship as the basic parameter of population structure, his use of migration matrices, and his formulation of isolation by distance have been seminal. The clarity of their work is in contrast to later attempts to ignore either stochastic or systematic forces. The first approach leads to computations of the time required for two gene pools to go halfway to panmixia in the absence of drift, calculations which can be grossly in error in the presence of drift. Ignoring systematic pressure, on the other hand, leads to concepts of isolate size and to construction of phylogenetic trees which can be justified only in the total absence of migration, the ubiquity and importance of which are attested by all available evidence. One has only to apply the logic of phylogenetic trees to historical populations, to ask for example whether Germany and France could reasonably be regarded as the fission products of a single tribe (which one?), to realize that phylogenetic trees are like flower arrangements: it is enough that they are pretty, without asking that they be meaningful.

I hope that this personal overview of the topics to be discussed today has at least signalized some of the major areas of controversy. Our understanding of many aspects of population genetics is only beginning, but we know enough to recognize the central role of polymorphism, mutation, and population structure in human biology. We are privileged in having as speakers today some of the principal architects of this theory.

REFERENCES

KIMURA, M. and OHTA, T. (1971: Protein polymorphism as a phase of molecular evolution. *Nature (Lond.), 229*, 467.

MALÉCOT, G. (1967): Identical loci and relationship. *Proc. Fifth Berkeley Symposium Mathematical Problems; IV*, pp. 317–332. University California Press, Berkeley, Calif.

WRIGHT, S. (1965): The interpretation of population structure by F statistics with special regard to systems of mating. *Evolution, 19*, 395.

L. S. Penrose

The Kennedy-Galton Centre for Mental Retardation Research and Diagnosis, Harperbury Hospital, Harper Lane, Shenley, Radlett, Hertfordshire, United Kingdom.

Stable equilibrium and human polymorphism

Rules for the effects of natural selection, worked out for theoretical populations, are not always easy to apply convincingly to the human population, which is seen at a moment of time or, at best, for a few generations. The assumption can reasonably be made that genetical processes, if they are observed, are probably in stable equilibrium, otherwise they would not exist. The two possible kinds of stable equilibrium are that which depends upon continuing spontaneous mutation and that which depends upon heterozygote advantage, sometimes called 'segregation' for brevity. The two methods may both act at the same time. Consequently, there are, in practice, difficulties in the interpretation of the frequencies of disadvantageous diseases, whether to attribute their presence to mutation or to segregation. The result is that estimates of mutation rates of hereditary diseases, especially in recessive conditions, must be considered as upper limits.

In man the biological mechanism of heterozygous advantage, when its presence can be suspected, is usually not dependent upon an obvious characteristic, like exceptional physical strength. The phenomenon is more subtle than that of traditional hybrid vigour in plants. The standard example is the advantage conferred on heterozygotes in certain haemoglobinopathies, which seems likely to be some kind of defense against malaria. There are, moreover, many different environmental circumstances which can bring about heterozygous advantage as, for instance, periodic temperature changes which might reduce the fitness of different homozygotes at different times.

In the present paper I am mainly concerned with some formal aspects of the classical situation of stable equilibrium in a randomly mating population, as first indicated by Fisher (1922) and described again by him in 1930. The first suggestion that the principle might be applied in human genetics came from Haldane (1939) and he later elaborated his ideas (Haldane, 1947, 1957). In particular he emphasized the possible relationship of this principle to the stability of quantitative traits, such as stature, birth weight and intelligence, all of which are, in part, genetically determined and subject to selection. There is evidence, as Galton (1869) mentioned, that extremes tend to be biologically unfavourable. Giants and dwarfs, as well as men of genius and imbeciles, tend to have reduced fertility. The greatest degree of fitness lies near the average.

Fitness, from the biological point of view, is measured by fertility, that is, by number of offspring. Fisher's suggestion was that, in man, fitness should be measured by the number of offspring who reach the mean reproductive age. This is a very difficult thing to measure in human populations and it is usually necessary to be satisfied with crude data, such as comparison of number of surviving children in a family, as a relative measure of fertility. The difficulty in human data is emphasized by the great variations in family size which occur in consequence of environmental influences. In order to establish credibly the existence of hetero-

zygous advantage, say 11%, which would keep a lethal recessive, which had 1% frequency, in stable equilibrium we would need about 100 families, with a known heterozygote as parent, to compare with the mean family size in a basic control population. However, much information can be obtained by using entirely different methods of analysis, either in single gene characters or in quantitative traits.

The neatest way of writing the equilibrium conditions in a randomly mating population is shown in Table 1. Here one pair of alleles is considered, **A** and **a**. The mean fitness is 1 and the heterozygote has a relative fitness, $1 + C$, where C stands for the coefficient of selection. The fitness of any given mating, expressed as number of offspring, is assumed to be proportional to the product (or sum) of the fitness values of the two parents. The distribution of that part of the population, which supplies the parents, is obtained by counting a parent once for one child, twice for two, and so on. The total population numbers are irrelevant and all the figures are relative. In the case of a recessive lethal, $1 - Cp/q = 0$, so $C = q/p$. This shows at once that, if $q = 1/10$, as just suggested, $C = 1/9$ or 11%.

TABLE 1 *Conditions for stable equilibrium with heterozygous advantage and random mating*

Genotype	Quantitative value	Relative fitness	Frequency	
			(*i*) at birth	(*ii*) in parents
AA	+1	$1 - Cq/p$	p^2	$p^2 - Cpq$
Aa	0	$1 + C$	$2pq$	$2pq + 2Cpq$
aa	−1	$1 - Cp/q$	q^2	$q^2 - Cpq$
Total			1	1
Mean		1	$p - q$	$p - q$
Variance		C^2	$2pq$	$2pq - 2Cpq$

Note: C is the coefficient of selection and variance (*ii*)/variance (*i*) $= 1 - C$.

With quantitative characters, the other test which can be applied is to compare the variance of the trait in parents with the variance in all children. If the genes are perfectly additive (intermediate) then the selection coefficient can be calculated from the comparison of the two variances for $C = 1 - V_{((ii)\text{parents})}/V_{((iii)\text{children})}$. When a number of genes at different loci, all additive in their effects, are combined, the fitness values can be theoretically combined, cumulatively, by multiplying (or by adding). In multifactorial systems, the relation of quantitative values to fitness will then be less obvious than when only one gene pair is considered because, except at the extremities of the distribution, each quantitative value represents more than one kind of genotype. Nevertheless, if all the genes which belong to the system are comparable with one another in frequency, in fitness value and in quantitative effect, an estimate of the average value of their individual coefficients of selection can be obtained from this comparison of variances.

Examples of metrical traits, which show reduced variance in parents as contrasted with that in the population at birth (Penrose, 1950), include birth weight (Karn and Penrose, 1950), where survivors can be treated as potential parents, cephalic index and intelligence. Calculation shows that, in all these, the coefficient of selection must be considerable for genes which contribute to the causation (see Table 2).

When there is dominance or recessivity, the means of the parental generation will tend to differ from that in the total population. It can be shown, after substituting +d for +1, +h for 0 and −1 for −1 in Table 1, that the amount of the difference depends upon the values of h/d, of C and of p and q. If **a** is recessive, the difference is always negative and it is positive if **A** is recessive. The differences in Table 2 suggest that some genes which determine low birth weight and low intelligence are recessive and that low cephalic index is dominant over high

TABLE 2 *Analysis of some metrical characters for estimating selection coefficients*

Character	All children			Parental generation			Differences		Estimate of C
	Mean_i	σ_i	σ_i^2	Mean_{ii}	σ_{ii}	σ_{ii}^2	$\text{Mean}_i - \text{mean}_{ii}$	$\sigma_i^2 - \sigma_{ii}^2$	$(\sigma_i^2 - \sigma_{ii}^2)/\sigma_i^2$
Birth weight ♂	7.27	1.32	1.75	7.35	1.17	1.37	−0.08	0.38	0.217
Birth weight ♀	7.06	1.22	1.49	7.13	1.10	1.21	−0.05	0.28	0.188
Cephalic index	80.80	3.29	10.72	80.10	3.01	9.06	+0.70	1.66	0.157
Intelligence	92.84	17.64	311.17	93.45	13.71	187.96	−0.61	123.21	0.394

cephalic index. In addition to producing this effect, dominance and recessivity exaggerate the magnitude of the estimate of C, as it is made here, especially for a lethal recessive.

Contrasting with the results shown in Table 2, the values of C, obtained from family data on total ridge-count of finger-prints, are close to zero; in the table of parent and child pairs, given by Holt (1955, Table V), C = –0.027 and the means (i) and (ii) are very close, as would be expected in the absence of selection and of dominance.

If it is conceded that stable segregational equilibrium is a genuine phenomenon in man, it is worth while to consider some of its formal consequences. At equilibrium point, correlation coefficients, for likeness between relatives for additive characters, are reduced according to the magnitude of the selection coefficient. For example, for perfectly additive gene effects (Penrose, 1964),

$$r_{(\text{parent}-\text{child})}=\tfrac{1}{2}(1-C)^{\frac{1}{2}} \text{ and } r_{(\text{sib}-\text{sib})}=\tfrac{1}{2}(1-C).$$

More remarkable are the peculiarities which are concerned with fitness as measured by number of offspring or by size of sibship, if this property is investigated as a genetical character. The striking result that, in parent and child, fertility, as measured by number of offspring, is uncorrelated, was obtained by Haldane (1947). Naturally, this is also true neutral equilibrium, in the absence of selection, when C = 0. In sibs the correlation for fertility is not zero in stable equilibrium but is less than $\frac{1}{4}$.

Attention was drawn by Fisher (1930) to the problem of the inheritance of fertility and much eugenical speculation has been based upon the assumption of its significance in human families. However, if a positive correlation between fertility of parent and fertility of child should be observed and if it were genetically determined, it is difficult to imagine how the population could still be in equilibrium with respect to any given gene which was responsible. If a gene increases fertility, it will tend to increase in frequency, and *vice versa*. Nevertheless, when fertility is measured by family size, that is by sib number, some curious results follow although there is stable equilibrium.

One direct consequence of heterozygous advantage is a negative correlation between sib number and any genetical quantitative character. When the homozygote **aa** is lethal, the correlation has the approximate value –(1–C)/2. A negative relationship between size of sibship has been found both with stature and intelligence, and in both cases this can be shown to be consistent with stable equilibrium (Penrose, 1955). Neglect of this possibility has led people to suppose that decline in mental ability and in physique is imminent (Cattell, 1937).

There is a practical point of interest here because parent-child correlation coefficients for sibship size were actually calculated by Pearson (1899) in a large sample of families. He obtained values of $+0.21 \pm 0.02$ for mother and daughter sibships. This would superficially suggest that some genes for fertility were being strongly positively selected. Such a value, however, can be shown to be consistent with equilibrium of a gene with a specially fertile heterozygote and with frequency of the infertile homozygote of about 1/25.

At the present time, several examples are known of polymorphisms which depend upon many genes at one locus. In these cases, the proportion of heterozygotes is increased. The effects of mutation in the absence of selection have been studied by Wright (1966) and by Kimura (1968). The conditions of stable equilibrium with heterozygote advantage have not been very intensively examined except in the case of three alleles with special reference to the haemoglobins A, S and C (Penrose *et al.*, 1956).

Not only genes but chromosomal peculiarities also give rise to equilibrium problems. In particular, certain balanced translocation carriers have special risks of producing offspring with unbalanced karyotypes, most of which are lethal. Just as with a lethal condition caused by a gene, the upper limit of the rate of spontaneous mutation can be estimated directly on the basis of frequency of loss, from the population, of a given translocation when it occurs in a lethal phenotype. The mode of inheritance has essentially the same appearance as that of an irregular dominant trait. However, the possibility must be considered that some chromosomal

translocations may actually be beneficial, as found in some wild species of *Drosophila*. If a given translocation of the D/D or D/G type were found to be beneficial and to increase fitness, this could make the assumption of a mutation rate, as indicated by the incidence of unbalanced types, too high. At present, not very many translocations and other structural changes in man, which produce lethal effects, have been recorded, but more will be found as methods of identifying chromosomes improve. At least it is fairly certain at present that the rate of structural mutation of the D/G translocation cannot be much higher than that estimated directly for dominant gene mutations in some well defined clinical types, such as epiloia and acrocephalosyndactyly.

REFERENCES

CATTELL, R. B. (1937): *The Fight for our National Intelligence*. P. S. King and Son, London.

FISHER, R. A. (1922): On the dominance ratio. *Proc. roy. Soc. Edinb.*, *62*, 321.

FISHER, R. A. (1930): *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.

GALTON, F. (1869): *Hereditary Genius*. Macmillan and Co., London.

HALDANE, J. B. S. (1939): The spread of harmful autosomal recessive genes in human populations. *Ann. Eugen. (Lond.)*, *9*, 322.

HALDANE, J. B. S. (1947): Parental and fraternal correlations for fitness. *Ann. Eugen. (Lond.)*, *14*, 288.

HALDANE, J. B. S. (1957): Natural selection in man. *Acta genet. (Basel)*, *6*, 321.

HOLT, S. B. (1955): Genetics of dermal ridges: parent-child correlations for total finger ridge-counts. *Ann. hum. Genet.*, *20*, 270.

KARN, M. N. and PENROSE, L. S. (1950): Birth weight and gestation time in relation to maternal age, parity and infant survival. *Ann. Eugen. (Lond.)*, *16*, 147.

KIMURA, M. (1968): Genetic variability maintained in a finite population due to mutational production of neutral and nearly neutral isoalleles. *Genet. Res.*, *11*, 247.

PEARSON, K. (1899): Mathematical contributions to the theory of evolution. *Phil. Trans. A*, *192*, 257.

PENROSE, L. S. (1950): Propagation of the unfit. *Lancet*, *2*, 425.

PENROSE, L. S. (1955): Evidence of heterosis in man. *Proc. roy. Soc. B*, *144*, 203.

PENROSE, L. S. (1964): Some formal consequences of genes in stable equilibrium. *Ann. hum. Genet.*, *28*, 159.

PENROSE, L. S., MAYNARD-SMITH, S. and SPROTT, D. A. (1956): On the stability of allelic systems with special reference to haemoglobins A, S and C. *Ann. hum. Genet.*, *21*, 90.

WRIGHT, S. (1966): Polyallelic random drift in relation to evolution. *Proc. nat. Acad. Sci. USA*, *55*, 1074.

Gustave Malécot

Université de Lyon I, Mathématiques Appliquées, Villeurbanne-Charpennes, France

Structure géographique et variabilité d'une grande population

INTRODUCTION

1. La structure d'une population étendue (humaine ou non) est commandée par la manière dont se fait le tirage des 'gamètes utiles': étant donné que chaque individu diploïde (chaque 'parent', mâle ou femelle) est producteur, potentiel ou réel, d'un très grand nombre de gamètes, seule une faible proportion de ces gamètes sera utilisée pour produire les diploïdes de la génération suivante (que nous appellerons F_n). Il y a donc un tirage au sort des 'gamètes utiles' parmi un nombre beaucoup plus grand de 'gamètes produits'.

2. S'il advenait que la population soit à la fois nombreuse et panmictique dans son ensemble, c'est-à-dire qu'elle constitue 'un seul groupe panmictique infiniment nombreux' (comme dirait le mathématicien) les gamètes utiles (très nombreux) seraient tirés au sort, au hasard et indépendamment, dans un 'réservoir gamétique' contenant un nombre encore bien plus grand de gamètes produits: la fréquence q d'un certain gène nommé a (et de même la fréquence $p = 1 - q$ de l'ensemble, nommé A, de ses allèles) serait alors, d'après la loi des grands nombres, la même chez les gamètes utiles que chez les gamètes produits; et les associations au hasard des gamètes utiles 2 à 2 serait conforme à la loi de Hardy-Weinberg (modifiée par les mutations et sélections, qui ont pour effet de faire varier q au cours des générations et de faire tendre vers une limite $C = \bar{q}$, qui dépend de la pression de mutation et de la pression de sélection, comme nous le rappellerons plus loin).

3. Mais le fait que la population totale soit étendue sur une aire très grande (ou, dans le cas 'unidimensionnel', sur une ligne de grande longueur, fermée ou non) rend impossible que la population soit panmictique dans toute son étendue; elle peut alors être regardé ecomme divisée en 'groupes panmictiques', comprenant chacun N individus diploïdes résultant de la fusion 2 à 2 de 2N 'gamètes utiles', ces gamètes étant eux mêmes tirés au sort indépendamment dans un 'réservoir gamétique local' comprenant un nombre beaucoup plus grand de gamètes produits (réellement ou potentiellement) par les individus du voisinage. La constitution du réservoir gamétique, dans un emplacement ou 'site' donné (que nous désignerons par la lettre x pour le distinguer des autres sites, voisins ou non, que nous désignerons par z), répond en général à des conditions intermédiaires entre deux cas extrêmes:

a. Si le réservoir gamétique en x est constitué par des gamètes provenant en quantités égales de tous les sites z, voisins ou non, on se trouve dans le cas, indiqué ci-dessus (2), de 'panmixie générale': la distinction des sites et des réservoirs correspondants est inutile: le passage de chaque génération F_{n-1} à la génération suivante F_n se fait par un 'brassage général' de toute la population.

b. Si le réservoir gamétique en x est produit par un seul individu d'emplacement x (hermaphrodite), les gamètes tirés en x proviennent tous du même individu, c'est le cas extrême de

l'autofécondation exclusive: la population totale est séparée en lignées évoluant chacune sans échanges génétiques avec les autres.

Une variante, dans le cas des sexes séparés, est celle du croisement exclusif 'frère-sœur', où chaque site x est occupé par deux diploïdes, l'un mâle et l'autre femelle, dont les gamètes mélangés constituent le réservoir gamétique où sont tirés (toujours dans le site x) les deux géniteurs suivants.

c. La réalité est en général intermédiaire, en ce sens que les diploïdes qui fournissent des gamètes au réservoir gamétique en x peuvent occuper eux-mêmes, soit le site x, soit un site z voisin de x, mais avec une contribution d'autant plus faible que z est moins voisin de x: c'est ce que j'appelle la 'migration', qui peut être le fait des gamètes (pollen des plantes) ou des diploïdes eux-mêmes (par leurs déplacements depuis leur naissance, en z, jusqu'à leur reproduction en x): elle se caractérise mathématiquement par le 'taux de migration' l_{xz}, proportion dans le réservoir gamétique en x, de gamètes issus de l'emplacement z; l_{xx}, proportion de gamètes 'autochtones', est en général le plus important numériquement; de toutes façons, $\sum_z l_{xz} = 1$. Les l_{xz} peuvent être disposés en 'matrices de migration' (Malécot, 1950). (La somme, étendue théoriquement à la totalité des sites z occupés par l'espèce, ne comprend en fait qu'un petit nombre de termes non nuls, ceux qui correspondent à 'z voisin de x').

La structure de cette 'distribution' l_{xz} (qui est aussi la distribution des 'probabilités de provenance' d'un gamète utilisé en x) est tout à fait essentielle; elle me paraît être (conjointement avec le nombre 2N de gamètes utilisés en x) l'élément majeur de la 'structure de la population': *trop souvent*, on *réduit* ce problème à la manière dont les 'conjoints' se choisissent, c'est-à-dire à la manière dont s'accouplent 2 à 2 les gamètes utiles mâles et femelles (consanguinité, homogamie, etc). Mais, si l'on admet que les *N couples* de gamètes utiles sont tirés au sort indépendamment dans le réservoir gamétique en x, il importe alors assez peu que les deux gamètes qui s'unissent présentent au sein du réservoir gamétique une corrélation locale (en général positive, mais négative dans le cas d'hétérogamie): l'homogamie totale équivaudrait à tirer N couples de gamètes 2 à 2 identiques, donc à réduire de 2N (cas de la 'panmixie locale') à N le nombre des tirages au sort indépendants, autrement dit à diviser par 2 l'effectif diploïde local N: cette correction est en général d'importance numérique faible, ce qui fait que la panmixie locale (tirages indépendants des 2N gamètes utiles de tous sexes) représente une bonne approximation: on montre aisément que, si l'on désigne par α la corrélation locale entre les gamètes qui s'unissent ($\alpha = 0$ dans le cas de panmixie locale) le nombre 2N de tirages au sort doit être remplacé par $2N/(1+\alpha)$ [1].

d. Ce qui est essentiel (outre la loi de migration définie par l_{xz}) c'est l'ordre de grandeur de N (donc de $2N/(1+\alpha)$): c'est en effet ce qui fixe d'après le théorème de Bernoulli l'ordre de grandeur de l'écart aléatoire entre la fréquence q de a (fréquence mesurée sur les 2N gamètes utiles seulement) et la probabilité q', pour chaque gamète, de porter a (probabilité qui ne serait mesurée que par la fréquence mesurée en *épuisant* les très nombreux gamètes produits, c'est-a-dire en épuisant le réservoir gamétique local). C'est donc la distinction entre probabilité (fréquence mesurée sur un échantillon de taille infinie) et fréquence mesurée sur un échantillon fini de taille 2N qui est la clef de la 'dérive gamétique' ou 'dérive fortuite' dont l'importance, pour l'évolution des petits groupes locaux, a été soulignée par Sewall Wright: J'ai montré moi-même l'importance du jeu conjoint de cette dérive génétique locale (définie par N) et de la migration (définie par l_{xz}); la différenciation locale est entravée par une forte migration, mais elle est facilitée par un faible effectif N; certes la sélection, si elle s'exerce dans des sens différents sur différents sites, peut être un facteur considérable de différenciation locale (à condition que son taux ne soit pas d'ordre de grandeur inférieur au taux de migration entre les groupes les plus proches et à l'inverse 1/N de l'effectif local): à ce moment là la différenciation géographique peut aller jusqu'à la différenciation de sous espèces, comme l'a montré Mayr, mais *seulement* si la dérive génétique agissant dans un isolement relatif a au préalable créé un bouleversement génétique suffisant). Qu'il s'agisse de la préparation de la différenciation de sous espèces, ou seulement de la variation, sur une grande étendue, de la fréquence

d'un gène appartenant à un locus polymorphique de l'espèce humaine (par exemple groupes sanguins, étudiés par Rosin (1956) et Morton (1970) la migration tend toujours à égaliser les probabilités locales et la petitesse de l'effectif N tend toujours à développer des fluctuations locales par rapport à ces probabilités; les fluctuations par rapport aux probabilités et aux moyennes, et les corrélations à distance, ont fait l'objet déjà de nombreuses recherches. La recherche de l'équilibre statistique entre migrations et dérive génétique peut être faite à l'aide du modèle suivant, qui nécessite des précisions préalables sur 'probabilités et fréquences'.

I. RELATION ENTRE PROBABILITÉS ET FRÉQUENCES

La génétique de population s'intéresse depuis longtemps à la distribution, dans l'espace, et dans le temps, d'un gène particulier: distribution géographique du gène sanguin O, étudié par Rosin et Morton en Suisse: propagation en Bretagne du gène de la luxation congénitale de la hanche, étudié par le regretté Sutter.

Si l'on désigne par a le gène étudié (et, s'il y a lieu, par A l'ensemble de ses allèles) il est d'usage de désigner par q sa fréquence dans un groupe local (un village), par exemple; mais il faut spécifier le site géographique par un indice x variant de façon discontinue (pour numéroter les villages sur une carte) ou de façon continue (dans l'approximation d'une 'population continue', avec une densité constante ou lentement variable): il faut aussi spécifier la date, en introduisant l'approximation (permise) qui numérote les générations (F_n) par des entiers (n).

On désignera alors par q_{nx} la fréquence du gène a dans le site x et dans la génération F_n.

Les variations de q_{nx} dans l'espace et dans le temps, c'est-à-dire en fonction de x et en fonction de n, dépendent d'une part des causes systématiques (mutations, sélections, migrations) et d'autre part d'une 'dérive fortuite' qui fait de q_{nx} une fonction *aléatoire*. Cette dérive fortuite provient de fait que la fréquence locale q_{nx} n'est mesurée que sur N individus, résultant de fusion 2 à 2 de 2N gamètes utiles. Or chaque gamète utile doit être regardé comme tiré au sort dans un 'réservoir gamétique' ensemble des gamètes (très nombreux) produits par la génération précédente F_{n-1}, ou des gamètes (infiniment nombreux) qui pourraient être produits (avec des probabilités déterminées).

Et ceci nous amène à définir la probabilité pour qu'un gamète (pris au hasard dans la production issue de F_{n-1}) porte le gène a:

1. Si la génération F_{n-1} avait une constitution gamétique connue (avec fréquence $q_{n-1,x}$ pour le gène a) *et* s'il n'y avait ni mutations, ni sélections, ni migrations, la probabilité 'conditionnée' pour qu'un gamète porte a serait $q_{n-1,x}$; si par contre il y avait des causes de variation systématique, la probabilité conditionnée serait

$$q'_{nx} = q_{n-1,x} + \delta(q_{n-1,x}) \tag{1}$$

2. Mais, si l'on ne connaît pas la constitution de la génération F_{n-1} mais seulement celle de la génération initiale F_0, on ne peut définir que la 'probabilité a priori' pour qu'un gamète issu de F_{n-1} (et donc à l'origine de F_n) porte a, probabilité que nous appellerons $\overline{q_{nx}}$ (dans le site x); cette notation est justifiée par le fait que cette probabilité a priori est aussi l'espérance mathématique a priori de la fréquence aléatoire q_{nx}: en effet cette aléatoire est la moyenne arithmétique de 2N aléatoires 'indicatrices' X_j ($1 \leqslant j \leqslant 2N$), chacune définie comme attachée à l'un Γ_j des 2N gamètes utiles constituant F_n, et comme égale à 1 ou à 0 suivant que Γ_j porte le gène a ou non. L'espérance a priori de q_{nx} est donc:

$$E(q_{nx}) = \frac{\sum_{j=1}^{j=2N} E(X_j)}{2N} \tag{2}$$

or $E(X_j) = \overline{q_{nx}}$; d'où $E(q_{nx}) = \overline{q_{nx}}$

Dorénavant la notation $\overline{q}$ désignera donc, suivant l'utilisation, soit la probabilité a priori relative à un gamète tiré au sort, soit l'espérance a priori d'une fréquence aléatoire.

3. D'après le théorème de l'espérance conditionnée,

$$\overline{q_{nx}} = \overline{q'_{nx}} = \overline{q_{n-1,x}} + \overline{\delta(q_{n-1,x})} \tag{3}$$

qui peut être résolu par récurrence dans les cas suivants:

a. Dans le cas où il n'y a pas de migration et où la sélection est linéarisée, c'est-à-dire où la variation systématique $\delta(q)$ est, en chaque site x, de la forme:

$$\delta(q) = -k(q - C_x)^{(2)} \tag{4}$$

L'équation (3) qui relie les espérances a priori relatives à 2 générations consécutives en un même site x donne alors:

$$\overline{q_{nx}} - \overline{q_{n-1,x}} = -k(\overline{q_{n-1,x}} - C_x)$$

D'où:

$$\overline{q_{nx}} - C_x = (1-k)(\overline{q_{n-1,x}} - C_x) = (1-k)^n(\overline{q_{0x}} - C_x) \tag{5}$$

La probabilité (ou espérance de fréquence) $\overline{q_{nx}}$ tend donc, quand le nombre de n de générations tend vers l'infini, vers la limite C_x.

b. La sélection étant toujours linéarisée et donnant donc lieu à la formule (4), on peut supposer qu'elle est précédée d'une migration par laquelle les fréquences $q_{n-1,z}$ des sites z voisins de x sont mélangées, dans des proportions l_{xz} ($\sum_z l_{xz} = 1$) pour donner naissance à une fréquence $q^*_{nx} = \sum_z l_{xz}\, q_{n-1,z}$ sur laquelle la sélection et les mutations opèrent suivant la formule (1), donnant une probabilité conditionnée:

$$q'_{nx} = q^*_{nx} + \delta(q^*_{nx}) = C_x + (1-k)(\sum_z l_{xz}\, q_{n-1,z} - C_x) \tag{6}$$

En prenant l'espérance a priori $\overline{q'_{nx}}$ qui, comme dans (3), est égale à $\overline{q_{nx}}$, on obtient la récurrence:

$$\overline{q_{nx}} = C_x + (1-k)(\sum_z l_{xz}\, \overline{q_{n-1,z}} - C_x) \tag{7}$$

qui permet de montrer que, quand n augmente indéfiniment, ces probabilités a priori (ou espérances) tendent vers des limites, que nous noterons $\overline{q_x}$, et qui sont naturellement définies par:

$$\overline{q_x} = C_x + (1-k)(\sum_z l_{xz}\, \overline{q_z} - C_x)$$

$$\overline{q_x} = k\, C_x + (1-k) \sum_z l_{xz}\, \overline{q_z} \tag{8}$$

Les $\overline{q_x}$ ne sont donc plus, comme dans le cas (*a*) d'isolement absolu, égales aux valeurs d'équilibre locales C_x: la migration produit un effet de nivellement sur les $\overline{q_x}$. Ce n'est que lorsque C_x est indépendant de x que l'équation (8) fournit la solution $\overline{q_x} = C_x$.

1. Dans le cas particulier d'un modèle de migration unidimensionnelle symétrique entre sites adjacents, c'est-à-dire lorsque les sites possibles sont repérés par l'ensemble Z des entiers z, on a $l_{x,x+1} = m$, $l_{xx} = 1-2m$, $l_{xz} = 0$ si $|z-x| \geqslant 2$, et le second membre de (8) fait intervenir la différence seconde $\Delta^2\overline{q_x} = \overline{q_{x+1}} + \overline{q_{x-1}} - 2\overline{q_x}$, car (8′) $k\, q_x = kC_x + (1-k)m\Delta^2\overline{q_x}$, ce qui montre que la variation avec x de $\overline{q_x}$ dépend à la fois de celle de C_x et des valeurs aux bornes

$\bar{q}_{x_1}$ et $\bar{q}_{x_2}$, valeurs qui sont définies par l'immigration sur la frontière de l'intervalle $[x_1, x_2]$ sur lequel sont répartis tous les sites.

(Ce résultat peut s'étendre au modèle bidimensionnel de migration dans un plan avec fréquences connues sur une frontière, lorsque les sites sont répartis sur un réseau rectangulaire avec migration symétrique entre colonies adjacentes).

2. (8′) montre en outre que, dans le cas particulier où k est petit par rapport à m, la variation de C_x influe peu sur celle de $\bar{q}_x$: $\Delta^2 \bar{q}_x$ est alors sensiblement nul, donc $\overline{q_x}$ est sensiblement une fonction linéaire définie par ses valeurs sur la frontière.

3. Dans la pratique, si l'on observe une réalisation expérimentale relevant approximativement d'un tel modèle (par exemple si l'on mesure les frequences q_{nx} du groupe sanguin O sur un ensemble de villages répartis sur l'aire de la Suisse), on peut tenter, comme l'a fait Morton (1968), un ajustement expérimental des espérances $\overline{q_x}$ (les limites étant supposées atteintes, donc n étant supposé suffisamment grand), en regardant les écarts q_{nx}-$\overline{q_x}$ comme des erreurs aléatoires d'espérances nulles (Morton a adopté pour l'espérance $\overline{q_x}$ une fonction simple des coordonnées du site x). Une telle méthode est applicable toutes les fois que la variation de C_x – et donc celle de $\overline{q_x}$ – résulte d'un gradient géographique ('cline'); mais elle cesse d'être valable lorsque C_x varie de façon aléatoire d'un lieu à un autre, c'est-à-dire dans le cas de 'sélection par les micromilieux' telle qu'elle peut se manifester, selon Caïn et Sheppard, dans les variations d'environnement auxquelles sont sujettes les colonies de l'escargot *Cepaea nemoralis*.

Rappelons une fois de plus que l'estimation ainsi obtenue pour $\overline{q_x}$ fournit à la fois l'espérance de la fréquence locale dans le site x, et la probabilité a priori pour qu'un gamète pris au hasard dans ce site porte le gène a auquel nous nous intéressons (le gène noté a). Ainsi se trouvent précisés les concepts théoriques de fréquences et de probabilités qui sont à la base de tous nos modèles.

II. VARIANCE ET COEFFICIENT DE PARENTÉ DANS LE SITE X

Puisque la valeur observée de q_{nx} est regardée comme une réalisation d'une aléatoire dont l'espérance mathématique est $\overline{q_x}$, il est important de déduire du modèle une prévision de l'écart aléatoire q_{nx}—$\overline{q_x}$.

Cette prévision sera fournie par la 'variance a priori':

$$\sigma_x^2 = E\left[(q_{nx} - \bar{q}_x)^2\right]$$

Or on a vu que la fréquence aléatoire q_{nx} a été définie par la formule

$$q_{nx} = \frac{\sum_j X_j}{2N},$$

à partir des 2N aléatoires indicatrices X_j (qui ont chacun $\overline{q_x}$ pour espérance).

Les aléatoires X_j seraient mutuellement indépendantes dans le cas où les gamètes utiles Γ_j de F_n auraient des lois de probabilité a priori indépendantes, ce qui exige qu'ils ne soient pas des copies (réalisées par descendance mendélienne et sans aucune mutation) d'un même gamète utile d'une génération F_h antérieure à F_n (de telles copies fourniraient des gènes 'identiques par descendance' ou, en bref 'identiques'). On peut supposer que réciproquement l'indépendance a priori est assurée pour deux gamètes qui:

a. ou bien descendent de gamètes utiles différents aussi loin qu'on remonte, c'est-à-dire 'jusqu'à F_0' et en supposant que F_0 est une génération 'complètement panmictique' (c'est-à-dire où deux gamètes différents sont indépendants en probabilité);

b. ou bien descendent d'un même gamète utile d'une génération F_h ($0 \leqslant h < n$) mais qu'il

est survenu dans le descendance gamétique depuis F_h au moins une 'betamutation' transformant le gène porté par le gamète en un gène présentant, quel que soit son état avant mutation, la probabilité C_x d'être le gène a [3].

Dans le cas (*a*) et (*b*), les gènes portés par les deux gamètes sont indépendants en probabilité [4].

Sous ces hypothèses, les gènes portés par deux gamètes distincts Γ_j et Γ_l (et les aléatoires indicatrices correspondantes X_j et X_l) ne peuvent présenter que deux alternatives: identité *ou* indépendance. Je désigne par φ, 'coefficient de parenté de Γ_j et Γ_l', la probabilité a priori pour que leurs gènes soient identiques; la probabilité pour qu'ils soient indépendants est $1-\varphi$ (φ peut dépendre du site x où sont tirés au sort [5] les deux gamètes, et de leur génération F_n: les notations correspondantes seront précisées plus tard).

La covariance (a priori) entre X_j et X_l, $E(X_j X_l) - E(X_j) E(X_l)$ est donc:

$$\varphi \overline{q_x} + (1-\varphi)\overline{q_x}^2 - (\overline{q_x})^2 = \varphi \overline{q_x}(1-\overline{q_x})$$

Il suffit de la diviser par la variance commune de X_j et X_l, $\overline{q_x}(1-\overline{q_x})$, pour obtenir ce que Wright (1931) a appelé le 'coefficient de corrélation gamétique' (a priori), qui est donc égal au coefficient de parenté φ.

On obtient alors pour la variance a priori dans le site x:

$$\sigma_x^2 = E\left[(q_{nx} - \overline{q_x})^2\right] = E\left\{\left[\sum_j (X_j - \overline{q_x})/2N\right]^2\right\}$$

$$= \sum_j E\left[(X_j - \bar{q}_x)^2\right]/4N^2 + 2\sum_{(jl)} E\left[(X_j - \bar{q}_x)(X_l - \bar{q}_x)\right]/4N^2$$

la dernière somme étant étendue aux $2N(2N-1)/2$ combinaisons sans répétition de j et de l; tous ses termes sont d'ailleurs égaux à la covariance que nous venons de calculer; comme la première somme fait apparaître la variance, on a:

$$\sigma_x^2 = \bar{q}_x(1-\bar{q}_x)\left[\frac{1}{2N} + \left(1 - \frac{1}{2N}\right)\varphi\right] \qquad (1)$$

Cette formule fournit, en fonction du coefficient de parenté φ de deux gamètes tirés au sort dans le site x, la variance de la fréquence q_{nx} du gène a calculée sur les N diploïdes présents dans ce site. Mais la formule reste valable si l'on calcule la fréquence de a sur un échantillon au hasard de s diploïdes, ou de 2s gamètes; il suffit de remplacer N par s.

Quand N (et éventuellement s) est suffisamment grand pour que 1/2N (ou 1/2s) soit négligeable par rapport à φ, on obtient la formule de Wahlund-Wright:

$$\sigma_x^2 = \bar{q}_x(1-\bar{q}_x)\,\varphi \qquad (1')$$

qui ramène la variance locale au coefficient de parenté local (la nullité de cette variance quand $\varphi = 0$ traduit la convergence de la moyenne arithmétique d'un grand nombre d'épreuves indépendantes).

III. COVARIANCES ET COEFFICIENT DE PARENTÉ ENTRE SITES DIFFÉRENTS

Considérons maintenant les générations F_n appartenant à deux sites différents x et y (d'effectifs diploïdes N et N'), avec leurs fréquences

$$q_{nx} = \frac{\sum_j X_j}{2N} \quad \text{et} \quad q_{ny} = \frac{\sum_l X'_l}{2N'};$$

les espérances mathématiques de ses fréquences sont (dans l'état stationnaire) les quantités $\bar{q}_x$ et $\bar{q}_y$ définies au para. I. La covariance

$$v_{xy} = E[(q_{nx} - \bar{q}_x)(q_{ny} - \bar{q}_y)] = \sum_{j=1}^{N} \sum_{l=1}^{N'} E[(X_j - \bar{q}_x)(X'_l - \bar{q}_y)]/4NN'$$

se réduit à la covariance entre les aléatoires indicatrices X_j et X'_l attachées à deux gamètes Γ_j et Γ'_l tirés au sort respectivement sans les sites x et y. On peut encore définir le coefficient de parenté de ces gamètes – qui sera noté φ_{xy} [6] – comme la probabilité d'identité de leurs gènes (la probabilité complémentaire étant celle de leur indépendance); ce n'est plus (sauf si $\bar{q}_y = \bar{q}_x$) qu'une valeur approchée du 'coefficient de corrélation gamétique' entre X_j et X'_l. Néanmoins c'est la valeur de ce coefficient de parenté que nous allons étudier dorénavant, en raison de la simplicité de l'équation de récurrence à laquelle elle obéit. On en déduira la covariance v_{xy} entre fréquences par la formule approchée:

$$v_{xy} = \varphi_{xy} \sqrt{\bar{q}_x (1 - \bar{q}_x) \bar{q}_y (1 - \bar{q}_y)} \qquad (1)$$

et le coefficient de corrélation ρ_{xy} entre les fréquences q_{nx} et q_{ny}, $v_{xy}/\sigma_x\sigma_y$, sera donc, dans la mesure où la formule de Wahlund [II, (1′)] est valable:

$$\rho_{xy} = \frac{\varphi_{xy}}{\sqrt{\varphi_{xx}\varphi_{yy}}} \qquad (2)$$

qui est aussi la 'probabilité d'identité normalisée' introduite par Nei (1971) dans le cas de multiallélisme.

IV. DÉCROISSANCE DU COEFFICIENT DE PARENTÉ ET DE LA CORRÉLATION EN FONCTION DE LA DISTANCE

Deux gamètes Γ_j et Γ'_l tirés au sort respectivement dans les sites x et y (à l'origine de la génération F_n) sont identiques si et seulement si ils proviennent sans betamutation, soit de deux gamètes identiques, soit d'un même gamète (à l'origine de F_{n-1}).

Comme la probabilité de non betamutation sur chaque chaînon de descendance est 1–k [II,(*b*), note 3], la probabilité conjointe pour que Γ_j et Γ'_l proviennent sans betamutation des sites z et w est $(1-k)^2 l_{xz} l_{yw}$; ils ne peuvent provenir du même gamète que si les sites z et w coïncident, avec au surplus une probabilité conditionnée qui est alors égale à 1/2N, 2N étant le nombre de gamètes distincts utilisés, dans ce site, à l'origine de F_{n-1} (nous supposerons dorénavant que les effectifs diploïdes N sont les mêmes dans tous les sites). On a donc (Malécot, 1950):

$$\varphi_{xy} = (1-k)^2 \sum_z \sum_w l_{xz} l_{yw} \varphi_{zw} + (1-k)^2 \sum_z l_{xz} l_{yz} \frac{1 - \varphi_{zz}}{2N} \qquad (1)$$

En fait, les coefficients de parenté figurant dans le 2ème membre sont relatifs à la génération F_{n-1}, alors que celui qui figure dans le 1er membre est relatif à la génération F_n; mais on peut démontrer que, quand n augmente indéfiniment, ces coefficients tendent vers des limites indépendantes de n, limites qui définissent 'l'état de parenté stationnaire'. Nous retarderons l'équation (1) comme définissant ces limites, indépendantes de la génération. On peut en outre démontrer que, si la probabilité de migration l_{xz} ne dépend que de la distance (scalaire ou vectorielle) z–x, il en est de même des limites φ_{xy}, qui ne dépendent que de y–x = d et que nous noterons dorénavant $\varphi(d)$. L'équation (1) s'écrit alors:

$$\varphi(d) = (1-k)^2 \sum_z \sum_w l_{xz} l_{x+d,\,w}\, \varphi(w - z) + (1-k)^2 \sum_z l_{xz} l_{x+d,\,z} \frac{1 - \varphi(0)}{2N} \qquad (2)$$

Dans le cas particulier de migration unidimensionnelle symétrique défini en para. I(3)(b)(1), et en supposant m assez petit pour que son carré soit négligeable (nous négligerons aussi k^2, mk, k/N et m/N), il n'y a à retenir du 2ème membre de (2) qu'une partie des termes pour lesquels $|z-x| \leqslant 1$ et $|w-x-d| \leqslant 1$. On obtient (au 2ème ordre près en m et en k):

$$\varphi(d) = (1-2k)\{(1-4m)\varphi(d) + 2m[\varphi(d+1) + \varphi(d-1)]\} + \frac{1-\varphi(0)}{2N}\delta(d)$$

$$k\varphi(d) = \delta(d)\frac{1-\varphi(0)}{4N} + m\Delta^2\varphi(d) \qquad (3)$$

$\delta(d)$ étant une 'fonction delta de Kronecker' (nulle quand $d \neq 0$, et égale à 1 quand $d = 0$).

L'équation (3) est de même forme que l'équation (8′) de I, mais il y a deux différences importantes: la fonction donnée C_x est remplacée par une fonction delta; et, surtout $\varphi(d)$ est une fonction paire de la distance algébrique $d = y-x$, et en outre une fonction bornée, puisque $\varphi(d) \leqslant 1$ (comme probabilité) et même $\varphi(d) \leqslant \varphi(0)$ (d'après la formule (2) de para. III). Il en résulte que, pour $d \geqslant 1$, l'équation (3) est homogène, et que sa solution $\varphi(d) = \lambda b_1{}^d + \mu/b_1{}^d$, b_1 étant la racine <1 de l'équation $b^2-(2+(k/m))b+1=0$, ne peut comporter d'exponentielle croissante, donc $\mu = 0$; en outre la formule $\varphi(d) = \lambda b_1{}^d$ est encore valable pour $d = 0$, comme on le voit en écrivant (3) pour $d = 1$.

Compte tenu de ce que $\varphi(-d) = \varphi(d)$, le coefficient de parenté $\varphi(d)$ entre sites de distance d est donné, quel que soit d, par la formule:

$$\varphi(d) = \lambda b_1^{|d|} = \lambda\left(1 + \frac{k}{2m} - \sqrt{\frac{k}{m} + \frac{k^2}{4m^2}}\right)^{|d|} \qquad (4)$$

En particulier la constante λ – qui reste à calculer – est égale à $\varphi(0)$, ce qui démontre immédiatement que le coefficient de corrélation entre fréquences de sites distants de d, coefficient fourni d'après para. III (2) par $\varphi(d)/\varphi(0)$, se réduit à $b_1{}^{|d|}$: il décroit (à partir de 1) en fonction exponentielle de la distance absolue $|d|$.

$\lambda = \varphi(0)$ se calcule en écrivant pour $d = 0$ l'équation (3) qui est alors non homogène:

$$k\varphi(0) = \frac{1-\varphi(0)}{4N} + 2m(b_1 - 1)\varphi(0)$$

$$\varphi(0) = \frac{1}{1 + 4N\sqrt{4mk + k^2}} \qquad (5)$$

et les formules (1) ou (1′) de II permettent d'en déduire la variance de la fréquence locale q_{nx}. Lorsqu'il n'y a pas de migration ($m = 0$) on déduit de (5) la formule de Sewall Wright:

$$\varphi(0) = \frac{1}{1 + 4Nk} \qquad (5')$$

Nous allons maintenant constater la grande diversité des conséquences numériques suivant les valeurs de m (migration depuis chaque groupe adjacent), de k (qui tient compte des mutations, de la sélection constante, et, comme l'a souligné Morton, de la 'migration à grande distance' regardée comme provenant d'un vaste réservoir de population d'effectif pratiquement infini); la formule (5) donne le coefficient de parenté local (intragroupe) duquel se déduit (para. II, 1′) la variance (intergroupe) $\sigma^2{}_x$ en multipliant par $\bar{q}_x(1-\bar{q}_x)$; et le calcul de $b_1{}^{|d|}$ nous donnera la décroissance de la parenté $\varphi(d)$ et de la corrélation $v_{xy}/v_{xx} = \varphi(d)/\varphi(0)$ en fonction de la distance $y-x = d$. Les 3 applications numériques qui suivent correspondent à 3 cas notablement distincts:

a. Celui où k est très petit par rapport à m: dans ce cas la migration à courte distance m maintient, contre la différenciation locale traduite par k et N, une grande ressemblance entre groupes même non adjacents.

b. Celui où k est de l'ordre de m, comme dans le cas des colonies d'escargots *Cepaea nemoralis*.

c. Celui où m est très petit par rapport à k: l'influence de la migration à courte distance est alors si faible, que le modèle unidimensionnel reste numériquement valable pour un réseau à 2 dimensions (Morton).

(*a*) Prenons m = 100 k. Alors

$$\frac{\varphi(d)}{\varphi(0)} = b_1^{|d|} \# (0{,}9)^{|d|}$$

La décroissance de la corrélation ne devient importante que lorsque |d| est de l'ordre de 10, c'est-à-dire lorsqu'on a franchi une dizaine de groupes intermédiaires.

$$\varphi(0) = \frac{1}{1 + 80Nk}$$

La comparaison avec la formule (5′) qui correspondrait à l'isolement complet ('modèle de l'île'), montre que la variance de chaque groupe par rapport à la moyenne générale (variance intragroupe) est la même que celle d'un groupe isolé dont l'effectif serait 20 fois plus grand: l'importance (par rapport à k) de la migration entre groupes voisins équivaut à une très grande augmentation de l'effectif local.

Remarquons d'ailleurs que l'effectif local N n'intervient (par le produit Nk) que dans la variance locale, et par contre n'influe nullement sur la corrélation entre groupes différents.

(*b*) m = k

$$\frac{\varphi(d)}{\varphi(0)} = \left(1 - \frac{\sqrt{5-1}}{2}\right)^{|d|}$$

La corrélation est réduite de 60% chaque fois que la distance augmente d'une unité: deux groupes distants de 4 unités peuvent déjà être regardés comme fluctuant indépendamment: c'est ce qui se passe chez *Cepaea*.

$$\varphi(0) \# \frac{1}{1 + 9Nk}$$

Une telle migration n'équivaut donc guère plus qu'à un doublement de l'effectif local.

(*c*) 2m = 0,01, k = 0,2 (Morton), donc k = 40 m:

$$\frac{\varphi(d)}{\varphi(0)} = (21 - \sqrt{440})^{|d|}$$
$$\# \left(\tfrac{1}{40}\right)^{|d|}$$

La corrélation entre deux groupes immédiatement adjacents est déjà quasi négligeable; on doit s'attendre à ce que le 'modèle de l'île' (5′) soit sensiblement valable; en fait, on trouve ici

$$\varphi(0) = \frac{1}{1 + 4Nk\sqrt{11/10}}$$

V. LE CAS 'BIDIMENSIONNEL' DE MIGRATION

Le cas 'bidimensionnel' de migration, dans le plan, sur les sommets d'un réseau rectangulaire, peut être également traité jusqu'au bout lorsqu'il y a seulement migration entre sommets adjacents: si l'on repère un sommet par ses coordonnées x_1 et x_2 (supposées entières grâce à un choix convenable des unités), il est possible de calculer le coefficient de parenté $\varphi(d_1, d_2)$ entre le site de coordonnées x_1, x_2 et le site de coordonnées $z_1 = x_1 + d_1$, $z_2 = x_2 + d_2$, en passant par l'intermédiaire de la 'fonction génératrice' $K(\alpha_1, \alpha_2) = \sum_{d_1} \sum_{d_2} \alpha_1^{d_1} \alpha_2^{d_2} \varphi(d_1, d_2)$, fonction qui existe si α_1 et α_2 sont des nombres complexes de module 1. La loi de migration est définie elle-même par la probabilité $l(d_1, d_2)$, pour qu'un gamète utilisé dans le site x_1, x_2 provienne du site $x_1 + d_1, x_2 + d_2$, et nous supposons que $l(\pm 1, 0) = m$, $l(0, \pm 1) = m'$, $l(0, 0) = 1 - 2m - 2m'$.

La fonction génératrice correspondante est alors

$$L(\alpha_1, \alpha_2) = 1 + m(\alpha_1 + 1/\alpha_1 - 2) + m'(\alpha_2 + 1/\alpha_2 - 2)$$

et il est aisé d'obtenir la formule:

$$[1 - (1 - k)^2 L(\alpha_1, \alpha_2) L(1/\alpha_1, 1/\alpha_2)] K(\alpha_1, \alpha_2) = \\ = (1 - k)^2 \frac{1 - \varphi(0, 0)}{2N} L(\alpha_1, \alpha_2) L(1/\alpha_1, 1/\alpha_2)$$

dont la 'formule d'inversion de Fourier' permit de tirer:

$$\varphi(d_1, d_2) = \frac{1}{(2\pi i)^2} \int_{C_1} \int_{C_2} \alpha_1^{d_1 - 1} \alpha_2^{d_2 - 1} K(\alpha_1, \alpha_2) \, d\alpha_1 d\alpha_2 \qquad (1)$$

C_1 et C_2 désignant, dans le plan complexe, les cercles $|\alpha_1| = 1$ et $|\alpha_2| = 1$.

Or la première des intégrations, par rapport à α_1 par exemple, peut être effectuée par la méthode des résidus; car

$$\alpha_1^{d_1 - 1} K(\alpha_1, \alpha_2) = \frac{1 - \varphi(0)}{2N} \left[- \alpha_1^{d_1 - 1} + \frac{1 + k_1}{2} \frac{\alpha_1^{d_1 - 1}}{1 + k_1 - L} + \frac{1 + k_1}{2} \frac{\alpha_1^{d_1 - 1}}{1 + k_1 + L} \right] \qquad (2)$$

(en posant, pour simplifier, $1/1 - k = 1 + k_1$).

Or $\dfrac{\alpha_1^{d_1 - 1}}{1 + k_1 - L}$

peut s'écrir

$$\frac{\alpha_1^{d_1}}{- m\alpha_1^2 + [k_1 + 2m - G(\alpha_2)] \alpha_1 - m}$$

en posant $G(\alpha_2) = m'(\alpha_2 - 2 + 1/\alpha_2)$ et la dernière fraction se transforme de façon analogue.

Si nous désignons par p_1 le pôle de module < 1 de la première fraction, par r_1 son résidu (et par p_2 et r_2 le pôle et le résidu de la dernière fraction), nous obtenons (sauf dans le cas $d_1 = d_2 = 0$ où il faut retrancher $[1 - \varphi(0)]/2N$ du second membre):

$$\varphi(d_1, d_2) = \frac{1 - \varphi(0)}{2N} \frac{1 + k_1}{4i\pi} \int_{C_2} \alpha_2^{d_2 - 1} (r_1 + r_2) \, d\alpha_2$$

Il suffit, pratiquement, de faire le calcul dans l'une des deux directions rectangulaires, par exemple dans la direction définie par $d_1 = 0$; en posant alors $d_1 = 0$, $d_2 = p$, et $\varphi(d_1, d_2) = \varphi(p)$,

on est ramené à une somme de deux intégrales elliptiques:

$$\varphi(p) = \frac{1-\varphi(0)}{2N}\frac{1+k_1}{4i\pi}\left[\int_{C_2}\frac{\alpha_2^{p-1}d\alpha_2}{\sqrt{y^2-4m^2}} + \int_{C_2}\frac{\alpha_2^{p-1}d\alpha_2}{\sqrt{z^2-4m^2}}\right] \tag{3}$$

en posant $y = k_1 + 2m - G(\alpha_2)$ et $z = -2 + 2m - k_1 - G(\alpha_2)$.

Il ne reste qu'à calculer chacune des intégrales figurant dans le crochet, intégrales que nous résumerons par la notation:

$$I_p = \int_C \frac{\alpha^{p-1}d\alpha}{\sqrt{[b + c(\alpha + 1/\alpha)]^2 - 4m^2}} \tag{4}$$

L'intégrale possède 4 points critiques $\alpha_1, \alpha_2 = 1/\alpha_1, \alpha_3, \alpha_4 = 1/\alpha_3$, dont 2 seulement, soient α_1 et α_3, sont à l'intérieur du cercle C d'équation $|\alpha| = 1$.

Dans le cas où m, m′, et k_1 sont petits – ce que nous supposerons – la première intégrale du deuxième membre de (3) est grande, alors que la seconde est bornée et donc négligeable par rapport à la première.

Il nous suffit donc de réduire le crochet qui est dans le second membre de (3) au calcul de:

$$I_p = \int_C \frac{\alpha^{p-1}d\alpha}{\sqrt{[k_1 + 2m' - m'(\alpha + 1/\alpha)]\,[k_1 + 4m + 2m' - m'(\alpha + 1/\alpha)]}} \tag{4′}$$

$$I_p = \frac{1}{m'}\int_C \frac{\alpha^p d\alpha}{\sqrt{\left[(\alpha^2+1) - \left(\frac{k_1}{m'} + 2\right)\alpha\right]\left[\alpha^2 + 1 - \left(\frac{k_1}{m'} + 4\frac{m}{m'} + 2\right)\alpha\right]}} =$$

$$= \frac{1}{m'}\int_C \frac{\alpha^p d\alpha}{\sqrt{P_1(\alpha)\,P_2(\alpha)}} = \frac{1}{m'}\int_C \frac{\alpha^p d\alpha}{\sqrt{(\alpha-\alpha_1)(\alpha-\alpha_2)(\alpha-\alpha_3)(\alpha-\alpha_4)}} \tag{4″}$$

qui peut d'ailleurs s'écrire aussi, compte tenu de la symétrie $I_{-p} = I_p$:

$$I_p = \frac{1}{2m'}\int_C \frac{(\alpha^p + \alpha^{-p})\,d\alpha}{\sqrt{P_1(\alpha)\,P_2(\alpha)}} \tag{5}$$

$P_1(\alpha)$ et $P_2(\alpha)$ ont chacun 2 racines inverses, soient respectivement α_1 ; $\alpha_2 = 1/\alpha_1$; α_3 ; $\alpha_4 = 1/\alpha_3$ [$|\alpha_1| < 1, |\alpha_3| < 1$] ce qui conduit à poser $\alpha = (\beta+1)/(\beta-1)$; β décrivant (vers le bas) l'axe des imaginaires lorsque α décrit C (dans le sens direct):

$$I_p = -\frac{1}{m'}\int_{+i\infty}^{-i\infty} \frac{[(\beta+1)^{2p} + (\beta-1)^{2p}]/(\beta^2-1)^p}{(\beta-1)^2\sqrt{P_1\left(\frac{\beta+1}{\beta-1}\right)P_2\left(\frac{\beta+1}{\beta-1}\right)}}\,d\beta \tag{6}$$

En revenant aux notations de la formule (4), l'intégrand peut aussi s'écrire comme le produit par 2m′ de:

$$\frac{[(\beta+1)/(\beta-1)]^p\,d\beta}{\sqrt{(2c+b+2m)\beta^2 - (b+2m-2c)}\sqrt{(2c+b-2m)\beta^2 - (b-2m-2c)}} \tag{6′}$$

Les points critiques sont alors

$$\pm \beta_1 = \pm \sqrt{\frac{b + 2m - 2c}{b + 2m + 2c}}$$

et

$$\pm \beta_2 = \pm \sqrt{\frac{b - 2m - 2c}{b - 2m + 2c}}$$

et l'on peut écrire:

$$I_p = \frac{4}{\sqrt{(2c + b)^2 - 4m^2}} \int_{\beta_1}^{\beta_2} \frac{\left(\frac{\beta - 1}{\beta + 1}\right)^{-p} d\beta}{\sqrt{(\beta^2 - \beta_1^2)(\beta^2 - \beta_2^2)}}$$

La symétrie, aisément vérifiée, $I_{-p} = I_p$, permet d'ailleurs de 'symétriser' le numérateur en

$$\frac{1}{2}\left[\left(\frac{\beta - 1}{\beta + 1}\right)^{-p} + \left(\frac{\beta - 1}{\beta + 1}\right)^{p}\right]$$

Calcul de I_0: Posons $\beta/\beta_1 = \lambda$ et $\beta_1/\beta_2 = \mu$. On a:

$$I_0 = \frac{4}{\sqrt{(b + 2m + 2c)(b - 2m - 2c)}} \int_1^{1/\mu} \frac{d\lambda}{\sqrt{(1 - \lambda^2)(1 - \mu^2\lambda^2)}}$$

et l'intégrale n'est autre que la 'période imaginaire' de la fonction elliptique sn, d'où

$$I_0 = \frac{4i}{\sqrt{(k_1 + 4m)(k_1 + 4m')}} K(\alpha)^*$$

(en se souvenant que $b = k_1 + 2m + 2m'$ et $c = -m'$)

Calcul de I_1:

$$I_1 = -\int_{+i\infty}^{-i\infty} \frac{\left[\frac{\beta + 1}{\beta - 1} + \frac{\beta - 1}{\beta + 1}\right] d\beta}{\sqrt{(2c + b)^2 - 4m^2}\sqrt{(\beta^2 - \beta_1^2)(\beta^2 - \beta_2^2)}}$$

On se ramène à une variable réelle en posant $\beta = i\beta_1 t$:

$$I_1 = i \int_{-\infty}^{+\infty} \frac{2(\beta_1^2 t^2 - 1)/(\beta_1^2 t^2 + 1)}{\beta_2 \sqrt{(2c + b)^2 - 4m^2}\sqrt{(1 + t^2)(1 + \mu^2 t^2)}} dt$$

ou, en posant $t = \operatorname{tg} \varphi$:

$$I_1 = \frac{4i}{\beta_2 \sqrt{(2c + b)^2 - 4m^2}} \int_0^{\pi/2} \frac{\beta_1^2 \operatorname{tg}^2 \varphi - 1}{\beta_1^2 \operatorname{tg}^2 \varphi + 1} \sqrt{\frac{1 + \operatorname{tg}^2 \varphi}{1 + \mu^2 \operatorname{tg}^2 \varphi}} d\varphi$$

$$I_1 = \frac{4i}{\beta_2 \sqrt{4mk_1 + k_1^2}} \int_0^{\pi/2} \frac{(\beta_1^2 + 1)\sin^2 \varphi - 1}{(\beta_1^2 - 1)\sin^2 \varphi + 1} \frac{d\varphi}{\sqrt{1 - (1 - \mu^2)\sin^2 \varphi}}$$

* Les notations employées sont celles d'Abramowicz et Stegun (1966), en posant $1 - \mu^2 = \sin^2 \alpha$.

En posant $1-\mu^2=\sin^2\alpha$, on peut introduire l'intégrale de 3ème espèce

$$\int_0^{\pi/2} \frac{1}{1-(1-\beta_1^2)\sin^2\varphi} \frac{d\varphi}{\sqrt{1-\sin^2\alpha\sin^2\varphi}} = \pi\left[(1-\beta_1^2)\backslash\alpha\right]$$

D'ailleurs $\pi(0\backslash\alpha)=K(\alpha)$ et

$$I_0 = \frac{4i}{\beta_2\sqrt{4mk_1+k_1^2}} K(\alpha)$$

$$I_1 = \frac{\beta_1^2+1}{\beta_1^2-1} I_0 + \frac{8i\beta_1^2}{(1-\beta_1^2)\beta_2\sqrt{4mk_1+k_1^2}} \pi\left[(1-\beta_1^2)\backslash\alpha\right]$$

Le calcul du rapport I_1/I_0, qui d'après (3) est aussi le coefficient de corrélation $\varphi(1)/\varphi(0)$ entre groupes adjacents, nécessite des transformations sur l'intégrale de 3ème espèce, car $1-\beta_1{}^2=n$ est ici négatif; il faut (Abramowicz et Stegun, 1966, p. 600) poser

$$N = \frac{\sin^2\alpha - n}{1-n} = 1 - \frac{\mu^2}{\beta_1^2} = 1 - \beta_2^{-2}$$

et l'on a alors

$$\pi\left[(1-\beta_1^2)\backslash\alpha\right] = \frac{(\beta_1^2-1)\mu^2}{\beta_1^2(\beta_1^2-\mu^2)} \pi(N\backslash\alpha) + \frac{1-\mu^2}{\beta_1^2-\mu^2} K(\alpha)$$

D'ailleurs, comme N est compris entre $\sin^2\alpha$ et 1, on a:

$$\pi(N\backslash\alpha) = K(\alpha) + \tfrac{1}{2}\pi\delta_2\left[1 - \Lambda_0(\varepsilon\backslash\alpha)\right]$$

d'où:

$$\pi\left[(1-\beta_1^2)\backslash\alpha\right] = \frac{1}{\beta_1^2} K(\alpha) + \frac{\beta_1^2-1}{\beta_2^2(\beta_1^2-\mu^2)} \frac{\pi}{2} \delta_2\left[1 - \Lambda_0(\varepsilon\backslash\alpha)\right]$$

D'où, par transformation de la formule donnant I_1:

$$I_1 = I_0 + \frac{8i\beta_1^2}{(1-\beta_1^2)\beta_2\sqrt{4mk_1+k_1^2\beta_2^2}} \frac{\beta_1^2-1}{(\beta_1^2-\mu^2)} \frac{\pi}{2} \delta_2\left[1 - \Lambda_0(\varepsilon\backslash\alpha)\right]$$

avec

$$\delta_2 = \sqrt{\frac{N}{(1-N)(N-\sin^2\alpha)}} = \sqrt{\frac{\beta_2^2-1}{\mu^2-\beta_2^{-2}}} = \beta_2\sqrt{\frac{\beta_2^2-1}{\beta_1^2-1}}$$

et

$$\varepsilon = \operatorname{Arc}\sin\sqrt{\frac{1-N}{1-\sin^2\alpha}} = \operatorname{Arc}\sin\frac{1}{\beta_1}$$

Les tables permettent alors de comparer la corrélation I_1/I_0 à la valeur

$$1 + \frac{k}{2m} - \sqrt{\frac{k}{m} + \frac{k^2}{4m^2}}$$

obtenue précédemment, en para. IV, dans le cas unidimensionnel.

VI. DÉCROISSANCE ASYMPTOTIQUE DE LA CORRÉLATION

La récurrence entre les intégrales I_p, pour les grandes valeurs de p, peut s'obtenir en récrivant V (4) sous la forme:

$$I_p = \int_C \frac{\alpha^p \, d\alpha}{\sqrt{p(\alpha)}}$$

$P(\alpha)$ étant un polynôme du 4e degré $P(\alpha)=[b\alpha+c(\alpha^2+1)]^2-4m^2\alpha^2$ dont 2 racines seulement, celles que nous avons appelées α_1 et α_3, sont à l'intérieur du cercle C (les autres en étant les inverses); il en résulte que

$$\int_C \frac{d(\alpha^p \sqrt{P})}{d\alpha} d\alpha$$

est nul, ce qui fournit la récurrence homogène:

$$\left[p\,P(E) + \frac{E}{2}\frac{dP}{dE}\right] I_{p-1} = 0$$

E étant l''opérateur translation' tel que $E\,I_{p-1}=I_p$. Ce qui donne (en diminuant p d'une unité, puis divisant par p):

$$c^2(1+1/p)\,I_{p+2} + 2bc(1+1/2p)\,I_{p+1} + (b^2+2c^2-4m^2)\,I_p + \\ + 2bc(1-1/2p)\,I_{p-1} + c^2(1-1/p)\,I_{p-2} = 0$$

Cette récurrence n'est pas à coefficients constants, mais se ramène sensiblement, pour p assez grand, à une récurrence à coefficients constants en posant $\sqrt{p}I_p=J_p$.

Ce qui donne:

$$[1+0(p^{-3/2})]\sqrt{p}(1+h/p)\,I_{p+2h} = J_{p+2h}$$
$$c^2J_{p+2} + 2bcJ_{p+1} + (b^2+2c^2-4m^2)\,J_p + 2bcJ_{p-1} + c^2J_{p-2} = 0 \qquad (7)$$

avec une erreur relative de l'ordre de $p^{-3/2}$.

La solution générale de la récurrence (7) est une combinaison linéaire de 4 exponentielles 2 à 2 inverses; mais, comme I_p et J_p restent bornées quand $|p|\to+\infty$, il suffit, quand $p>0$, de considérer les 2 exponentielles dont les bases sont de modules <1. La comparaison de (7) avec le polynôme $P(\alpha)$ montre que ces bases sont précisément les racines α_1 et α_3 de modules <1 de ce polynôme, lesquelles vérifient respectivement les équations:

$$\alpha_1^2 + \frac{b-2m}{c}\alpha_1 + 1 = 0 \quad \text{et} \quad \alpha_3^2 + \frac{b+2m}{c}\alpha_3 + 1 = 0$$

$$\alpha_1 = -\frac{b-2m}{2c} - \sqrt{\left(\frac{b-2m}{2c}\right)^2 - 1}; \qquad \alpha_3 = -\frac{b+2m}{2c} - \sqrt{\left(\frac{b+2m}{2c}\right)^2 - 1}$$

Pour la première intégrale figurant dans V (3), il faut prendre

$$b + c(\alpha + 1/\alpha) = y = k_1 + 2m + 2m' - m'(\alpha + 1/\alpha)$$

d'où:

$$\alpha_1 = 1 + \frac{k_1}{2m'} - \sqrt{\frac{k_1}{m'} + \frac{k_1^2}{4m'^2}}$$
$$\alpha_3 = 1 + \frac{k_1 + 4m}{2m'} - \sqrt{\frac{k_1 + 4m}{m'} + \frac{(k_1 + 4m)^2}{4m'^2}} \tag{8}$$

Dans le cas général, la demi-somme $1+(k_1/2m')$ de α_1 et $1/\alpha_1$ est beaucoup plus voisine de 1 que la demi-somme $1+(k_1/2m')+2(m/m')$ de α_3 et $1/\alpha_3$; on obtient, comme expression asymptotique de la première intégrale figurant dans V(3):

$$I_p = \frac{1}{\sqrt{p}} J_p \sim \frac{cte}{\sqrt{p}} \alpha_1^p \ (p > 0) \tag{9}$$

Alors que, pour la deuxième intégrale figurant dans V(3), on prend:

$$b + c(\alpha + 1/\alpha) = z = -2 - k_1 + 2m + 2m' - m'(\alpha_2 + 1/\alpha_2)$$

$$\alpha_1' = \frac{-2 - k_1 + 2m'}{2m'} + \sqrt{\left(1 - \frac{2 + k_1}{2m'}\right)^2 - 1}$$

$$\alpha_3' = \frac{-2 - k_1 + 4m + 2m'}{2m'} + \sqrt{\left(1 - \frac{2 + k_1 - 4m}{2m'}\right)^2 - 1}$$

On vérifie que les modules de ces racines sont beaucoup plus éloignés de l'unité que les précédents, car leurs demi-sommes avec leurs inverses sont de bien plus grandes valeurs absolues.

Les formules (3), (8) et (9) donnent donc, pour p grand:

$$\varphi(0, p) \sim \frac{cte}{\sqrt{p}} \left[1 + \frac{k_1}{2m'} - \sqrt{\frac{k_1}{m'} + \frac{k_1^2}{4m'^2}}\right]^p$$

mais le facteur $1/\sqrt{p}$ n'intervient pas asymptotiquement dans le rapport $\varphi(0, p+d)/\varphi(0, p)$, qui a une valeur équivalente à celle fournie par $\varphi(p+d)/\varphi(d)$ dans le cas unidimensionnel [IV, formule (4)].

Par ailleurs, pour les petites valeurs de p, cette formule asymptotique peut devenir très inexacte, comme le montre la comparaison numérique des intégrales elliptiques I_0 et I_1 définies au V.

En particulier, lorsque $m=m'$ est petit par rapport à k_1, il est possible (Malécot, 1971) d'approcher la formule V(4″) en y remplaçant α-α_2 et α-α_4 (qui sont alors grands) par des constantes, et l'on trouve alors:

$$\frac{\varphi(d)}{\varphi(0)} \sim \left(\frac{m}{k_1}\right)^d$$

ce qui définit une décroissance simplement exponentielle, dont on vérifie aisément qu'elle est numériquement la même que dans le cas unidimensionnel.

Ceci explique pourquoi (comme Morton l'a souligné) il est rare que la bidimensionalité apparaisse dans les ajustements statistiques.

RENVOIS

1. La parenté locale entre deux individus pris au hasard dépend essentiellement de la struc-

ture du réservoir gamétique local, mais fort peu de la manière dont les gamètes s'unissent 2 à 2, comme l'a montré Morton (1971) à propos des mariages croisés entre clans.

2. C_x est une 'fréquence d'équilibre', qui dépend du site x si la sélection en dépend; k est un coefficient de rappel vers l'équilibre qui, s'il n'y a pas de sélection, se réduit à la somme $u+v$ du taux de mutation u de a vers A et du taux de mutation v de A vers a (C_x est alors égal à $v/(u+v)$).

3. Si l'on désigne par k la probabilité de survenue, sur un chaînon de descendance, d'une telle 'betamutation', la probabilité $k\,C_x$ pour qu'elle aboutisse au gène a, doit être égale à v (taux de mutation réel observé vers a depuis un gène différent), d'où $k=v/C_x$: d'après la note (2), k est donc égal à $u+v$ s'il n'y a pas sélection; et, s'il y a sélection linéarisée, k est le coefficient de rappel figurant dans la formule (4) de para. I(3) (*a*), car, si Γ_j descend d'un gamète Γ' de F_{n-1} dont l'indicatrice est X', l'espérance, conditionnée par X', de X_j est $(1-k)X'+k\,C_x$; cette valeur est aussi la probabilité conditionnée pour que $X_j=1$ c'est-à-dire pour que Γ_j porte le gène a, ce qui est en accord avec les formules (1) et (4) de para. I (*cf.* Malécot, 1948, 1969*a*).

4. La probabilité k de betamutation est susceptible d'une autre interprétation, plus conforme à la biologie moléculaire et au multiallélisme qu'elle introduit; au lieu de déterminer k (lorsqu'il n'y a pas de sélection) comme somme de 2 taux de mutation observés, u de a vers A et v vers a (ce qui suppose que les mutations soient récurrentes) on peut plutôt regarder k comme la probabilité de survenue, dans l'un quelconque des nucléotides du gène considéré, d'une mutation biochimique quelconque, transformant en général a en un gène nouveau (comme l'a fait remarquer Kimura (1968)): la survenue d'une telle mutation assure donc pratiquement l'indépendance entre le gène nouveau et l'ancien; le calcul de Kimura (1968) permet de vérifier que k est bien alors la somme des probabilités des mutations d'un gène *vers* tous les allèles possibles; il peut d'ailleurs y avoir lieu de mesurer, comme le suggère Kimura (1968) la parenté, non pas sur des caractères phénotypiques, mais sur le pourcentage de substitutions d'amino-acides dans une chaîne protéinique: cette dernière parenté doit être plus grande, le taux de mutation par amino-acide étant plus faible que le taux de mutation par gène.

5. Si les gamètes utiles Γ_j et Γ_l sont ceux qui fusionnent pour donner naissance à un diploïde, leur probabilité d'identité, qui est le 'coefficient de consanguinité' F de ce diploïde peut être différente de φ (si les gamètes qui s'unissent ont une corrélation conditionnée non nulle); nous ne tiendrons pas compte de cette différence dans le calcul de la variance (la différence entre F et φ est étudiée dans Wright (1965) et Malécot (1969*b*); elle équivaut à une modification de l'effectif N, comme nous l'avons expliqué dans l'introduction).

6. Le coefficient de parenté φ défini en para. II à l'intérieur du site x devra donc dorénavant être noté φ_{xx}.

BIBLIOGRAPHIE

ABRAMOWICZ, M. et STEGUN, I. (1966): *Handbook of Mathematical Functions*, 1046 p. National Bureau of Standards, Washington, D.C. (U.S. Government Printing Office).

GILLOIS, M. (1964): *La Relation d'Identité en Génétique*. Thèse, Faculté des Sciences, Paris.

IMAIZUMI, Y., MORTON, N. et HARRIS, E. (1970): Isolation by distance in artificial populations. *Genetics*, *66*, 569.

KIMURA, M. (1964): The stepping stone model of population structure. *Genetics*, *49*, 561.

MALÉCOT, G. (1948): *Les Mathématiques de l'Hérédité*, 60 p. Masson et Cie, Paris.

MALÉCOT, G. (1950): Quelques schémas probabilistes sur la variabilité des populations naturelles. *Ann. Univ. Lyon, Sci. A*, *13*, 37.

MALÉCOT, G. (1969*a*): *The Mathematics of Heredity*, 86 p. W. H. Freeman and Co., San Francisco, Calif.

MALÉCOT, G. (1969*b*): Consanguinité panmictique et consanguinité systématique. *Ann. Génét. et Sélect. anim.*, *1/3*, 237.

MALÉCOT, G. (1971): Etude du coefficient de parenté. *Ann. Génét. et Sélect. anim.*, sous presse.
MORTON, N. (1968): Population structure of ABO groups in Switzerland. *Amer. J. hum. Genet.*, *20*, 420.
MORTON, N. (1971): Clans as genetic barriers. *Amer. Anthrop.*, *73*, 1005.
NEI (1971): Communication au 4ème Congrès International de Génétique Humaine.
ROSIN, S. (1956): Die Verteilung der ABO Blutgruppen in der Schweiz. *Arch. Klaus-Stift. Vererbungsforsch.*, *31*, 1.
WRIGHT, S. (1931): Evolution in mendelian populations. *Genetics*, *16*, 97.
WRIGHT, S. (1965): Interpretation of population structure by F-statistics. *Evolution*, *19*, 395.

James F. Crow

Genetics Laboratory, University of Wisconsin, Madison, Wis., U. S. A.

Some effects of relaxed selection and mutation*

I should like to discuss three aspects of human population genetics. The first of these is the demographic framework, the pattern of birth and death rates, within which natural selection operates. Secondly, I shall mention briefly some of the consequences of a relaxation of natural selection, in particular the magnification of the relative importance of mutation. Thirdy, I shall discuss the possible impact of the mutation load on the population, relying partll on Drosophila data.

THE OPPORTUNITY FOR SELECTION IN A POPULATION

It is not possible from census records alone to infer what is happening genetically. But it is within this framework that genetic changes occur, so I look first at demographic data.

Inherent in any schedule of birth and death rates is a certain maximum capacity for genetic selection. Every animal breeder is painfully aware that intrinsic biological properties of the species limit his ability to select for desirable traits. This is especially important if he tries to select for several traits simultaneously. He finds that progress is disappointingly slow. He can improve the efficiency, provided he knows what he wishes to attain, by using a selection index that assigns optimal weight to each trait or measurement, or by special techniques such as artificial insemination.

The amount of selection going on in a human population at any time is limited by the amount of *differential* viability and fertility in the population. Of course, the actual amount of genetically effective selection will be less than this limit and perhaps very much less because of environmental differences, simultaneous selection in different directions, balanced polymorphisms, and other causes of low heritability.

I have suggested previously (Crow, 1958, 1961) an *Index of Opportunity for Selection.* It is essentially the discrete generation analog of Fisher's (1930) fundamental theorem of natural selection. It measures the proportion by which fitness would increase with a specified schedule of birth and death rates if these were all selective and the (narrow sense) heritability of fitness were complete. It also tells how much change in some other character would occur if all the selection were concentrated on that trait.

Of course, such an index can tell us nothing that is not already contained in the vital statistics. It is descriptive, not analytical. In particular, without additional genetic information it can only set an upper limit on the amount of genetic change that could occur within this

* Paper Number 1511 from the Genetics Laboratory, supported in part by the National Institutes of Health (GM15422).

system of vital statistics. Nevertheless, it describes the population by the same measure that selection utilizes, *i.e.* the variance. Also it can be separated into components associated with differences in viability or fertility, or to differences at various ages in the life cycle.

Basically, the index is the variance in number of descendants, counted at the same age as the parents, divided by the square of the mean number of descendants. It is invariant with respect to the total rate of growth of the population, depending only on the relative fitnesses of the different individuals in the population.

The *Index of Opportunity for Selection* is defined as:

$$I = I_m + \frac{1}{S} I_f$$

where

$$I_m = \frac{1-S}{S}$$

and

$$I_f = \frac{V_x}{\bar{x}^2}.$$

The symbols are as follows:

I_m is the index due to mortality,
I_f is the index due to fertility differences,
S is the proportion surviving through the reproductive period,
V_x and $\bar{x}$ are the variance and mean of the number of progeny per adult.

This index could easily be refined by taking into account such factors as deaths during the reproductive period, the age of the parent at the time of reproduction, and the continuous nature of the change in most populations. For the present purposes this hardly seems necessary.

There are two important limitations of most census data. One is that the data are usually given only as children per female; any departure from strict monogamy introduces uncertainty and errors. The second is that it is difficult to correct for persons who die during the reproductive period, since mortality data and data on the size of completed families are usually in separate tables.

TABLE 1 *The effect of demographic differences in three contemporary Chilean populations on the index of opportunity for selection*

	Nomads	Village	Town
Mean number of children, $\bar{x}$	6.1	5.9	4.3
Variance in number of children, V_x	6.4	7.5	8.5
Proportion surviving, S	0.42	0.75	0.87
Mortality index, I_m	1.38	0.33	0.15
Fertility index, I_f	0.17	0.22	0.45

Table 1 gives data from a Chilean study by Cruz-Coke *et al.* (1961). They illustrate the striking changes in the indices as we compare nomadic, village, and town populations. In general, the index due to mortality decreases sharply as the living standard increases in the villages and still more in the cities. On the other hand, the index due to fertility differences increases with the higher living standard. Despite a reduction in mean number of children from 6.1 to 4.3, the index *increased* from 0.17 to 0.45. Somehow the reduction in average number of children is accompanied by an increase in the variance so that the index rises.

TABLE 2 *Mean and variance of the number of children ever born to women age 45–49 in designated years, and the fertility index of opportunity for selection (source: U.S. Census)*

Year when age 45–49	Year when age 25–29	Mean number of children, $\bar{x}$	Variance in number of children, V_x	Index for fertility
1925	1905	3.06	10.60	0.82
1930	1910	3.36	9.68	0.86
1935	1915	3.18	8.18	0.87
1940	1920	2.95	7.77	0.90
1945	1925	2.66	6.86	0.97
1950	1930	2.41	6.00	1.03
1955	1935	2.26	5.35	1.04
1960	1940	2.31	4.95	0.93
1965	1945	2.55	4.79	0.74
1969	1949	2.82	4.77	0.56
1974	1954	3.01	4.87*	0.54*
1979	1959	3.12*	4.30**	0.44**

* Some of these families are not yet complete, so the figures may be slight underestimates.

** These parameters may be appreciably underestimated since large families, which include children born late in the life of the mother, contribute disproportionately to the variance.

Miss Linda Weimer of the University of Wisconsin has been examining U.S. Census reports to ascertain some of the recent trends in the fertility index. These values are shown in Table 2. As can be seen, the index rose to a maximum for the cohort that was age 25–29 in 1930 to 1935. This corresponds to a period of low birth rate. As with the Chilean data, a reduction of fertility was accompanied by a rise in the index. This is even more striking if we compare the U.S. population born in 1839 ($\bar{x} = 5.5$, $I_f = 0.23$) or non-contraceptive-practising current populations, such as rural Quebec ($\bar{x} = 9.9$, $I_f = 0.20$).

Table 3 shows the Malthusian parameter (Fisher, 1930) for the age-specific birth and death rates for selected recent years in the United States (Crow and Kimura, 1970, p. 19). (This measure includes deaths as well as births, but the death rate changes have been relatively small and uniform so the shape of the curve is determined mainly by birth rates.) The table shows that the cohorts now reproducing have a considerably lower birth rate than those a few years ago. We can therefore expect $\bar{x}$ to go down as these women reach the 45–49 age period and are recorded in completed family studies.

TABLE 3 *The Malthusian parameter for the United States population corresponding to age-specific birth and death rates at selected years*

Year	Malthusian parameter
1940	0.001
1945	0.004
1950	0.014
1955	0.020
1960	0.021
1965	0.012
1966	0.010
1967	0.007

As mentioned before, there has been a negative association between $\bar{x}$ and V_f in the past. However, there are signs that it may now be different in the United States. Notice in Table 2 that the cohort of women age 25–29 in 1954 has a birth rate, 3.01, equivalent to that between 1915 and 1920. Yet the index was about 70% higher at the earlier time. The data so far available for current populations indicate that this trend is continuing, but extrapolation from incomplete families is particularly uncertain for the fertility index. This is because the variance is strongly influenced by very large sibships and the largest sibships occur with women who continue reproduction to later ages; a woman can hardly have her 15th child at a young age.

In very recent years the birth rate in the United States has fallen precipitously. Probably this is associated with a low variance, and therefore with a low index of opportunity for selection, but one cannot be sure until the families are completed, which will be some time in the future for women now in the early reproductive ages.

The index can be extended to include deaths at more than one period. For example, we may wish to compare the opportunity for selection during pre- and postnatal periods. The extended index is:

$$I = I_{me} + \frac{1}{S_e} I_{m1} + \frac{1}{S_e S_1} I_f$$

where the subscripts me and ml designate early and late mortality indices and S_e and S_1 are the survival probabilities for these periods. The equation may also be written in the form,

$$1 + I = (1 + I_{me})(1 + I_{m1})(1 + I_f),$$

which more clearly reveals the multiplicative, rather than additive, nature of the index components.

Johnston and Kensinger (1971) have applied this to data on a small population of Cashinahua Indians in Peru. The measured rate of spontaneous abortion was about 26%. Table 4 gives the index and its components for this population and for a recent U.S. population, using the same estimate for abortion rate.

TABLE 4 *Mortality and fertility indices for two contrasting populations*

	Prenatal mortality I_{me}	Postnatal mortality I_{ml}	Fertility I_f	Total I
Cashinahua	0.26	0.79	0.11	1.50
U. S. women, age 45–49 in 1965	(0.26)	0.09	0.74	1.38

The data show that the roles of postnatal mortality and fertility are almost exactly reversed in these two populations. They also show that the index for prenatal mortality is intermediate. The mean number of children per woman is more than five in the Cashinahua, but the uniformity of this number from family to family means that there is little differential contribution from fertility differences.

Although these demographic details are interesting in their own right, our main concern as geneticists is the effect of any such trends on the genetic composition of the population. It is likely that the pattern for prenatal death has changed the least in the recent past. Making reasonable allowances for early losses that are not detected, perhaps 30% of zygotes die prenatally. The genetic component in these must be quite high; for deaths caused by detectable chromosome rearrangements are a substantial fraction of the total, and these cannot be all

the kinds of genetic defects that lead to embryonic death. So I think it is safe to conclude that considerable genetic selection takes place through embryonic death.

The role of genetic factors in determining fertility differences is much less certain. There are known genetic conditions that cause sterility or reduced fertility, but these account for only a minute fraction of the total variability in family size. Although there are undoubtedly genetic factors that influence family size, these have increasingly more to do with things that determine social and economic status or the desire to have children than with biological fertility. Despite the fact that the fertility index has stayed relatively high, and is actually higher than it was a century ago, there is no reason to think that it reflects a greater degree of genetic selection.

Postnatal, prereproductive death rates are now very low in many countries. The opportunity for selection through this mechanism is far less than in the past and there is every reason to think that this represents a real decline in the intensity of genetic selection.

SOME CONSEQUENCES OF RELAXED SELECTION

If, as the data given above suggest, postnatal death selection is greatly reduced and if fertility differences are not based on biologically important factors (although they may be of very great social importance), then natural selection is considerably reduced in many human societies. I should like to discuss briefly some of the more obvious consequences.

Consider first the consequence of successful treatment of a disease. If the disease is caused by a rare dominant gene that previously impaired survival and fertility by a fraction s and if, as a result of the treatment, the impairment is only t, then the frequency of inherited mutant genes next generation is $(1-t)p$ rather than $(1-s)p$ where p is the frequency this generation. Thus, if there would otherwise have been a frequency p' of mutants, there will now be $p' + (s-t)p = p' + ip$, where i is the improvement in fitness brought about by the treatment.

If p and p' are about the same, then the proportion by which the incidence increases is simply equal to i. In the extreme case of a formerly dominant lethal condition which is completely repaired, $i = 1$ and the incidence is doubled, as expected since the previous incidence was caused entirely by new mutants. Therefore, each generation there is a new increment equal to the mutation rate per zygote (or twice the rate per gamete). For dominant mutants with milder effects or a less complete cure the increase is correspondingly less.

The situation is greatly different when the disease is caused by a recessive gene. The gene frequency added to the next generation as a result of the treatment is ip^2 (making the usual random mating assumption) and the allele frequency next generation is $p' + ip^2$. Again if p and p' are nearly the same, the proportion by which the gene frequency is increased is ip. The proportion of recessive homozygotes is increased by a fraction $[p^2(1+ip)^2 - p^2]/p^2$ or approximately 2ip. For a typical recessive gene that is lethal or sterilizing and with frequency 0.002 a complete repair would enhance the incidence next generation by a fraction of about 0.004. It would require some 175 generations for the incidence to double.

I do not find either of these to be a matter of great immediate concern. With genetic counselling a person with a dominant disease will know of the 50% risk for each child and can make a rational decision based on whether his own life has been miserable or fulfilling. The burden for future generations can be calculated in terms of the cost of the treatment. For the recessive disease the increase is too small to worry about in the foreseeable future. I assume here that our main concern is with the next few generations – not the far distant future.

Along with the possibility of cure or repair has come the possibility of carrier identification. This can lead, perhaps because of genetic counselling, to abstaining from reproduction or, when embryonic detection is possible, to abortion of abnormal fetuses.

The most extreme possibility would be for all persons who are found to be carriers not to reproduce. This is out of the question for more than a few diseases, of course, since each of us

carries a substantial number of such genes. However, let me consider only matings where both parents are heterozygous and where, in consequence, there is an immediate risk of 1/4 for an affected child.

If the parents have no children, then none of the mutant genes is transmitted. If there is complete selection by abortion or death or sterility of the child, half the mutant alleles are eliminated, the other half being transmitted by heterozygous children. Practically the same result occurs if the woman is artificially inseminated with sperm from a non-carrier male. Finally, if there is complete selection but also complete compensation, so that each family has the same number of surviving and reproducing children, 1/3 of the mutant genes are eliminated. Thus, selection is only 2/3 as effective with compensation, and complete abstention from reproduction only doubles the effectiveness. These results are given in Table 5. With rare recessive genes these differences are very small. In particular, humanitarian arguments for therapeutic abortion with severely deleterious conditions must surely outweigh any increase in incidence in later generations through partial compensation.

TABLE 5 *Change in gene frequency in future generations with various effects of genetic counselling*

	Gene frequency		Numerical example p = 0.01	
	After one generation	After n generations	One generation	10 generations
1. Complete cure, or avoidance of marriage between heterozygotes	p	p	0.0100	0.0100
2. Selection by death, sterility or abortion	$\frac{p}{1+p}$	$\frac{p}{1+np}$	0.0099	0.0091
3. Artificial insemination from non-carrier male	$p(1-p)$	$\sim p(1-np)$	0.0099	0.0090
4. No children from marriages between carriers	$\frac{p}{1+2p}$	$\frac{p}{1+np}$	0.0098	0.0083
5. Abortion with compensation	$p(1-0.67p)$	$\sim p(1-0.67np)$	0.0099	0.0093

The recessive gene frequency in adults of the current generation is p (so that the two reproducing genotypes are Aa with frequency 2p and AA with frequency 1-2p). The table gives the change in the absence of other factors, such as mutation.

This subject has recently been reviewed by Motulsky *et al.* (1971) who consider the important fact that most carriers are not detected until they have an affected child.

As embryonic detection is extended to other kinds of diseases it can be very important in reducing the burden of dominant diseases, particularly those that cause a great individual and social burden, but which do not greatly impair fertility. The incidence of the most severe types, whose current frequency depends largely on new mutants, will not be influenced much unless there are ways of doing mass screenings.

The most broadly general statement about reproductive compensation that I know of comes from consideration of within-family *versus* total genetic variance. With random mating

exactly half of the genic (additive genetic) variance is within families. Thus, selection within families, which is what would occur with perfect reproductive compensation, should change the phenotypic average just as fast as if there were zero compensation.

The effect of relaxed selection for more complexly inherited traits depends, of course, on the manner of inheritance of the trait and the manner in which selection is working. Removal of malaria will cause the hemoglobin S gene to decrease; curing anemia while leaving malaria alone would have the opposite effect. For traits such as cystic fibrosis, where the homozygous gene is highly deleterious, but where there is the possibility of a slight balancing selection on the heterozygote, the effect of our present environment is hard to predict. One possibility is that whatever heterozygous selection once operated no longer does. Alternatively, the high incidence in Caucasians may be a drift or founder effect.

One consequence of relaxed selection is highly predictable, however. Although the effects on different genotypes in a system will usually be uneven, the overall effect as selection approaches zero will be to keep all gene frequencies constant. There will be random drift, adjustment of linkage relationships, and effects of migration – all of which will have more or less random phenotypic effects. Increased exogamy and decreased consanguineous mating will have a systematic effect on the phenotype. But the major influence on gene frequencies that will continue unabated as selection ceases is mutation. To whatever extent man is successful in providing environments in which all genotypes survive and reproduce equally, mutation will become relatively more important as a cause of gene frequency change. This is accentuated if the mutation rate is itself enhanced by these environmental influences.

I turn therefore to a discussion of mutation and the mutation load. We can study dominant mutations in man and mice. We can also study chromosome rearrangements. The assessment of the mutational impact of these is relatively accurate. The area of great uncertainty is recessive mutations, in particular those of a small effect, and the extent to which these are expressed in the heterozygous condition. For this, most of the information comes from Drosophila.

MUTATION LOAD STUDIES IN DROSOPHILA

The spontaneous rate of recessive lethal mutations in Drosophila is about 0.015 per gamete per generation. This is based mainly on experiments in which a lethal-free chromosome is kept heterozygous with as little selection as possible for several generations during which mutations accumulate and can later be detected by making the chromosome homozygous.

The same experiments can also reveal the accumulated effect of viability-reducing mutants. The homozygous load caused by such mildly deleterious mutations accumulates at a rate of about 0.004 per generation. This is shown graphically in Figure 1 for 40 generations of accumulation, tested by homozygosing every 10 generations. This is the rate per chromosome; the rate per gamete is about 0.01 per generation.

Figure 1 shows two replicate experiments (Mukai *et al.*, 1972). These show no curvilinearity. However, when data from another experiment lasting more generations are included there is an appreciable downward curvature. Also, a fourth experiment in which the initial viability was lower showed a steeper downward slope. Putting all these together there is evidence for a quadratic component, but not of such a magnitude to be important at the low levels of inbreeding in natural populations of either Drosophila or man.

Let me emphasize that the accumulation of mutations under relaxed selection really occurs. By about 30–40 generations as many mutants have occurred as are normally found in a population—enough to reduce homozygous viability by about 10%. As the lines were continued longer much larger numbers were accumulated until after 60 or more generations the viability was low enough to make it difficult to distinguish between accumulated minor genes and lethals.

Translating this load into an actual mutation rate is uncertain because there is always the

possibility of a large number of mutations with nearly zero effect on viability. Any estimate is therefore necessarily minimal. Also dominant mutants with a large effect on viability are not counted. From the variance among replicate lines the rate of occurrence of mildly deleterious mutants is estimated to be at least 10 times as high as lethals, more likely 20 times, and perhaps many more times as high if there is a large class of very nearly neutral mutants. It is not known whether these mild mutants are at the same loci as lethals or not. I prefer the explanation that they are and that the more drastic impairment produces a lethal effect while the more numerous lesser impairments are only mildly detrimental. This view gains some support from experiments with Salmonella where only a small fraction of mis-sense mutations were detected as conditional lethals, relative to chain terminators (Whitfield *et al.*, 1966).

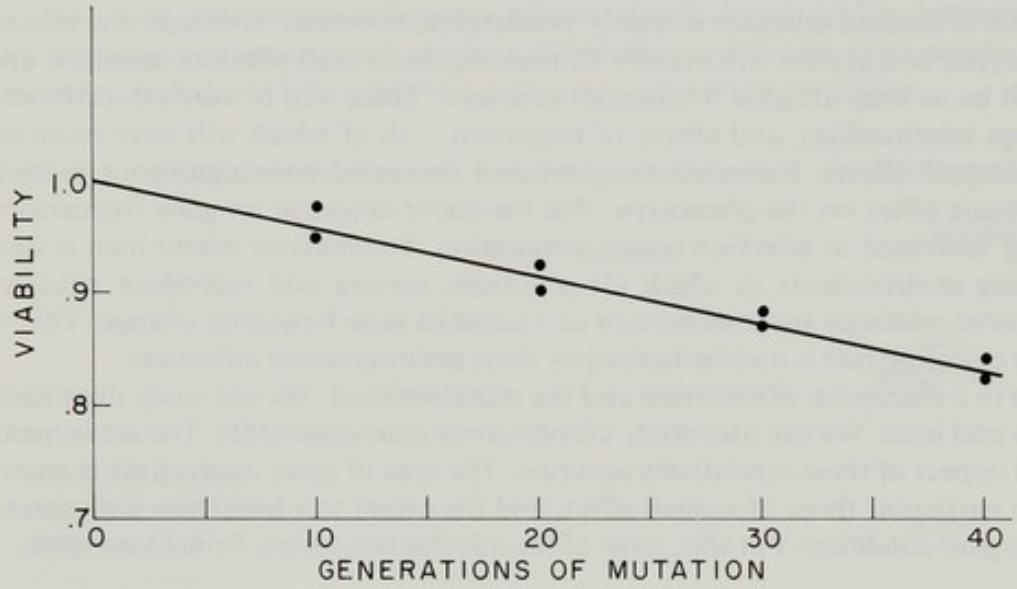


Fig. 1. The relative viability of flies homozygous for a chromosome that had been allowed to accumulate mutations for many generations while being kept heterozygous.

There is good evidence from direct measurements that the average newly arisen recessive lethal has an appreciable heterozygous effect – some 4% viability depression. There is also evidence that those 'recessive' lethals that are found in natural populations also have a deleterious effect in heterozygotes, although only 1 or 2 %. This is to be expected since those with the largest heterozygous effects are the most quickly eliminated and hence are less likely to be found in natural populations. The evidence for heterozygous effects comes partly from direct tests and partly from population analysis (Hiraizumi and Crow, 1960; Crow and Temin, 1964) that the inbred lethal load is largely mutation-maintained and, indeed, the lethal mutation rate can be estimated by the population gene frequency and the measured heterozygous effect.

An exactly analogous procedure has been used by Morton and his associates to estimate the mutation rate for severe recessive genes in man. Neglecting the products of small quantities the equilibrium frequency of a recessive gene is given by

$$q = \frac{u}{s(h+F+q)}$$

where u is the mutation rate, s is the selective disadvantage of the mutant homozygote, h is the heterozygous effect as a fraction of the homozygous effect, and F is the inbreeding coefficient during the time the equilibrium was attained. For Drosophila lethals $z = h+F+q$ averages about 0.015. It has been estimated to be 0.01–0.02 for recessive lethals in man. As an example, cystic fibrosis in orientals has an estimated allele frequency of 0.0033, consistent with mutational maintenance by a mutation rate of $3.3–6.6 \times 10^{-5}$ (Morton, 1969; Wright and Morton, 1968).

In Drosophila the inbred load is easily separated into lethal and detrimental components. The lethal component is about 2–3 times as large. The non-additive component (the 'phenodeviant' load in Morton's usage) of the minor detrimentals is measurable at high levels of inbreeding, but is not an appreciable factor at low F values. The same phenomenon is found in man. The inbred load is separable into major and minor genes by segregation analysis. The major inbreeding component is major genes. There is no significant non-linearity at low doses; in fact there is ordinarily no measurable minor component. The average person is heterozygous for some 2–3 lethal equivalents, the bulk of which are probably monogenic lethals.

The mutation load theory accounts very well for low level inbreeding effects in both Drosophila and man. Most of the effect is from individually rare, highly deleterious genes.

It is much less clear what is happening with mildly selected alleles. It is likely that much, perhaps most, of the variability and load in randomly mating populations is segregational – a possibility that I suggested 20 years ago (Crow, 1948, 1952).

At one time I thought that the mutational and segregational components could be separated by the ratio of the inbred to the random load. In practice this has not worked very well because of weaknesses in the data and difficulties in accounting for all components of fitness.

Morton has used genetic load theory to analyze human inheritance. In combination with segregation analysis to separate low- and high-risk families, he has gotten results that are very similar to those in Drosophila, and mutation rate estimates made this way are in agreement with more direct measures.

Another feature of the Drosophila data, for which no equivalent human information is available, arises from comparison of the mildly detrimental load in newly arising mutants and in a population at equilibrium. The relevant data for chromosome II in Drosophila are shown in Table 6.

TABLE 6 *Homozygous loads in Drosophila for newly arising mutants and for a natural population*

	New mutants per generation	Natural population	Ratio
Mild, D_m	0.004	0.095	24
Lethal, L	0.006	0.247	41
D_m/L	0.67	0.39	

The striking fact is that the ratio of the load in the natural population, presumably somewhere near equilibrium, to that for newly arising mutants is about the same for mild as for lethal mutants (24 *vs* 41). One would expect the mild mutants to persist many times longer and therefore to have a much higher ratio. I can only conclude that the mild mutants are eliminated from the population at rates comparable to or faster than those for lethals. Neither inbreeding nor homozygous elimination is sufficient so there must be substantial selection against these mutants as heterozygotes.

If man is like Drosophila a major share of the expressed mutation load (but not of the inbred load) is in this form. How this can be translated in terms of human welfare can only be guessed. In Drosophila it seems likely that much of the elimination of these mutants is through selective differences in fertility.

In man h is probably considerably higher than q for most highly deleterious recessive mutants. This means that most recessive mutants of this sort never become homozygous in large populations but exert their impact on the population through heterozygous effects. If this is

also true for a much more frequent class of mild mutants, then the typical impact on the population of these and of lethals is much the same.

An enhanced mutation rate should have an effect on fitness of immediately ensuing generations through heterozygous effects of many minor mutants. However, as Newcombe has emphasized at this Congress, heavy radiation in mice has shown almost no measurable effects of any kind on the first few generations (for a review, see Green, 1968). EMS treatment in Drosophila has produced about 5% heterozygous depression of viability per treated chromosome, but the frequency of lethals is high enough that most or all of this can be accounted for by heterozygous effects of lethals. So a strong empirical basis for a large immediate effect of a mutation increase does not exist except in Drosophila, and this is mainly from indirect inference.

Such Drosophila studies would suggest that mutations with mild homozygous effects occur at a very high rate and are quickly eliminated from the population. When and how this occurs I have no idea. Perhaps they are eliminated in large groups through some form of truncation selection, but there is no direct evidence for this.

If the relatively large heterozygous effect is true, then an enhanced mutation rate should show in early generations some effect on fitness components because of heterozygous effects. Yet radiation studies in mice have failed to show this. I join Howard Newcombe in being puzzled about this and regard the effect of minor mutants as the major dilemma in assessing the impact of mutation on human well-being.

HOW MANY GENES ARE THERE?

As mentioned before, the recessive lethal mutation rate in Drosophila is about 0.015 per gamete per generation. What is the corresponding rate per gene? If we consider the total DNA as made up of genes of some 500-1000 nucleotides each this means an average lethal rate of 10^{-7}–10^{-8}. On the other hand observed single locus rates are considerably higher.

Much more reasonable is to estimate the gene number in another way. Judd and his colleagues, studying a small, clearly demarcated region of the X chromosome of Drosophila, found 16 complementing lethal or visible producing loci (Shannon *et al.*, 1970). The region appears to have been saturated, for no new mutant that does not fall into one of the 16 complementation groups has been found for some time. This region also contains exactly 16 salivary chromosome bands, so the temptation to equate the number of chromosome bands, or interband regions, with complementation units (genes?) is overwhelming. Similar data for another chromosomal region are reported by Lifschitz (1971). This places the operational gene number at about 6000 and the lethal mutation rate becomes 2.5×10^{-6}. The rate for mild detrimentals, some 10 or more times higher, then is at least 2×10^{-5}.

If we take the estimate of 1–2 lethal equivalents per human gamete and $z = 0.01$–0.02, the lethal mutation rate is estimated as 0.01–0.04 per gamete. The uncertainties in interpretation are too well known and have been too widely discussed to need any further elaboration by me, but I will note the similarity to the Drosophila rate. If the number of lethal-producing loci is of the order of 10^4 per gamete, then this corresponds to a mutation rate of $1\text{–}4 \times 10^{-6}$ per locus. If there are 10 or more times as many mild mutants per locus, the rate is in the range 10^{-5}–10^{-4}.

If there are only this many genes, what is all the rest of the DNA doing? I join the group who suspect that the bulk of the DNA is non-informational. It is the 'silent majority'. It may be purely structural or mechanical. It may be regulatory. It may have a timing or synaptic function. It may once have been informational but have lost this function after duplication. It may or may not be transcribing, but it does not encode information in its linear sequence.

If one were looking for an intracellular structural material one would like for it to replicate itself in synchrony with the cell division process so as to be present in a constant amount, to

have a cellular mechanism already present for doing this, to have a structure of constant shape and rigidity, and, most important, to maintain its structural integrity and replicative capacity regardless of random chemical alterations of the molecule. Clearly, I have written a prescription for DNA.

Non-informational DNA would have very little mutation load. Its function would presumably depend on average properties such as the overall AT:GC ratio, but not on the specific sequence. Mutations increasing AT pairs and those decreasing them would be largely cancelling in their effects.

In this discussion I have assumed that the typical mutation is deleterious in both homozygous and heterozygous conditon. The effects of mutation on the population are roughly proportional to the mutation rate although dominance and epistasis may change the proportionality constant. Particularly, extreme epistasis models such as truncation selection reduce the constant greatly, but evidence for such extreme epistasis is lacking, especially in inbreeding data.

Two other kinds of mutation deserve mention. One is mutations so nearly neutral that their fate is determined mainly by random drift. It has been suggested that many of the amino acid changes observed in evolution of homologous proteins are of this type. Whether any fractions of polymorphisms have this explanation is an open question.

The other class of mutant is one that enters into some form of selectively maintained polymorphism. Such a mutant may persist indefinitely and be the major cause of genetic variability and genetic load in randomly mating populations. Since the number of possible mutational alterations of a gene far exceeds the number of alleles maintained in balanced polymorphism, the existence of such polymorphisms does not greatly change the principle that the majority of mutants are harmful in all combinations (or at best neutral).

Our practical problem, as selection is relaxed, is to estimate what the effect is in terms of human welfare and we have hardly made a beginning.

REFERENCES

Crow, J. F. (1948): Alternative hypotheses of hybrid vigor. *Genetics*, *33/9*, 447.

Crow, J. F. (1952): Dominance and overdominance. In: *Heterosis*, pp. 282–297. Editor: J. W. Gowen. Iowa State College Press, Ames, Iowa.

Crow, J. F. (1958): Some possibilities for measuring selection intensities in man. *Hum. Biol.*, *30/1*, 1.

Crow, J. F. (1961): Mechanisms and trends in human evolution. *Daedalus*, *90*/3, 416.

Crow, J. F. and Kimura, M. (1970): *An Introduction to Population Genetics Theory*. Harper and Row, New York, N.Y.

Crow, J. F. and Temin, R. G. (1964): Evidence for the partial dominance or recessive lethal genes in natural populations of Drosophila. *Amer. Natur.*, *98/1*, 21 and *98/6*, 447.

Cruz-Coke, R., Cristoffanini, A. P., Aspillaga, M. and Biancani, F. (1961): Evolutionary forces in human populations in an environmental gradient in Africa, Chile. *Hum. Biol.*, *38/4*, 421.

Fisher, R. A. (1930): *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford. (*Second Edition* (1958). Dover Press, New York, N.Y.)

Green, E. L. (1968): Genetic effects of radiation on mammalian populations. *Ann. Rev. Genet.*, *2*, 87.

Hiraizumi, Y. and Crow, J. F. (1960): Heterozygous effects on viability, fertility, rate of development, and longevity of Drosophila chromosomes that are lethal when homozygous. *Genetics*, *45/8*, 1071.

Johnston, F. E. and Kensinger, K. M. (1971): Fertility and mortality differentials and their implications for microevolutionary change among the Cashinahua. *Hum. Biol.*, *43/3*, 356.

Lifschitz, E. (1971): Fine-structure analysis of the chromosome recombinational patterns at the base of the X-chromosome of *Drosophila melanogaster*. *Mutation Res.*, *13/1*, 35.

Morton, N. E. (1969): Human population structure. *Ann. Rev. Genet.*, *3*, 53.

MOTULSKY, A. G., FRASER, G. R. and FELSENSTEIN, J. (1971): Public health and long-term genetic implications of intrauterine diagnosis and selective abortion. In: *Birth Defects: Original Article Series*, *7/5*, pp. 22–32. The National Foundation-March of Dimes, White Plains, N.Y.

MUKAI, T., CHIGUSA, S. I., METTLER, L. E. and CROW, J. F. (1972): Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*. *Genetics*, submitted for publication.

SHANNON, M. P., KAUFMAN, T. C. and JUDD, B. H. (1970): Lethality patterns of mutations in the zeste-white region of *Drosophila melanogaster*. *Genetics*, *64/s*, 58.

WHITFIELD, H. J., MARTIN, R. G. and AMES, B. N. (1966): Classification of mutants in the C gene of the histidine operon. *J. molec. Biol.*, *21/2*, 335.

WRIGHT, S. W. and MORTON, N. E. (1968): Genetic studies on cystic fibrosis in Hawaii. *Amer. J. hum. Genet.*, *20/2*, 157.

Chapter IV Cytogenetics

Bent Pedersen

Cancer Research Institute, Danish Cancer Society, The Radium Station, Aarhus, Denmark

Karyotype evolution in human leukaemia – the relation between karyotypes, cellular phenotypes and clinical progression

For more than 10 years we have accumulated detailed information on cytogenetic abnormalities in a variety of malignant cells. Although certain correlations have been suggested between cytogenetic and phenotypic parameters in these cells, it is not clear whether or not karyotype abnormalities play any essential role for the malignant character of the host cells. Thus, in the absence of convincing evidence the accumulated cytogenetic information remains of academic interest rather than of practical importance. The uncertainty concerning the relation between karyotype and essential phenotypic characters is possibly stronger in human leukaemic research than anywhere else. Whereas solid tumours may be removed surgically with curative effects, the leukaemias are almost entirely referred to chemotherapeutic treatment in which the principle is still largely trial and error in attempting to develop drugs which hit malignant cells maximally, and normal haemopoietic cells minimally. In this situation knowledge about the biochemical differences between normal and leukaemic cells is of crucial importance. Although relevant information is increasing, progress is seriously impeded by the fact that the leukaemic cells may change their biochemical profiles during the course of the disease. In this situation cytogenetics might be of paramount importance if certain cytogenetic characters were known to be firmly linked to other important aspects of the leukaemic phenotype.

However, if this goal is to be reached, the cytogenetic technique must fulfil some elementary demands. First, the investigator must know whether he is analysing normal or leukaemic cells. Secondly, he has to be sure that a karyotype without demonstrable abnormalities is in fact normal, and that cells with observed karyotype abnormalities do not contain additional unobserved abnormalities. Thirdly, in addition to determining the karyotype he should be able to investigate other relevant parameters in the same cell.

In the acute leukaemias the situation is particularly difficult: there is no chromosomal marker to indicate whether a given metaphase is normal or leukaemic. In addition, the fact that only about 50% of the patients show chromosome abnormalities (Krogh Jensen, 1969) poses the question as to whether the remaining 50% are cytogenetically normal or harbour unobserved aberrations in their karyotypes. Whether or not karyotype abnormalities are of significant importance for the establishment and propagation of the leukaemic cell population depends on the answer. Chronic myeloid leukaemia (CML) possesses the marker, namely the Philadelphia chromosome; it is present in 90–100% of the proliferating bone marrow cells in almost all clinically typical cases of CML (Court Brown and Tough, 1963; Tough *et al.*, 1963; Whang *et al.*, 1963; Pedersen, 1969) indicating that this chromosome abnormality is essential for the establishment and propagation of the leukaemic cell population.

On the other hand, the relation between cytogenetic and other cellular characters is diffi-

cult to approach because 2 of the three demands mentioned cannot be fulfilled with the present techniques. Few chromosome pairs can be identified individually on morphological criteria, the others can only be referred to chromosome groups of varying sizes. Consequently, an apparently normal karyotype may in fact be abnormal and an abnormal one may contain unobserved additional aberrations, because it is almost impossible to discover a combined trisomy and monosomy inside the same chromosome group. This applies in particular to the C group. There are good reasons to believe that the quinacrine mustard fluorescence technique developed by Caspersson and his colleagues (Caspersson *et al.*, 1971) will eventually eliminate this problem. To combine karyotype studies with the investigation of other biological parameters in the same cell is still impossible because all cellular characters are destroyed in the process of preparing the metaphase. Improved techniques for cell cloning may eventually alleviate this problem. At present it can be approached indirectly by investigating whether given characters show mutual correlations in a cell population. However, such correlations may be suggestive but are rarely more than that.

In spite of these depressing conditions the challenge is there: leukaemia is still an invariably fatal disease and, although far from conclusive, such investigations might contribute to a better understanding of the factors provoking and promoting the disease.

In the following I want to discuss the relations between cytogenetic and other cellular features in human leukaemic cells. Due to the limited amount of substantiated information much of the discussion is bound to have a hypothetical character. I want to restrict the discussion to classical Ph^1-positive CML, partly for the reasons already mentioned and partly because CML is a gradually progressive disease. It is well known that in typical cases the patients spend 2–3 years after the disease is diagnosed in the so-called chronic phase. On small chemotherapeutic doses they are kept in relatively good clinical and haematological conditions. Later they grow less responsive to therapy, increasing numbers of primitive cells occur in their haemopoietic tissues and ultimately they are in blastic crisis showing falling haemoglobin and thrombocyte counts and bone marrows packed with primitive and frequently morphologically atypical cells.

This biphasic course of disease makes CML specially favourable for studying the relation between the karyotype abnormalities and the variety of biochemical, physiological, kinetic and cytological abnormalities present in the leukaemic cells. In the chronic phase the patients present the Philadelphia chromosome in all or practically all proliferating marrow cells even in the best of remissions (Tough *et al.*, 1963; Whang *et al.*, 1963; Tjio *et al.*, 1966; Pedersen, 1969), but rarely any other cytogenetic abnormalities. Consequently, the morphologically mature granulocytes are probably all of them progeny of Ph^1-positive cells. As the morphologically mature granulocytes are characterized by certain biochemical and physiological abnormalities, the question arises: are these cellular abnormalities caused by the Ph^1-deletion or in any other way directly related to the Philadelphia chromosome? In connection with the transition to the acute phase when increasing numbers of primitive cells make their appearance in marrow and blood, additional karyotype abnormalities develop in the haemopoietic cells. This coincidence raises the question: is the primitivity of the haemopoietic cell population caused by these additional karyotype abnormalities or otherwise directly related to them?

The phenotypic abnormalities of the chronic myeloid leucocytes, already occurring in the chronic phase, concern the morphologically mature granulocytes. Best known are the very low alkaline phosphatase activities in the leukaemic polymorphonuclear granulocytes. The other abnormalities include their phagocytic capacities, their intravascular circulation times and finally the mechanism regulating granulocyte production.

THE CHRONIC PHASE OF CML

It is very reasonable to assume that a regulatory system has broken down in CML as this

could explain the grotesque overproduction of granulocytes which is characteristic of the disease. This assumption has some experimental support (Perry and Marsh, 1964). It has been shown that if mature granulocytes from normal individuals are homogenized and added to a culture of CML cells, the latter respond with reduced DNA synthesis. If culture medium in which large numbers of normal mature granulocytes had been incubated for some hours was added to the leukaemic culture, the same thing happened. Apparently, the granulocytes contain and release a substance capable of depressing the proliferation of leukaemic precursor cells. This substance is possibly identical to the granulocytic chalone identified recently (Rytömaa and Kiviniemi, 1968). However, if the same experiment was carried out using medium in which large numbers of chronic myeloid granulocytes had been incubated, no depression of the DNA synthesis followed. If, on the other hand, homogenized leukaemic cells were used, DNA synthesis was again depressed, although to a lesser extent. Apparently, the leukaemic polymorphs contained the chalone-like substance but were not able to release it from the cells. Clearly, this inability could explain the overproduction of granulocytes in the leukaemic organism and apparently reflect the basic malignancy of the cells. However, an alternative interpretation might be that physiologically the leukaemic polymorphs were not completely mature. Incomplete physiological maturation might have the consequence that although synthesis of the factor had been started, too small quantities were available for release from the cells. This alternative interpretation is supported by the following experiment (Pedersen, 1970).

In peripheral blood cultures from Ph^1-positive patients with CML the mitotic activity observed after 48 hours *in vitro* was found to be inversely related to the frequencies of polymorphonuclear granulocytes in the cultured population; with more polymorphs present less proliferation was observed in the cultures. In this system the polymorphonuclear granulocytes were apparently capable of limiting the *in vitro* cell production. As it is well established that chronic myeloid leucocytes mature well *in vitro* (Gunz, 1948; Boll, 1964; Quaglino *et al.*, 1964), the simplest explanation of the discrepancy among these investigations is that the granulocytes completed their physiological maturation during the period of *in vitro* incubation, and consequently became competent to regulate proliferation among their precursors. Indirectly, the results suggest that in CML granulocyte production is in principle an orderly regulated process. In addition, as indicated by the normal or almost normal marrow and peripheral blood pictures observed in complete remission, there are no signs of arrest or inhibition of cell differentiation in the chronic phase.

In discussing CML, 3 abnormalities of the polymorphonuclear granulocytes are frequently pointed out as stigmata of their basically abnormal nature: (1) their reduced phagocytic activities (Brandt, 1965; Penny and Galton, 1966), (2) a prolonged circulation time in the blood vessels before the cells enter the tissues (Raab *et al.*, 1962), and (3) their low alkaline phosphatase activities (Wachstein, 1946).

However, all 3 characters during remission approach and sometimes even reach normal values (Hayhoe and Quaglino, 1958; Galbraith, 1966; Penny and Galton, 1966; Brandt, 1967). If these physiological and biochemical defects are direct consequences of the pathogenetic factors, their relative normalization is very difficult to explain since even in remission the cells probably descend from Ph^1-positive ancestors. Another feature common to all 3 characters is the fact that they are restricted to the most mature granulocyte forms. Primitive precursor cells have even longer circulation times (Perillie and Finch, 1960; Boggs, 1960), are incapable of phagocytosis (Jersild, 1948) and are completely alkaline phosphatase negative (Hayhoe and Quaglino, 1958). On this basis it has been proposed that, like failure of the circulating leukaemic granulocytes to liberate a proliferation regulating factor, these 3 defects may all be consequences of incomplete physiological maturation (Pedersen and Hayhoe, 1971*b*). In most cases a minority of the leukaemic granulocytes show normal phagocytosis, whereas the majority are poorly active (Brandt, 1965; Penny and Galton, 1966). Similarly, a minority of the cells show alkaline phosphatase positivity (Hayhoe and Quaglino, 1958).

There is now experimental evidence that the actively phagocytic cells are the alkaline phosphatase positive ones (Pedersen and Hayhoe, 1971*a*). Studies on the mobilization of granulocytes from the bone marrow in response to injected bacterial endotoxins suggest a relative normalization of the process of cellular release from the marrow in remission (Marsh and Perry, 1964) and may indicate that in remission the granulocytes are retained in the bone marrow for a sufficient period to allow them a more complete maturation. This may explain the improved physiological and biochemical qualities of the cells in remission.

If this hypothesis is ultimately substantiated, we are in the situation that we cannot point out any unequivocally malignant stigmata in the granulocytes of the chronic phase and consequently may have to conclude that the Philadelphia chromosome apparently leaves no specific footprints in the phenotype of the leukaemic cells. On this basis it may be postulated that the chronic phase of CML is in fact not a leukaemic condition, but rather a preleukaemic state having a very high probability of terminating in an acute myeloid leukaemia, namely the blastic phase.

THE ACUTE PHASE OF CML

The blastic phase, on the other hand, is a highly malignant condition as indicated by the rapidly deteriorating clinical condition of the patients, their short survival time (a few months) in this phase and the progressively more primitive cytology of the leukaemic cells. The simultaneous emergence of a variety of cytogenetically abnormal clones of haemopoietic cells raises the question whether a causal link may exist between these abnormalities and the primitive cytological picture. If such a link does exist, the clinical deterioration and fatal outcome of the condition may conceivably be consequences of the cytogenetic aberrations.

As already mentioned this problem cannot be approached in any direct way, as it is not possible with the present techniques to determine from which type of cell a given metaphase originates when it has been prepared for karyotype analysis. Consequently, an indirect approach has to be employed, in which possible correlations between various cytogenetic and cytological characters are looked for.

An investigation along these lines was carried out in 56 blood cultures obtained from 27 patients with Ph^1-positive CML covering the chronic as well as the acute phase of the disease (Pedersen, 1971). The blood was cultured, harvested and prepared for karyotype analysis according to conventional methods (Moorhead *et al.*, 1960). A total of 1800 Ph^1-positive metaphases were completely karyotyped. From each culture at least 10 and on the average 32 Ph^1-positive cells were analysed. From the same blood samples smears were prepared before incubation. At least 300 leucocytes were differentially counted from each smear.

The possibility of an association between the chromosome count distributions and the cytological pictures was examined in the first instance. As shown by Figure 1, the percentages of hyperdiploid metaphases are positively correlated to the frequencies of granulocyte precursors in the circulating blood. Here, as well as later, the term 'granulocyte precursor' covers all cellular forms capable of proliferation, that is myeloblasts, promyelocytes and myelocytes. When plotted against the myeloblasts alone or against the combined promyelocyte + myelocyte frequencies the results showed more scatter, but a general positive correlation with the percentages of hyperdiploid metaphases was still clear. An interpretation of these results is to assume that the presence of supernumerary chromosomes is responsible for the association with the cytological picture, irrespective of the chromosome groups they belong to. If this interpretation is correct, a similar correlation could be expected to emerge between the frequencies of granulocyte precursors and pseudodiploid metaphases, because the latter contain supernumerary chromosomes as well. In Figure 2 only those cultures were included which contained no hyperdiploid cells. It appears that even in the presence of high frequencies of pseudodiploid cells the blood picture is relatively unaffected. This discrepancy could suggest

that different types of supernumerary chromosomes characterize hyperdiploid and pseudodiploid metaphases.

Comparing the populations of additional chromosomes in these two categories of metaphases it was found that whereas structurally normal G(21–22), E(16) and E(17–18) chromo-

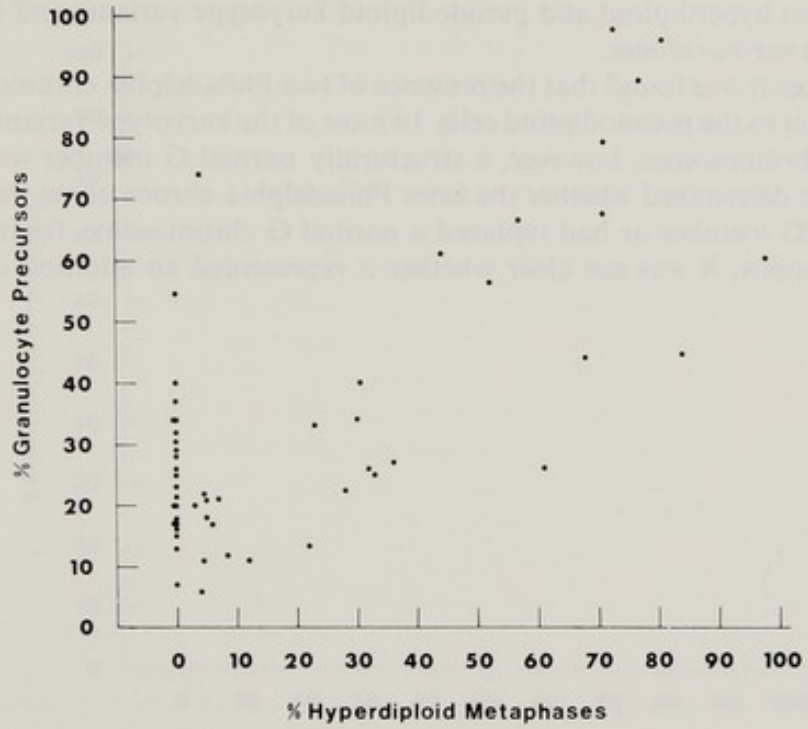


Fig. 1. Frequencies of nucleolated granulocyte precursors (myeloblasts, promyelocytes and myelocytes) in the peripheral blood related to the percentages of hyperdiploid metaphases in the corresponding blood cultures.

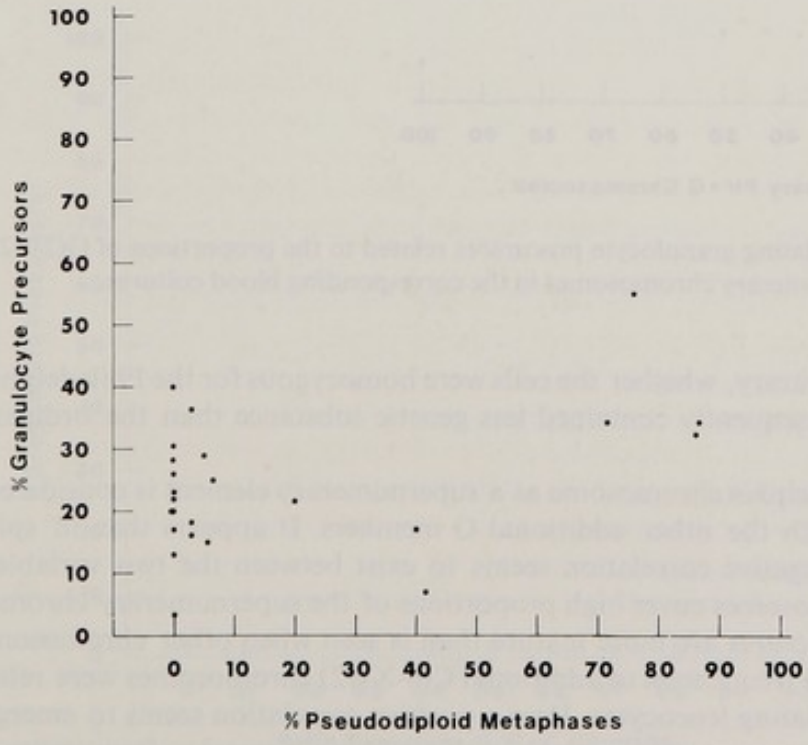


Fig. 2. Relation between circulating granulocyte precursors and blood culture frequencies of pseudodiploid metaphases.

somes occurred in excess statistically more frequently in pseudodiploid metaphases ($P < 0.01$, $P < 0.05$ and $P < 0.0005$, respectively), additional C(6-X-12) chromosomes were significantly more frequent in the hyperdiploid cells ($P < 0.005$). In this comparison each individual karyotype was counted only once in each patient irrespective of the numbers of metaphases in which it was actually found. Consequently, the comparison deals with the distribution of supernumerary chromosomes in hyperdiploid and pseudodiploid karyotype variants and is not influenced by the size of the various clones.

In addition to these differences it was found that the presence of two Philadelphia chromosomes was a more frequent event in the pseudodiploid cells. In most of the karyotype variants containing two Philadelphia chromosomes, however, a structurally normal G member was also missing. As it could not be determined whether the extra Philadelphia chromosome was due to a second deletion of a G member or had replaced a normal G chromosome, for instance by non-disjunction divisions, it was not clear whether it represented an addition of

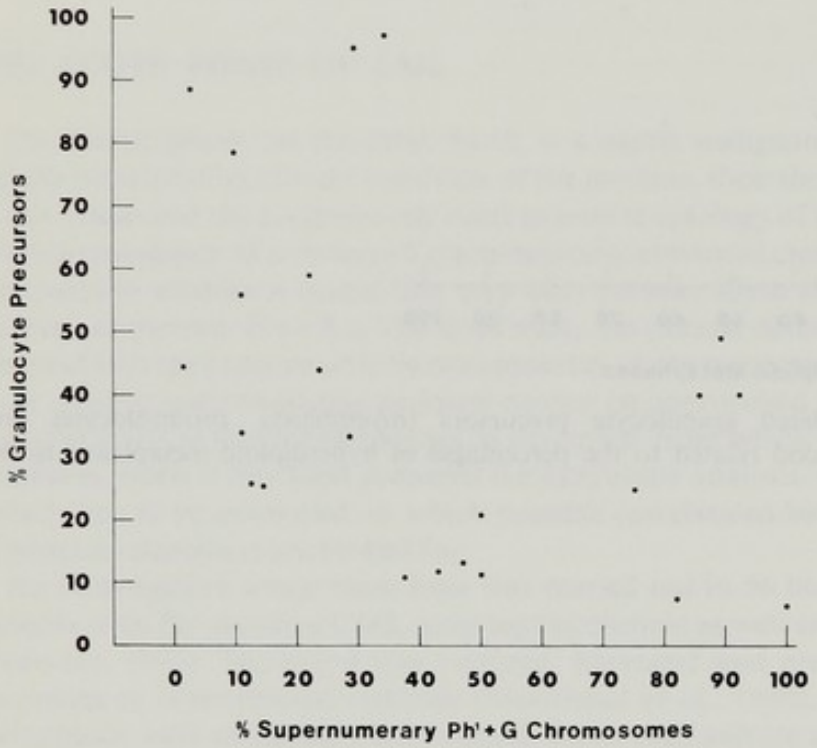


Fig. 3. The frequencies of circulating granulocyte precursors related to the proportions of G(21–22, Ph^1) members among the supernumerary chromosomes in the corresponding blood cultures.

genetic material or, on the contrary, whether the cells were homozygous for the Philadelphia chromosome deletion and consequently contained less genetic substance than the ordinary Ph^1-positive cell.

In Figure 3 the second Philadelphia chromosome as a supernumerary element is considered, and it is grouped together with the other additional G members. It appears that in spite of a considerable scatter, a negative correlation seems to exist between the two variables. When small acrocentric chromosomes cover high proportions of the supernumerary chromosome population, the blood pictures are more mature than is seen when other chromosome types dominate. In Figure 4 the frequencies of additional C(6-X-12) chromosomes were related to the cytology of the circulating leucocytes. Here a positive correlation seems to emerge. Increasingly primitive blood pictures are apparently associated with increasing frequencies of cells containing excess numbers of C chromosomes.

In agreement with the findings of other investigators (Levan, 1966; De Grouchy *et al.*, 1968;

Berger, 1970) G and C group chromosomes represented the majority of the total number of excess chromosomes in the present material. As a result, the cytological picture was related to the ratios between additional G and additional C chromosomes in cultures presenting both. As shown in Figure 5 the ratios of G to G plus C chromosomes are inversely related to the granulocyte precursor frequencies in the blood.

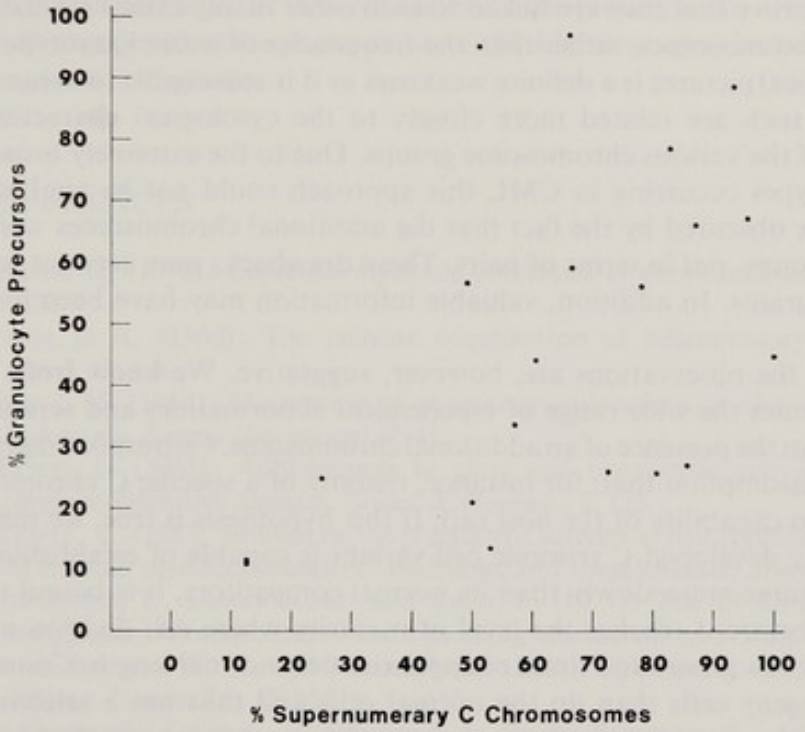


Fig. 4. Relation between the frequencies of circulating granulocyte precursors and the percentages of C(6-X-12) members among the supernumerary chromosomes of the corresponding blood cultures.

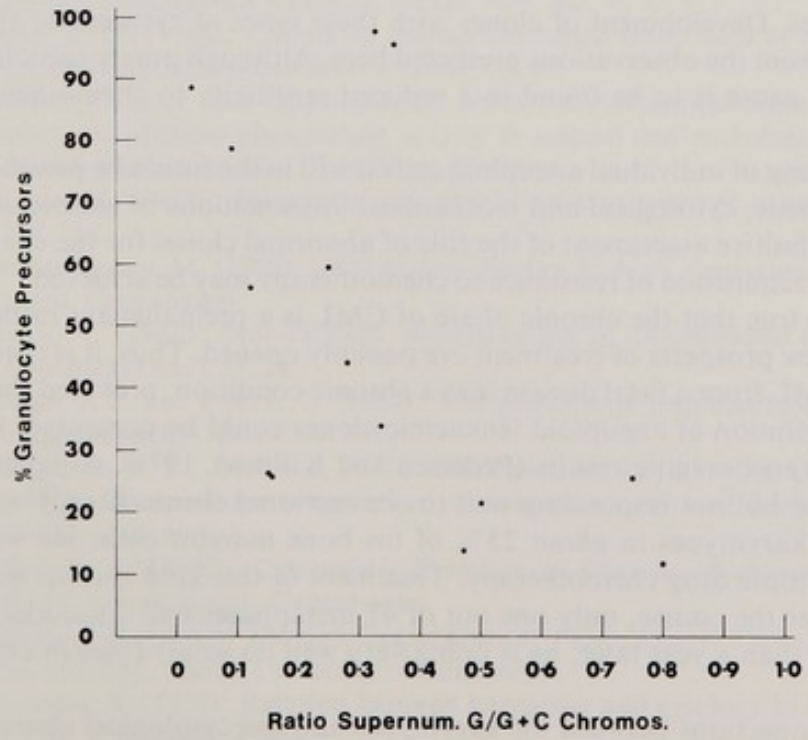


Fig. 5. The frequencies of circulating granulocyte precursors related to the ratios of supernumerary G/G + C chromosomes in the corresponding cultured cells.

The remaining categories of supernumerary chromosomes were too scantily represented to allow graphical representations. As far as missing chromosomes are concerned, it was found that whereas loss of C members occurs particularly in pseudodiploid karyotype variants ($P<0.05$), missing E(17–18) chromosomes characterize in particular the hyperdiploid variants ($P< 0.0005$). Again, however, these findings could not be illustrated graphically.

The correlations presented here indicate certain associations between cytogenetic and cytological parameters, but do not prove that they are linked to each other in any causal manner. The fact that supernumerary chromosomes, rather than the frequencies of entire karyotypes, were plotted against the cytological pictures is a definite weakness as it is reasonable to assume that abnormal karyotypes as such are related more closely to the cytological characters than are additional members of the various chromosome groups. Due to the extremely broad spectrum of abnormal karyotypes occurring in CML this approach could not be applied, however. The picture is further obscured by the fact that the additional chromosomes were only identifiable in terms of groups, not in terms of pairs. These drawbacks may account for some of the scatter of the diagrams. In addition, valuable information may have been lost and bias introduced.

In spite of these limitations the observations are, however, suggestive. We know from a number of constitutional trisomies the wide range of biochemical abnormalities and serious clinical symptoms resulting from the presence of an additional chromosome. Correspondingly, it appears to be a reasonable assumption that, for instance, trisomy of a specific C chromosome may affect the maturation capability of the host cell. If this hypothesis is true, we may be able to explain how a newly developed C trisomic cell variant is capable of establishing itself as a large clone. If it matures more slowly than its normal competitors, it is bound to carry out more cell divisions before it reaches the level of maturity where cell division no longer takes place, provided it has generation times of approximately normal length. Consequently, it produces more progeny cells than do the normal cells and thus has a selective advantage over these. This development brings about a more primitive cytology in the bone marrow, leads to haematological relapse and in the end possibly to blastic crisis.

A mechanism of retarded maturation may thus explain the relation between excess numbers of C chromosomes and the cytological picture, but not those connected with additional small acrocentric chromosomes. Development of clones with these types of cytogenetic abnormalities are not explained from the observations presented here. Although purely speculative, it is conceivable that the cause is to be found in a reduced sensitivity to chemotherapeutic agents.

It is to be hoped that by cloning of individual aneuploid cells it will in the future be possible to carry out combined cytogenetic, cytological and biochemical investigations of genetically identical cells. In this way a definitive assessment of the role of abnormal clones for the character of the disease and for the acquisition of resistance to chemotherapy may be achieved.

If eventually it proves to be true that the chronic phase of CML is a premalignant rather than a malignant condition, new prospects of treatment are possibly opened. Thus, it is theoretically possible to change CML from a fatal disease into a chronic condition, provided that by adequate chemotherapy evolution of aneuploid leukaemic clones could be prevented. In this respect we have had some encouraging results (Pedersen and Killman, 1971). A patient with CML in the chronic phase but not responding well to conventional chemotherapy was found to have pseudodiploid karyotypes in about 25% of his bone marrow cells. He was given a course of intensive multiple drug chemotherapy. Treatment of this kind is otherwise used only in blastic crisis. After the course, only one out of 47 metaphases had a pseudodiploid karyotype. Today, more than a year later, he is doing very well on small doses of conventional chemotherapy.

If in fact there are causal connections between the karyotypes and the cytological characters of leukaemic cells, the discipline of cytogenetics may occupy a central position in leukaemia research, contributing to a better understanding of the progression mechanism, as a medi-

ator between pharmacology and biochemistry on the one hand and the clinician on the other, and as a look-out signalling when genetically altered cells appear and strive for control of the cell population. It may also be considered as an observation post studying the impact of chemotherapy on the cytogenetic profile of the leukaemic cells.

The present contribution has presented hypotheses rather than facts. I apologise for that. On the other hand, at a time when scattered islets of knowledge are appearing here and there, it is important that all relevant ways of interpreting them are brought up for discussion. To me it seems that perhaps the most profitable view upon leukaemic cells is obtained through the geneticist's glasses.

REFERENCES

Berger, R. (1970): Leucémie myéloïde chronique et chromosomes. *Rev. europ. Etud. clin biol.*, *15*, 1000.

Boggs, D. R. (1960): The cellular composition of inflammatory exudates in human leukemias. *Blood*, *15*, 466.

Boll, I. M. (1964): Maturation of normal granulopoiesis *in vitro* compared with chronic myeloid leukaemia. *Sangre* (*Barcelona*), *9*, 41.

Brandt, L. (1965): Adhesiveness to glass and phagocytic activity of neutrophilic leukocytes in myeloproliferative diseases. *Scand. J. Haemat.*, *2*, 126.

Brandt, L. (1967): Studies on the phagocytic activity of neutrophilic leukocytes with special reference to chronic myeloproliferative conditions and megaloblastic anemia. *Scand. J. Haemat., Suppl. 2.*

Caspersson, T., Lomakka, G. and Zech, L. (1971): The 24 fluorescence patterns of the human metaphase chromosomes – distinguishing characters and variability. *Hereditas* (*Lund*), *67*, 89.

Court Brown, W. M. and Tough, I. M. (1963): Cytogenetic studies in chronic myeloid leukemia. *Advanc. Cancer Res.*, *7*, 351.

De Grouchy, J., Nava, C. Feingold, J., Bilski-Pasquier, G. and Bousser, J. (1968): Onze observations d'un modèle précis d'évolution caryotypique au cours de la leucémie myéloïde chronique. *Europ. J. Cancer*, *4*, 481.

Galbraith, P. R. (1966): Studies on the longevity, sequestration and release of the leukocytes in chronic myelogenous leukemia. *Canad. med. Ass. J.*, *95*, 511.

Gunz, F. W. (1948): Culture of human leukaemic blood cells *in vitro*. Normal and abnormal cell division and maturation. *Brit. J. Cancer*, *2*, 41.

Hayhoe, F. G. J. and Quaglino, D. (1958): Cytochemical demonstration and measurement of leucocyte alkaline phosphatase activity in normal and pathological states by a modified azo-dye coupling technique. *Brit. J. Haemat.*, *4*, 375.

Jersild, M. (1948): Phagocytic activities of various types of leucocytes. *Acta med. scand., Suppl. 213*, 238.

Krogh Jensen, M. (1969): *Chromosome Studies in Acute Leukaemia*, Chapter 3, pp. 24–25. Munksgaard, Copenhagen.

Levan, A. (1966): Non-random representation of chromosome types in human tumor stemlines. *Hereditas* (*Lund*), *55*, 28.

Marsh, J. C. and Perry, S. (1964): The granulocyte response to endotoxin in patients with hematologic disorders. *Blood*, *23*, 581.

Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M. and Hungerford, D. A. (1960): Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp. Cell Res.*, *20*, 613.

Pedersen, B. (1969): *Cytogenetic Evolution in Chronic Myelogenous Leukaemia*, Chapter 2, pp. 10–13. Munksgaard, Copenhagen.

Pedersen, B. (1970): *The Cytological Basis of Progression in Chronic Myeloid Leukaemia*, Chapter 7, pp. 153–154. Thesis, University of Cambridge.

Pedersen, B. (1971): Relation between karyotype and cytology in chronic myelogenous leukaemia. *Scand. J. Haemat.*, *8*, 494.

Pedersen, B. and Hayhoe, F. G. J. (1971*a*): Relation between phagocytic activity and alkaline phosphatase content of neutrophils in chronic myeloid leukaemia. *Brit. J. Haemat.*, *21*, 257.

PEDERSEN, B. and HAYHOE, F. G. J. (1971*b*): Annotation: cellular changes in chronic myeloid leukaemia. *Brit. J. Haemat.*, *21*, 251.

PEDERSEN, B. and KILLMANN, S. A. (1971): Leukaemic subclones in chronic myelogenous leukaemia. Differences in drug sensitivity and in differentiation capacity. *Acta med. scand.*, *190*, 61.

PENNY, R. and GALTON, D. A. G. (1966): Studies on neutrophil function. II. Pathological aspects. *Brit. J. Haemat.*, *12*, 633.

PERILLIE, P. E. and FINCH, S. C. (1960): The local exudative cellular response in human leukemias. *Blood*, *15*, 466.

PERRY, S. and MARSH, J. C. (1964): Uptake of tritiated thymidine by leukemic cells: effect of various leukocyte preparations. *Proc. Soc. exp. Biol. Med. (N.Y.)*, *115*, 51.

QUAGLINO, D., COWLING, D. C. and HAYHOE, F. G. J. (1964): Cytochemical and autoradiographic studies on normal and leukaemic cells in short-term tissue cultures. *Brit. J. Haemat.*, *10*, 417.

RAAB, S. O., ATHENS, J. W., HAAB, O. P., BOGGS, D. R., CARTWRIGHT, G. E. and WINTROBE, M. M. (1962): Leukokinetic studies in chronic myelocytic leukemia (CML) using radioactive diisopropylfluorophosphate (DFP^{32}). *Blood*, *20*, 803.

RYTÖMAA, T. and KIVINIEMI, K. (1968): Control of granulocyte production. I. Chalone and antichalone, two specific humoral regulators. *Cell Tiss. Kinet.*, *1*, 329.

TJIO, J. H., CARBONE, P. P., WHANG, J. and FREI, E. (1966): The Philadelphia chromosome and chronic myelogenous leukemia. *J. nat. Cancer Inst.*, *36*, 567.

TOUGH, I. M., JACOBS, P. A., COURT BROWN, W. M., BAIKIE, A. G. and WILLIAMSON, E. R. D. (1963): Cytogenetic studies on bone-marrow in chronic myeloid leukaemia. *Lancet*, *i*, 844.

WACHSTEIN, M. (1946): Alkaline phosphatase activity in normal and abnormal human blood and bone marrow cells. *J. Lab. clin. Med.*, *31*, 1.

WHANG, J., FREI, E., TJIO, J. H., CARBONE, P. P. and BRECHER, G. (1963): The distribution of the Philadelphia chromosome in patients with chronic myelogenous leukemia. *Blood*, *22*, 664.

*D. G. Harnden**

Department of Cancer Studies, University of Birmingham, Birmingham, United Kingdom

Specificity of acquired chromosome damage in man

Numerous environmental agents have been shown to cause chromosome aberrations. Although some patterns have emerged, these have been ill defined and have contributed little to our understanding of the role that such aberrations might play in the induction of heritable chromosome abnormalities, or in the genesis of malignant disease and the chromosomal changes associated with malignant disease. Nor has much been learned from a study of the way in which such aberrations are induced by various agents about the structure and function of the genetic material itself. New techniques are now available for studying chromosomes so that they can be identified with very much greater precision (Caspersson *et al.*, 1970*b*; Sumner *et al.*, 1971). It will be necessary to re-examine the evidence on chromosome aberrations using these techniques and, while this work is only beginning, it is useful at this stage to pinpoint the areas where re-examination might be most profitable. This paper is not intended as a comprehensive review and the examples are chosen to illustrate the main points.

'Acquired' chromosome damage is defined as damage occurring during the lifetime of a single individual and 'constitutional' chromosome aberrations which are present in all the cells at birth will not be discussed in any detail. It is recognised, however, that the possibility exists that chromosome damage to the gametes of one generation may contribute towards 'constitutional' chromosome abnormalities in the next generation. Some interesting observations have been made along these lines; for example, the clustering of the length of the deletion in Bp–chromosomes observed by Miller *et al.* (1969) could indicate the existence of a specific environmental factor which causes breakage at a particular point in these chromosomes. This is, however, a major and separate issue which will not be considered further at present.

ORIGIN OF ACQUIRED CHROMOSOME DAMAGE

Chromosome damage may arise in a number of different ways. It may occur without any obvious environmental stimulus in some genetically determined syndromes. It may arise following exposure to a particular stimulus, such as ionising radiation, or to biological agents such as viruses or mycoplasma, or following exposure to one of a variety of chemical agents. Lastly, chromosome damage may be seen in neoplastic or pre-neoplastic tissues, or in cells transformed *in vitro* by a number of environmental agents. The precise mode of origin of the chromosome aberration in these cases is not known, and is still a matter for much speculation. Chromosome aberrations are rarely seen in non-neoplastic cells in the absence of any

* Supported by a grant from the Cancer Research Campaign.

obvious environmental stimulus. The constancy of the normal karyotype in the cells of the body is remarkable.

The various types of acquired chromosomal aberration are (*a*) loss or gain of chromosomes, (*b*) marker chromosomes, (*c*) specific lesions at particular points in the genome. In each of these classes I will consider chromosomal aberrations that have arisen in each of the ways

TABLE 1 *The origin of acquired chromosomal aberrations*

1. 'Spontaneous'

Genetically determined syndromes, ageing, other unknown causes

2. Induced by specific environmental agencies

(*a*) Ionising radiation
(*b*) Chemical agents, *e.g.* analogues, antibiotics, carcinogens
(*c*) Biological agents, *e.g.* viruses and mycoplasma

3. Neoplasia

(*a*) Associated with tumour induction
(*b*) Instability of karyotype following tumour induction
(*c*) Following cellular transformation *in vitro*

described above, but before presenting the details it is necessary to give some consideration to what is meant by a specific lesion. This depends to some extent on the nature of the aberration. It might also depend on what observations have been made, *e.g.* it is important that an aberration is seen in more than one individual. The frequency is also important. Ferguson-Smith *et al.* (1962) in discussing the frequency of secondary constrictions claim that if a lesion is seen with a frequency of only 3% of the available chromosomes it should be scored as a specific locus for a secondary constriction. Obviously, if time is available to examine sufficient cells one can be quite certain of a specificity at a frequency as low as this, but in practice 3% would be too low except for bizarre aberrations. It is probable that one should consider a specificity as something which occurs in more than 10% or so of the cells of a number of different individuals or independent cultures. If one applies this criterion to Ferguson-Smith's data it can be seen that he has picked up very successfully those points which on staining with the Giemsa banding technique (Sumner *et al.*, 1971) are the widest non-staining regions. It has been almost universally agreed that chromosomes number 1, 9 and 16 carry secondary constrictions fairly near to the centromere, but it has not been so generally agreed that secondary constrictions also exist about half way along the short arm of chromosome number 6, near the centromere of chromosome number 11 and about a third of the way along the long arm of chromosome number 17. However, in 1962 Ferguson-Smith reported secondary constrictions at these sites in 23%, 7% and 23% of the cells, respectively.

LOSS OR GAIN OF CHROMOSOMES

A. SPONTANEOUS LOSS OR GAIN OF CHROMOSOMES

There are several reports of the specific loss of the X chromosome in elderly females and of the Y chromosome in elderly males (Court Brown *et al.*, 1966; Hamerton *et al.*, 1965). The latter phenomenon has been found to be particularly marked in a small number of specific cases rather than as an overall reduction (O'Riordan *et al.*, 1970). No explanation has yet been given for either of these phenomena, except a rather vague suggestion of increasing instability of the genome with increasing age. It is not even certain that the increased loss of a

C group chromosome with age in elderly females is due to the loss of the X and this is one area that could be re-examined using either quinacrine or Giemsa staining. The absence of the Y in some males has been confirmed by Rowley (1971) using quinacrine staining.

B. ENVIRONMENTALLY INDUCED LOSS OR GAIN OF CHROMOSOMES

This has not been reported in human cells.

C. CHROMOSOME LOSS OR GAIN IN ASSOCIATION WITH TRANSFORMATION OR NEOPLASIA

There have been reports of gain of chromosomes, particularly C group chromosomes, in some pre-malignant conditions (Rowley *et al.*, 1966). There have been reports of loss of particular chromosomes in several different tumours. Spiers and Baikie (1970) have drawn attention to an excess of lymphoreticular tumours in which an E group chromosome is lacking. The low numbers of B, D and G group chromosomes in carcinomas of the cervix, bowel and ovary are of interest, but it is important to realise that this is a deficiency of these chromosome groups in sub-tetraploid cells and not a loss of chromosomes in diploid cells (Levan, 1966; Atkin and Baker, 1969; Atkin, 1970). The average deficiency is around 25% below the level found in diploid cells and there are some compensating excesses of other chromosomes. Atkin points out that there is a tendency towards a deficiency of chromosomes which have terminal or near terminal centromeres, and that the apparently preferential loss could have a purely mechanical explanation. It is possible, however, that it is in some way related to the proposal put forward by Hitotsumachi *et al.* (1971) that a balance of a variety of different chromosomal factors controls the balance between normal and malignant behaviour of cells. Now that it is possible to recognise each of the chromosomes individually a re-examination of these data will be necessary to see whether the specificities are indeed real and whether or not they are accompanied by a balancing excess of specific chromosomes in accord with the predictions of Hitotsumachi on the basis of his experimental model system.

Specific loss of chromosomes has been reported also in cells transformed in culture by SV40 virus (Shein and Enders, 1962; Yerganian *et al.*, 1962; Moorhead and Saksela, 1963). All agreed that there is a deficiency of G group chromosomes, but while Yerganian reports a loss of E chromosomes Saksela and Moorhead report the loss of both E and D group chromosomes. There is one report of an excess of chromosome 16 by Minkler *et al.* (1970) on established tumour cell lines and freshly isolated tumours. However, the uncertainties of the technique which they used and the fact that established cell lines are notoriously susceptible to contamination suggest that these findings should at present be treated with the greatest of caution. The feeling does remain, however, that the suggestion of Hitotsumachi *et al.* (1971) should be examined carefully using primary human tumours and the new techniques for chromosome identification. Preliminary observations in our laboratory suggest, however, that this is going to be a very long and difficult process. To determine the basic karyotype is rather easy, but detailed aberration analysis on tissues with a low mitotic rate when only a proportion of the good mitoses are suitable for analysis will be difficult.

MARKER CHROMOSOMES

A. SPONTANEOUS OCCURRENCE OF MARKER CHROMOSOMES

Spontaneous occurrence of marker chromosomes has been reported only in the involved tissues of a number of non-neoplastic conditions, *e.g.* Waldenström's macroglobulinaemia (Broustet *et al*,. 1967), polycythaemia vera (Millard *et al.*, 1968). In the latter case, however, treatment of the patients with ^{32}P complicates the matter.

TABLE 2 *Specific loss or gain of chromosomes in human cells*

Group	Specific chromosome		Frequency		Authority
	Loss	Gain	No. of cases	% of cells	
Spontaneous					
(*a*) Lymphocytes of normal women (increase with age)	X	–	–	up to 7% average	Court Brown *et al.*, 1966
(*b*) Marrow of normal men and men with Ca bronchus (age?)	Y	–	4/32	up to 20%	O'Riordan *et al.*, 1970
Environmental agents	nil				
Neoplasia					
(*a*) Lymphoid cell lines	–	C	4/5	up to 97%	Kohn *et al.*, 1967
(*b*) Pre-leukaemia	–	C	3	up to 55%	Rowley *et al.*, 1966
	C	–	3	up to 62%	Rowley *et al.*, 1966
(*c*) Carcinomas of cervix, bowel and ovary	B, D and G	–	34	average 25%	Atkin and Baker, 1969
(*d*) Carcinoma	D and G	–	34/40 33/40	Av1–2/cell	Levan, 1966
	–	C	27/40	Av0–3/cell	Levan, 1966
(*e*) SV40 transformed cells *in vitro*	G	–			Shein and Enders, 1962
	G		–	38%	Yerganian *et al.*, 1962
	D		–	23.3%	Moorhead and Saksela, 1963
	E		–	14.8%	Moorhead and Saksela, 1963
	G		–	26.9%	Moorhead and Saksela, 1963

B. MARKER CHROMOSOMES INDUCED BY ENVIRONMENTAL AGENTS

The occurrence of marker chromosomes *in vivo* and *in vitro* following exposure to environmental stimuli is well documented, but here I will consider only marker chromosomes which appear repeatedly in different cases and these are rare. One example is the production of a Ph^1-like chromosome in human cells following exposure to rubidomycin (De Grouchy, 1963). Unusually long acrocentric chromosomes are found following many different treatments including radiation, but the frequency is low and the origin is probably heterogeneous.

C. MARKERS IN NEOPLASIA

The presence of marker chromosomes in tumour cells or in cells transformed *in vitro* has been reported on numerous occasions, but again the occurrence of the same abnormal chromosome in a number of cases of the same tumour is rare. Examples have been described in testicular tumours (Martineau, 1967) and in Burkitt's tumour (Jacobs *et al.*, 1963). In the former cases there is the occurrence of a long number 2-like marker chromosome, while in the latter cases there is repeated occurrence of a long D-like chromosome and a long B-like chromosome. A long chromosome like a number 2 has also been reported in some lymphosarcomas. Deletions of both long and short arms of chromosomes of the E group have been reported repeatedly in neoplasms of the lymphoreticular system (Spiers and Baikie, 1970). It has been pointed out (Tough *et al.*, 1968) that such marker chromosomes may not be the only abnormal chromosomes that are present in the karyotype. They stand out because there is no other chromosome in the varied human karyotype of this morphology and it follows, therefore, that these chromosomes may not always have the same origin. Steel (1971) has re-examined the long D-like marker of chromosomes in cultured lines established from Burkitt's tumour and concludes that, in all cases, the chromosome is of different origin. The classic case of a marker chromosome in malignant tissue is, however, the Philadelphia (Ph^1) chromosome in chronic myeloid leukaemia. It has now been shown by fluorescent techniques that this chromosome is different from the one involved in Down's syndrome and since Down's syndrome has become known as trisomy 21 it is suggested that the Philadelphia chromosome is derived from chromosome 22, although in practice this chromosome is slightly the longer of the 2 G group pairs (Caspersson *et al.*, 1970*a*; O'Riordan *et al.*, 1971). In spite of fairly intensive investigation we are at present no further forward in discovering the origin of the Philadelphia chromosome.

In vitro specific markers have been reported following SV40 transformation of human cells (Moorhead and Saksela, 1963), but the significance of their findings is obscured by the occurrence of similar abnormalities in control cells.

ABERRATIONS AT SPECIFIC LOCATIONS ON PARTICULAR CHROMOSOMES

A. SPONTANEOUS LESIONS

The breaks and gaps which occur in genetically determined syndromes such as Fanconi's anaemia, Bloom's syndrome*, ataxia-telangiectasia, are thought always to be randomly distributed throughout the karyotype.

* Evidence was presented in the discussion by Dr. T. M. Schroeder that the distribution of break points is non-random in Bloom's syndrome.

TABLE 3 *Occurrence of specific marker chromosomes in human cells*

Group	Morphology of abnormal chromosome	Frequency		Authority
		No. of cases	% of cells	
Spontaneous				
(*a*) Waldenström's macroglobulinaemia	long 2-like	–	7.5	Broustet *et al.*, 1967
(*b*) Polycythaemia vera	deleted F	–	22–100	Millard *et al.*, 1968
Environmental agents				
(*a*) Radiation	Cq— abnormally long acrocentric	5 –	2 –	Court Brown *et al.*, 1967 De Grouchy, 1963
(*b*) Chemical rubidomycin	unususally short acrocentric —Ph^1 like	'nombreux'		De Grouchy and De Nava, 1967
(*c*) Viral	nil			
Neoplasia				
(*a*) Chronic myeloid leukaemia	22q— (Ph^1)	90%	up to 100	Tjio *et al.*, 1966
(*b*) Burkitt's tumour	abnormally long acrocentric	5/9	up to 100	Jacobs *et al.*, 1963
(*c*) Testicular tumours	long 2-like	8/9	–	Martineau, 1967
(*d*) SV40 transformed cells *in vitro*	deleted 1 deleted 5*	7/8 8/8	up to 70 up to 25	Moorhead and Saksela, 1963 Moorhead and Saksela, 1963

* Also found in 1 control in 14 out of 40 cells (30%).

B. ABERRATIONS INDUCED BY ENVIRONMENTAL STIMULI

(*i*) *Radiation* There is good agreement that ionising radiation causes gaps and breaks that are randomly distributed throughout the karyotype. However, damage due to the incorporation of tritium-labelled thymidine is, as one might expect, distributed according to the region of the genome that is actively synthesising DNA at the time of administration of the ^{3}H thymidine. Similarly, treatment with tritiated uridine gives rise to non-random damage to the chromosomes. This is interpreted to suggest that some regions of the genome are more active than others in the synthesis of mRNA (Klevecz and Hsu, 1964).

(*ii*) *Chemical compounds* There are many reports of chemicals causing non-random damage to the chromosomes, although the specificity of none of these is very high. Bromodeoxyuridine (BUDR) specifically enhances secondary constrictions (Kaback *et al.*, 1964), while cytosine arabinoside and deoxyadenosine triphosphate cause a non-random distribution of breaks, particularly in the subterminal region of chromosomes of the C group and also chromosome 16 (Nichols *et al.*, 1964). Streptonigrin and mitomycin C both cause specific lesions. Nearly always the effect is to cause an enhancement of secondary constrictions, increased breakage at secondary constrictions, or even re-unions at these regions (Cohen and Shaw, 1964, 1965).

(*iii*) *Viruses* Many viruses cause chromosome damage, which is apparently randomly distributed throughout the karyotype. It is probable that some of these cells which show severe damage, sometimes amounting to pulverisation of all the chromosomes, are dying and that these aberrations can be of no future significance.

There are, however, some reports of specificity. For example, Aula (1965) shows considerable specificity for the breaks caused by chickenpox, and claims some specificity for the lesions caused by measles virus. There is, however, one situation which appears to be very different from the others, that is the specific lesions caused in chromosome number 17 by the administration of adenovirus 12. This was first reported by Zur Hausen (1967) and has recently been confirmed in my laboratory by McDougall (1970, 1971*a*, *b*). This lesion, which appears as a gap or a break in either one or both chromatids of a chromosome 17 and sometimes of both number 17 chromosomes in the same cell, may occur with a frequency as high as 60% of all the cells infected. There are a number of points that are well worth examining. Firstly, it is not the only lesion caused by adenovirus 12, there is also a specific breakage at a lower frequency on chromosome 1 near the end of the longer arms. Other apparently random breaks are also caused. The aberrations appear to be associated with an early event in virus infection. If a high multiplicity of infection is used then the random breaks appear to dominate so that there is no apparent specificity if one scores simply breakage on particular chromosomes. If, however, the damage in chromosome regions is analysed the specificity for the particular locus on chromosome 17 still remains. It seems possible, therefore, that in other situations where massive breakage of the chromosomes has been reported a detailed analysis would yield information about hitherto unrecognised specificities. The significance of the 17 lesion with adenovirus 12 is not clear. It is of interest, however, that a comparable lesion can also be caused by adenovirus 31 (McDougall, 1971*b*) and by adenovirus 18 but at a lower frequency. These three serotypes are the members of the group shown by Huebner (1967) to be highly oncogenic in hamsters. The thymidine kinase locus is on chromosome 17 (Weiss and Green, 1967) and it seems possible that the 17 locus is required by the virus for the production of thymidine kinase in connection with the synthesis of new viral nucleic acid.

The specific gap seen in a C group chromosome in a variable proportion of the cells in cultured lymphoblastoid cell lines derived from Burkitt's tumour and other sources (Kohn *et al.*, 1967) in some ways resembles the 17 gap produced by adenovirus 12. It cannot, however, be produced consistently and this has led some authors to conclude that it is a random effect (Whang-Peng *et al.*, 1970). The reality of the phenomenon cannot, however, be doubted and the lack of consistency must be related to a lack of understanding of the precise relationship between EB virus and the cells.

TABLE 4 *Occurrence of specific gaps or breakage points in human chromosomes*

Group	Site of lesion	Frequency	Authority
Spontaneous	nil*		
Environmental factors			
(*a*) Radiation	nil		
(*b*) Chemical			
(*i*) BUDR	secondary constriction sites (especially 1 and 9)	doubling?	Kaback *et al.*, 1964
(*ii*) Cytosine arabinoside	C, D and 16 (especially near end of long arms)	–	Nichols *et al.*, 1964
(*iii*) Streptonigrin	near centromeres of 1 and 2	–	Cohen *et al.*, 1963
(*iv*) Mitomycin C	secondary constriction sites (especially 1, 9 and 16)	33%,45%,12%	Cohen and Shaw, 1964 Cohen and Shaw, 1965
(*c*) Viruses			
(*i*) Chickenpox	2,B, D and E	7% or less	Aula, 1965
(*ii*) Rous sarcoma virus	centromeres of long chromosomes	–	Nichols *et al.*, 1965
(*iii*) Adenovirus type 12	long arm 17, near terminal 1	60%, 25%	Zur Hausen, 1967 McDougall, 1971*a*
type 31	long arm 17	31%	McDougall, 1971*b*
(*iv*) EB virus in lymphoid cell lines	long arm C	up to 49%	Kohn *et al.*, 1967
	long arm C	up to 10%	Tough *et al.*, 1968
Neoplasia			
SV40 transformed cells *in vitro*	secondary constriction sites and near centromeres		Moorhead and Saksela, 1963

* see footnote p. 137.

CONCLUSIONS

One can summarise these specific effects as follows. Loss of sex chromosomes tends to occur *in vivo* in old people, but other spontaneous or induced specific losses or gains are very rare except in malignant or premalignant tissues. In a number of different human tumours and in transformed human cells *in vitro* there is a deficiency of chromosomes in the D and G groups. Confirmation of these observations using the fluorescence and Giemsa banding techniques must be sought particularly to test the theory that chromosomal balance is critical in determining malignant potential.

Abnormal chromosomes, whether in non-neoplastic disease states, in cells treated with environmental agents or in tumours, only rarely occur in more than one case. One must conclude that most often the breakage which leads to their formation occurs at random and the question then remains as to whether or not the small remaining groups where an aberration occurs repeatedly are the results of specific breakage, or whether their resemblance is superficial and their origins diverse. Steel's work seems to show that the latter is more likely to be the case.

Chromosome breakage which occurs spontaneously at a low frequency in normal people and at a high frequency in some genetically determined syndromes as well as that induced by radiation appears to be random. Much virus and chemically induced chromosome damage is also random, but there are a number of general features which clearly show low grade specificities and two very striking examples of highly specific lesions. In the former category the sites of secondary constrictions of chromosomes 1, 9 and 16 and the centromere regions seem to be especially susceptible to the damaging effects of several different viruses and chemical agents. It is interesting to note that these are the regions which stain heavily with Giemsa following *in situ* denaturation of the DNA and which are thought to contain one of the two postulated types of constitutive heterochromatin (Gagne *et al.*, 1971; Arrighi and Hsu, 1971). Jones and Corneo (1971) have shown that human satellite II DNA localises on the secondary constrictions of chromosomes 1, 9 and 16. On the other hand, the specific lesion caused by adenoviruses 12 and 31 on chromosome 17 occurs at regions where Giemsa staining is particularly light, suggesting that this type of break is quite different from the other specific breakage.

REFERENCES

Arrighi, F. and Hsu, T. C. (1971): Localisation of heterochromatin in human chromosomes. *Cytogenetics*, *10*, 81.

Atkin, N. B. (1970): Cytogenetic studies on human tumours and premalignant lesions; the emergence of aneuploid cell lines and their relationship to the process of malignant transformation in man. In: *Genetic Concepts and Neoplasia*, p. 36. Williams and Wilkins Co., Baltimore, Md.

Atkin, N. B. and Baker, M. C. (1969): Possible differences between the karyotypes of preinvasive lesions and malignant tumours. *Brit. J. Cancer*, *23*, 329.

Aula, P. (1965): Virus associated chromosome breakage. *Ann. Acad. Sci. fenn. A*, *89*.

Broustet, A., Hartmann, L., Moulinier, J., Staeffen, J. and Moretti, G. (1967): Etude cytogénétique de dix-huit cas de maladie de Waldenström. *Nouv. Rev. franç. Hémat.*, *7*, 809.

Caspersson, T., Gahrton, G., Lindsten, J. and Zech, L. (1970*a*): Identification of the Philadelphia chromosome as a number 22 by quinacrine mustard fluorescence analysis. *Exp. Cell Res.*, *63*, 238.

Caspersson, T., Simonsson, E. and Zech, L. (1970*b*): UV-absorption and quinacrine mustard fluorescence patterns for chromosome aberration study in Chinese hamster. *Exp. Cell Res.*, *63*, 243.

Cohen, M. M. and Shaw, M. W. (1964): Effects of mitomycin C on human chromosomes. *J. Cell Biol.*, *23*, 386.

Cohen, M. M. and Shaw, M. W. (1965): Specific effects of viruses and antimetabolites on mamma-

lian chromosomes. In: *In Vitro, Vol. 1; The Chromosome: Structural and Functional Aspects*, p. 50. Waverley Press, Baltimore, Md.

COHEN, M. M., SHAW, M. W. and CRAIG, A. P. (1963): The effects of streptonigrin on cultured human leucocytes. *Proc. nat. Acad. Sci. USA*, *50*, 16.

COURT BROWN, W. M., BUCKTON, K. E., JACOBS, P. A., TOUGH, I. M., KUENSSBERG, E. V. and KNOX, J. D. E. (1966): Chromosome studies on adults. *Eugen. Lab. Mem. 42*, 1.

COURT BROWN, W. M., BUCKTON, K. E. and LANGLANDS, A. O. (1967): The identification of lymphocyte clones, with chromosome structural aberrations, in irradiated men and women. *Int. J. Radiat. Biol.*, *13*, 155.

DE GROUCHY, J. (1963): Cytogenetic studies in irradiated medulla and blood cells. *Proceedings, IX Congress of the European Society of Haematology, Lisbon*. S. Karger, Basel.

DE GROUCHY, J. and DE NAVA, C. (1967): Effets cytogénétiques de la rhubidomycine (ou daunomycine). *Ann. Génét.*, *11*, 39.

FERGUSON-SMITH, M. A., FERGUSON-SMITH, M. E., ELLIS, P. M. and DICKSON, M. (1962): The sites and relative frequencies of secondary constrictions in human somatic chromosomes. *Cytogenetics, 1*, 325.

GAGNE, R., TANGUAY, R. and LABERGE, C. (1971): Differential staining patterns of heterochromatin in man. *Nature New Biol.*, *232*, 29.

HAMERTON, J. L., TAYLOR, A. I., ANGELL, R. and MCGUIRE, V. M. (1965): Chromosome investigations of a small isolated human population. Chromosome abnormalities and distribution of chromosome counts according to age and sex among the population of Tristan Da Cunha. *Nature (Lond.)*, *206*, 1232.

HITOTSUMACHI, S., RABINOWITZ, S. and SACHS, L. (1971): Chromosomal control of reversion in transformed cells. *Nature (Lond.)*, *231*, 511.

HUEBNER, R. J. (1967): Adenovirus directed tumour and T antigens. In: *Perspectives in Virology, Vol. 5*, p. 147. Editor: M. Pollard. Academic Press, New York and London.

JACOBS, P. A., TOUGH, I. M. and WRIGHT, D. H. (1963): Cytogenetic studies in Burkitt's lymphoma. *Lancet*, *11*, 1144.

JONES, K. W. and CORNEO, G. (1971): Location of satellite and homogeneous DNA sequences on human chromosomes. *Nature new Biol., 233*, 268.

KABACK, M. M., SAKSELA, E. and MELLMAN, W. J. (1964): The effect of 5-bromodeoxyuridine on human chromosomes. *Exp. Cell Res., 34*, 182.

KLEVECZ, R. R. and HSU, T. C. (1964): The differential capacity for RNA synthesis among the chromosomes. A cytological approach. *Proc. nat. Acad. Sci. USA*, *52*, 811.

KOHN, G., MELLMAN, W. J., MOORHEAD, P. S., LOFTUS, J. and HENLE, G. (1967): Involvement of C group chromosomes in five Burkitt lymphoma cell lines. *J. nat. Cancer Inst., 38*, 209.

LEVAN, A. (1966): Non-random representation of chromosome types on human tumour stemlines. *Hereditas (Lund)*, *55*, 28.

MARTINEAU, M. (1967): Chromosomes in testicular tumours. *Lancet*, *i*, 386.

MCDOUGALL, J. K. (1970): Effects of adenoviruses on the chromosomes of normal human cells and cells trisomic for an E chromosome. *Nature (Lond.)*, *225*, 456.

MCDOUGALL, J. K. (1971*a*): Spontaneous and adenovirus type-12 induced chromosome aberrations in Fanconi's anaemia fibroblasts. *Int. Cancer*, *7*, 526.

MCDOUGALL, J. K. (1971*b*): Adenovirus induced chromosome aberrations in human cells. *J. gen. Virol.*, *12*, 43.

MILLARD, R. E., LAWLER, S. D., KAY, H. E. M. and CAMERON, C. B. (1968): Futher observations on patients with a chromosomal abnormality associated with polycythaemia vera. *Brit. J. Haemat., 14*, 363.

MILLER, D. A., WARBURTON, D. and MILLER, O. J. (1969): Clustering in deleted short-arm length among 25 cases with a Bp- chromosome. *Cytogenetics*, *8*, 109.

MINKLER, J. L., GOFMAN, J. W. and TANDY, R. K. (1970): A specific common chromosomal pathway for the origin of human malignancy. *Brit. J. Cancer*, *24*, 726.

MOORHEAD, P. S. and SAKSELA, E. (1963): Non-random chromosomal aberrations in SV40 transformed human cells. *J. cell. comp. Physiol.*, *62*, 57.

NICHOLS, W., LEVAN, A., HENEEN, W. and PELUSE, M. (1965): Synergism of the Schmidt-Ruppin strain of the Rous sarcoma virus and cytidine triphosphate in the induction of chromosome breaks in human cultured leucocytes. *Hereditas (Lund)*, *54*, 213.

NICHOLS, W. W., LEVAN, A. and KIHLMAN, B. A. (1964): Chromosome breakages associated with

viruses and DNA inhibitors. In: *Cytogenetics of Cells in Culture*, p. 255. Editor: R. J. C. Harris. Academic Press, New York, N. Y.

O'Riordan, M. L., Berry, E. W. and Tough, I. M. (1970): Chromosome studies on bone marrow from a male control population. *Brit. J. Haemat.*, *19*, 83.

O'Riordan, M. L., Robinson, J. A., Buckton, K. E. and Evans, H. J. (1971): Distinguishing between the chromosomes involved in Down's syndrome (trisomy 21) and chronic myeloid leukaemia (Ph^1) by fluorescence. *Nature (Lond.)*, *230*, 167.

Rowley, J. D. (1971): Personal communication.

Rowley, J. D., Blaisdell, R. K. and Jacobson, L. O. (1966): Chromosome studies in preleukaemia. 1. Aneuploidy of group C chromosomes in three patients. *Blood*, *27*, 782.

Shein, H. M. and Enders, J. F. (1962): Transformation induced by Simian virus 40 in human renal cell cultures. 1. Morphology and growth characteristics. *Proc. nat. Acad. Sci. USA*, *48*, 1164.

Spiers, A. S. D. and Baikie, A. G. (1970): The special role of the group 17, 18 chromosomes in reticuloendothelial neoplasia. *Brit. J. Cancer*, *24*, 77.

Steel, C. M. (1971): Non-identity of apparently similar chromosome aberrations in human lymphoblastoid cell lines. *Nature (Lond.)*, *233*, 555.

Sumner, A. T., Evans, H. J. and Buckland R. A. (1971): New technique for distinguishing between human chromosomes. *Nature New Biol.*, *232*, 31.

Tjio, J. H., Carbone, P. P., Whang, J. and Frei, E. III. (1966): The Philadelphia chromosome and chronic myelogenous leukaemia. *J. nat. Cancer Inst.*, *36*, 567.

Tough, I. M., Harnden, D. G. and Epstein, M. A. (1968): Chromosome markers in cultured cells from Burkitt's lymphoma. *Europ. J. Cancer*, *4*, 637.

Weiss, M. C. and Green, H. (1967): Human-mouse hybrid cell lines containing partial complements of human chromosomes and functioning human genes. *Proc. nat. Acad. Sci. USA*, *58*, 1104.

Whang-Peng, J., Gerber, P. and Knutsen, T. (1970): So-called C-marker chromosome and Epstein-Barr virus. *J. nat. Cancer Inst.*, *45*, 831.

Yerganian, G., Shein, H. M. and Enders, J. F. (1962): Chromosomal disturbances observed in human fetal renal cells transformed *in vitro* by Simian virus 40 and carried in culture. *Cytogenetics*, *1*, 314.

Zur Hausen, H. (1967): Virus-cell genome interactions. 1. Induction of specific chromosomal aberrations by adenovirus type 12 in human embryonic kidney cells. *J. Virol.*, *1*, 1174.

G. E. Moore and J. Minowada

Roswell Park Memorial Institute, Buffalo, N.Y., U.S.A.

Human lymphocyte cell lines for genetic and clinical studies*

The ease with which permanent lymphoid cell lines can be established from the peripheral blood, both from patients with various diseases and congenital abnormalities and from normal persons, provides a unique opportunity for accelerating human genetic research. In the last few years we have established over 700 human lymphoid cell lines; similar success has been reported from laboratories throughout the world. The permanence and stability of the lymphoid cell lines provide an excellent opportunity for establishing biochemical and genetic profiles that can subsequently be compared with other cell lines or with cells from the patient from whom the cell line was derived. Established lymphoid cell lines can be preserved with excellent viability at −75 to −200°C. The rapid growth of most lymphoid cell lines makes studies of their chromosome constitution relatively easy. Standard laboratory techniques can be used to grow large amounts of lymphoid cells for genetic and biochemical studies.

The purpose of this review is to summarize technical advances which make these cells of particular interest to geneticists.

METHODS OF ESTABLISHING HUMAN LYMPHOID CELL LINES

Permanent human lymphoid cell lines have been established successfully from buffy coat cultures of the blood (Moore *et al.*, 1967; Clarkson *et al.*, 1967) and cultures of lymphocytes from lymph nodes and spleen (Sinkovics *et al.*, 1967; Jensen *et al.*, 1967; Levy *et al.*, 1968), bone marrow (Benyesh-Melnick *et al.*, 1968) and even incidental to cultures of solid tumors. The most practical method of establishing such cell lines consists of harvesting peripheral venous blood in National Institutes of Health formula A of citric acid, sodium citrate buffer, dextrose (ACD) solution (1 part ACD to 10 parts blood in 10 to 50 ml syringes) or a similar citrate buffer solution. Heparin can be used as an anticoagulant if it is free of preservatives. The volume of blood necessary for the establishment of permanent cell lines depends on three factors: (1) the total white count and the number of the primitive lymphocytes included in the blood sample; (2) environmental effect of prior illness, for example, lymphocytosis due to various virus infections including infectious mononucleosis; and (3) the probable stimulating effect of the Epstein-Barr virus (EBV). Thirty milliliters of blood are sufficient to set up duplicate cultures in 4 oz. bottles with an initial total leukocyte population of approximately 3 to 4 million cells per ml of culture medium. Five to 10 ml samples of venous blood will suffice to establish cell cultures in test tubes by a modification of the technique described by Chang

* This study was supported by US Public Health Service Grant AI-08899-03 from the National Institutes of Health and a grant from the John A. Hartford Foundation, Inc.

(1970). This method utilizes tube cultures that are maintained either as closed cultures with the addition of 5% CO_2 and air, or as open cultures in a CO_2 incubator.

With either method it should be possible to establish permanent cell lines from one out of every 4 cultures from normal persons. In many instances it is very difficult to establish cultures from patients with lymphoid malignancies such as acute lymphatic leukemia and lymphomas. On the other hand, it is relatively easy to establish cell lines from patients with myelogenous leukemia, other cancerous diseases, and normal persons. It is easiest to establish lymphoid cell lines from children and young adults with infectious mononucleosis and other viral diseases that provoke release of immature lymphoid cells into the blood stream (Glade *et al.*, 1968; Pope, 1967).

Various scientists have suggested that the addition of feeder cells, conditioned media, Epstein-Barr virus (Gerber *et al.*, 1969), mitogens such as pokeweed and phytohemagglutinin (Stockman *et al.*, 1971; Elrod and Schrek, 1965; Fikrig *et al.*, 1966) or lysated established lymphocytes (Choi and Bloom, 1970*a*) may stimulate the growth of lymphocytes. We have been unable to demonstrate that any of these additives are necessary or desirable.

There is a lapse period of approximately 40 to 60 days before the cells develop into a self-sustaining cell line (Moore *et al.*, 1968*a*). During this lapse period the culture should be fed each week by removing approximately 50% of the culture media and replacing it with fresh media. We prefer the use of RPMI 1640 medium supplemented with 20% fetal calf serum (Moore *et al.*, 1967). The use of non-toxic fetal calf serum is very important. Lymphoid cell lines grow at an optimal rate with the pH between 6.8 and 7.2; hence it is important to maintain the cultures at slightly lower pH levels than are used with other kinds of cells.

During the first few weeks of culture the number of viable cells decreases alarmingly. We have recommended that duplicate cultures be added together, but others feel this is unnecessary. Establishment of a cell line is signaled by development of clumps of cells which hover in the media and a rapid lowering of the culture pH which reflects the increase in cell growth.

Methodology errors that should be avoided include prolonged delay (more than several hours) between the collection of the blood and separation of the lymphocytes from the plasma, the exposure of the blood to cold, and inclusion of excessive amounts of erythrocytes in the primary cultures. We have previously noted the need to maintain a pH of approximately 7.0. It is important, in our opinion, never to remove more than 50% of the medium of a culture in order to add fresh media.

CHARACTERIZATION OF HUMAN LYMPHOID CELL LINES

Cultured cell lines are valuable for extended genetic studies only if they are stable for the period of study and it is possible to confirm their identity with that of the donor. A majority of cultured human lymphoid cell lines have characteristics of normalcy (Moore, 1968). Most cell lines, including those derived from the patients with malignant diseases, are probably derived from normal primitive lymphocytes with a capacity to replicate under the conditions imposed by culture methods. All lymphoid cell lines should produce immunoglobulin (Moore and Minowada, 1969; Tanigaki *et al.*, 1966) or, if they are abnormal, either heavy or light peptide chain fragments of the immunoglobulin (Matsuoka *et al.*, 1967). Lymphoid cell lines contain a spectrum of cells ranging from very primitive lymphoblasts to macrophage-like and plasma cell-like forms and multinucleated cells (Moore *et al.*, 1968*a*, *b*; Rabin *et al.*, 1967).

The chromosome constitution is usually normal in 85% or more of the cells in culture. The majority of the cell lines maintain stable chromosome constitutions for many months and even a period of 5 or more years (Huang and Moore, 1969). A few cell lines, however, have become aneuploid after being cultured for over 10 to 100 generations. In some instances cell lines

derived from patients with hematopoietic malignancies have abnormal karyotypes as well as other characteristics of malignancy (Huang *et al.*, 1969).

The majority of these lymphoid cell lines contain herpes-like virus (EB virus) and, in an additional number of cell lines, EB virus-associated antigens can be identified by fluorescent staining. The effects of EB virus on the genetic constitution of the lymphoid cell lines is not known, although the insertion of the virus genome into the cellular nucleic acid has been reported (Gerber and Hoyer, 1971).

Isoantigen patterns (HL-A) can be determined by modification of the Terasaki cytotoxicity technique. Unfortunately, the method may yield variable results because of the lack of standardized test sera. Cultured lymphocytes may have additional detectable isoantigens and this makes it difficult to compare the antigen patterns of cell lines with those of the donor's fresh cells. In a few cell lines, where compared with fresh lymphocytes, there had been a loss of detectable isoantigens, but this is much less common than a gain in detectable isoantigens, and more positive reactions. We have noted changes in isoantigen patterns of some cell lines, whereas the isoantigen patterns of other cell lines have remained remarkably stable over a period of years despite changes in chromosome constitution of the cells (Moore and Woods, 1972).

The relative malignancy or normalcy of cell lines can be determined only by indirect means such as measurement of their relative cloning efficiency (Imamura and Moore, 1968) and ability to grow and form tumors in immunosuppressed heterologous hosts (Adams *et al.*, 1966). Both of these methods provide information subject to interpretation. For example, most normal cells may form clones and grow in heterologous hosts if a more favorable environment is provided. The only direct test of the malignancy of these lymphoid cell lines would be reinjection of them into the person from whom they were derived.

As a result of evidence derived from many clinical and experimental studies, lymphocytotherapy in patients with advanced malignancy was started and provided an opportunity to observe the growth of lymphoid cell lines as autochthonous grafts (Moore and Gerner, 1970). In brief, over 30 patients have been injected with from one to over 800 grams of autochthonous cultured lymphoid cells. There was no evidence of sustained growth of these lymphoid cells as malignant forms. No cell lines were used in these studies that had an abnormal chromosome constitution or that were derived from patients with hematopoietic malignancies.

EXAMPLES OF GENETIC STUDIES

A number of lymphoid cell lines have been established from individuals with chromosome and genetic disorders.

A young professional woman volunteered to provide a blood sample for our studies of the culture of normal cells. The cell line that developed had 47 chromosomes; the extra chromosome being a minute (Moore *et al.*, 1969*a*). An immediate study was made of her fresh lymphocytes incubated for 3 days after stimulation with phytohemagglutinin (PHA) and presence of the minute was confirmed. Subsequently, an additional lymphoid cell line was established which also contained the extra minute chromosome and a repeat study of her fresh lymphocytes again revealed that a majority of her cells contained the minute. This young lady has no evidence of any physical abnormalities and indeed she holds a responsible teaching position at the college level. There is no history of overt genetic disorders in the immediate members of the family.

A middle-aged man with a social history of multiple arrests and severe personal problems was found to have the XYY syndrome in studies of fresh lymphocytes. A sample of peripheral blood was used to establish permanent lymphoid cell lines in which all observed cells had the abnormal chromosome constitution (Moore *et al.*, 1969*b*). Similarly, permanent cell lines with the XO chromosome constitution were easily established from a patient with typical characteristics of Turner's syndrome.

These examples indicate the practical value of lymphocyte cultures for genetic studies. These cell lines with abnormal chromosome constitutions have remained stable for periods of 5, 4, and 3 years, respectively.

Blume *et al.* (1969) established lymphoid cell lines from a patient with the Chediak-Higashi syndrome. The presence of typical giant lysosome-containing granules was observed.

Conover *et al.* (1970) noted the usefulness of cultured lymphoid cells for studying the relationship of an individual's genotype *in vivo* and *in vitro*. They reported the persistence of the PGM phenotype in the cell lines.

Choi and Bloom (1970*b*) established lymphoid cell lines from a patient with the Lesch-

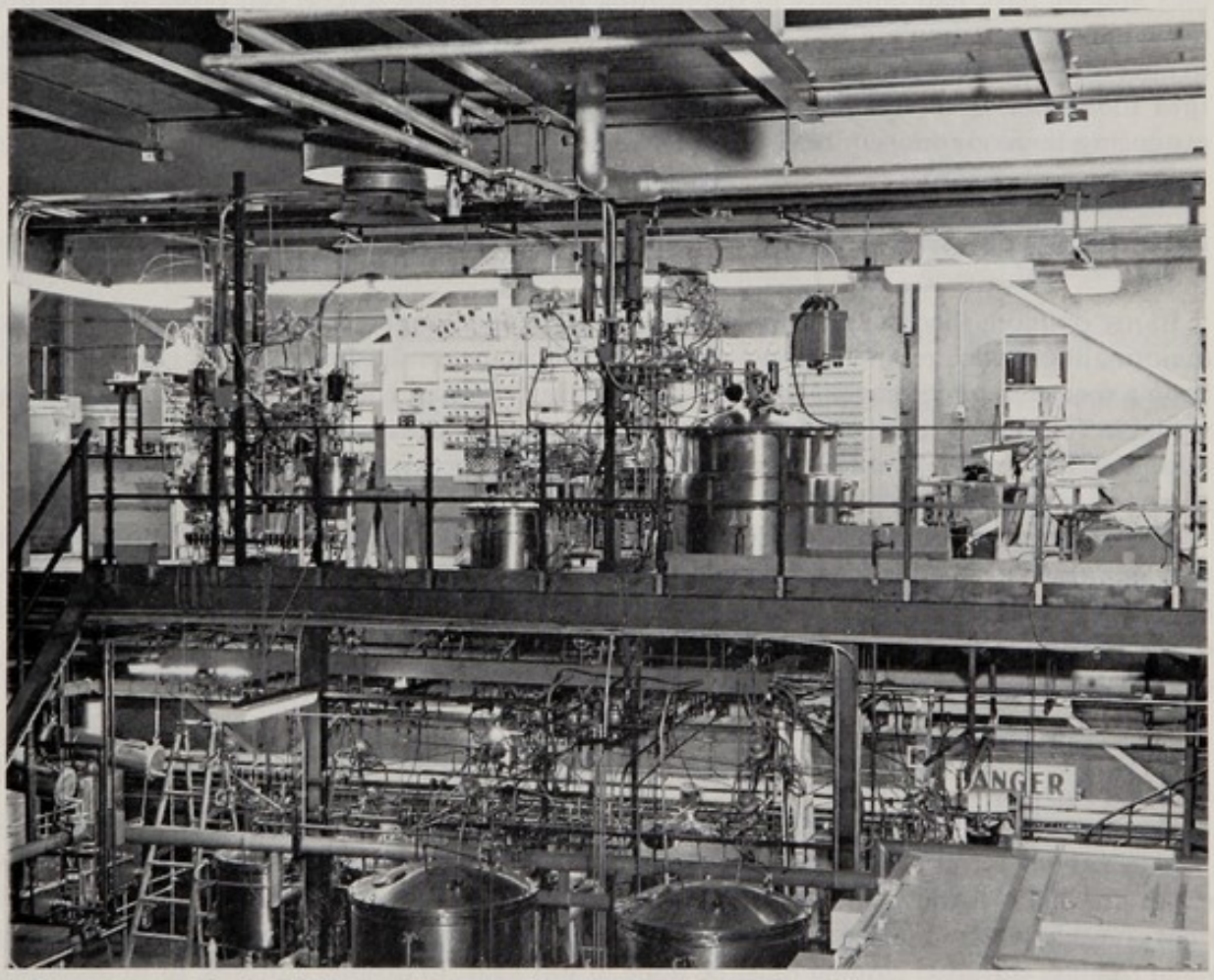


Fig. 1. Cell plant.

Nyhan syndrome and confirmed that the cells were deficient in hypoxanthine-guanine phosphoribosyltransferase.

If it was desirable to obtain a large number of such cells for nucleic acid studies or the separation of individual chromosomes, it would be possible to provide kilograms of cells with the abnormal chromosome pattern by use of large culture units, including a cell plant (Fig. 1).

The growth of a cell line from a patient with malignant myeloma demonstrates the feasibility of initiating cell lines yielding specific cell products. A peripheral blood sample from a patient with malignant myeloma was established as a permanent cell line with abnormal morphology (Moore and Kitamura, 1968), an abnormal chromosome constitution, and the production of lambda peptide light chains. Identical chains (Bence-Jones protein) also were the

predominant product of the abnormal cells in this patient (Yagi, 1970). Subsequent studies of this myeloma cell line permitted accurate determination that approximately 47,000 molecules of light chain peptides were being synthesized per cell per second. Similarly, large amounts of this peptide were harvested from suspension cultures for sequential analysis of the amino acids making up the peptide chain. This cell line would probably be very useful for attempts to isolate specific messenger RNA associated with the production of the light chain peptide.

Preliminary studies have been made of the use of established human lymphoid cells in attempts to provoke specific antibody production. Successful induction of specific antibody production would allow one to study cells with an additional label and provide evidence of their functional capacity (Kamei and Moore, 1967). Unfortunately, such studies are complex and we have had difficulty in duplicating them. Perhaps several cell types in a culture must interact to stimulate new antibody formation. Macrophage-like cells may be necessary to alter and introduce the antigenic material to other cells which are capable of synthesizing IgM molecules. Some lymphoid cell lines contain these forms as well as the capability of producing large amounts of IgM.

FUTURE USES OF CULTURED HUMAN LYMPHOID CELLS

In my opinion, there is little doubt but that all of the lymphoid cells with various genetic abnormalities which retain their capacity to divide can be established as permanent cell lines. Such a spectrum of permanent cell lines representing various genetic disorders should be very valuable for future research. In addition, these human cells would be available for cell-hybridization studies in which it may be possible to detect new relationships between chromosomes and cell products.

The stability and normalcy of cultured human lymphoid cells are noteworthy. Such cells may be valuable in carcinogen detection systems and in studies of the effect of various viruses on human cells. They may be particularly desirable for studies of the effect of radiation damage. Their function as producers of immunoglobulin and their known sensitivity to irradiation make them particularly desirable test subjects.

DISCUSSION

A recent review of gene expression in somatic cell hybrids by Davidson (1971) provides many examples of the kinds of research problems in which cultured human lymphoid cell lines could be used. One practical disadvantage of lymphoid cell lines is their lack of attachment to glass surfaces; cloning must be done in soft agar, in droplet cultures, or in immunosuppressed heterologous hosts.

There undoubtedly will be many instances in which cultured lymphoid cells will not reflect an expression of a genetic abnormality. However, those conditions that are expressed in fibroblasts should also be detectable in lymphoid cells. Some confirmation of these theses has been provided by Griffin and Gibbs (1971) who reported that lymphoid cells from patients with cystic fibrosis had a 30% reduction in beta-glucuronidase activity.

It would be interesting to establish lymphoid cell lines from patients with xeroderma pigmentosa. Temporary cultures of lymphocytes have been used for this purpose (Burk *et al.*, 1971).

Skin fibroblasts from patients with metachromatic leukodystrophy are deficient in arylsulfatase (Porter *et al.*, 1971); perhaps lymphoid cell lines could be used for further investigations.

It may be possible to use lymphoid cultures to study the initiation and repression of the expression of genetic traits.

SUMMARY

Lymphoid cell lines can be established from the peripheral blood with relative ease. The advantages of these cell lines are: (1) stability for many generations, (2) seemingly infinite life, (3) rapid growth and adaptability for massive cultures, and (4) retention of identification characteristics such as immunoglobulin products, isoantigen patterns, and the reflection of various genetic defects.

REFERENCES

Adams, R. A., Farber, S., Foley, G. E., Uzman, B. G., Lazarus, H. and Watrouse, P. (1966): Heterotransplantation of human leukemic cells and cell cultures in the lethally X-irradiated Syrian hamster. *Cancer Res.*, *26*, 2190.

Benyesh-Melnick, M., Fernbach, D. J., Dessy, S. and Lewis, R. T. (1968): Studies on acute leukemia and infectious mononucleosis of childhood. III. Incidence of spontaneous lymphoblastoid transformation in bone marrow cultures. *J. nat. Cancer Inst.*, *40*, 111.

Blume, R. S., Glade, P. R., Gralnick, H. R., Chessin, L. N., Hasse, A. T. and Wolff, S. M. (1969): The Chediak-Higashi syndrome: continuous suspension cultures derived from peripheral blood. *Blood*, *33*, 821.

Burk, P. G., Yuspa, S. H., Lutzner, M. A. and Robbins, J. H. (1971): Xeroderma pigmentosum and DNA repair. *Lancet*, *i*, 601.

Chang, R. S. (1970): The loss of growth vitality of human lymphoid cell lines derived from healthy adults. *Proc. Soc. exp. Biol. Med.* (*N. Y.*), *135*, 212.

Choi, K. W. and Bloom, A. D. (1970*a*): Cloning human lymphocytes *in vitro*. *Nature* (*Lond.*), *227*, 171.

Choi, K. W. and Bloom, A. D. (1970*b*): Biochemically marked lymphocytoid lines: establishment of Lesch-Nyhan cells. *Science*, *170*, 89.

Clarkson, B., Strife, A. and DeHarven, E. (1967): Continuous culture of seven new cell lines (SK-Ll to 7) from patients with acute leukemia. *Cancer* (*Philad.*), *20*, 926.

Conover, J. H., Hathaway, P., Glade, P. R. and Hirschhorn, K. (1970): Persistence of phosphoglucomutase (PMG) polymorphism in long-term lymphoid lines. *Proc. Soc. exp. Biol. Med.* (*N. Y.*), *133*, 750.

Davidson, R. L. (1971): Regulation of gene expression in somatic cell hybrids: a review. *In vitro*, *6*, 411.

Elrod, L. M. and Schrek, R. (1965): Production of lymphoblastoid cells from rat and human lymphocytes by rabbit serum and by phytohemagglutinin. *Exp. Cell Res.*, *38*, 418.

Fikrig, S., Gordon, F. and Uhr, J. W. (1966): Culture of leucocytes from rabbit blood and lymph: effect of phytohemagglutinin and growth of macrophages. *Proc. Soc. exp. Biol. Med.* (*N. Y.*), *122*, 379.

Gerber, P. and Hoyer, B. H. (1971): Induction of cellular DNA synthesis in human leucocytes by Epstein-Barr virus. *Nature* (*Lond.*), *231*, 46.

Gerber, P., Whang-Peng, J. and Monroe, J. H. (1969): Transformation and chromosome changes induced by Epstein-Barr virus in normal human leukocyte cultures. *Proc. nat. Acad. Sci. USA*, *63*, 740.

Glade, P. R., Kasel, J. A., Moses, H. L., Whang-Peng, J., Hoffman, P. F., Kammermeyer, J. K. and Chessin, L. N. (1968): Infectious mononucleosis: continuous suspension culture of peripheral blood leucocytes. *Nature* (*Lond.*), *217*, 564.

Griffin, G. D. and Gibbs, G. E. (1971): Lysosomal enzymes of cultured white blood cells in cystic fibrosis. *Proc. Soc. exp. Biol. Med.* (*N. Y.*), *137*, 438.

Huang, C. C. and Moore, G. E. (1969): Chromosomes of 14 hematopoietic cell lines derived from peripheral blood of persons with and without chromosome anomalies. *J. nat. Cancer Inst.*, *43*, 1119.

Huang, C. C., Imamura, T. and Moore, G. E. (1969): Chromosomes and cloning efficiencies of hematopoietic cell lines derived from patients with leukemia, melanoma, myeloma and Burkitt lymphoma. *J. nat. Cancer Inst.*, *43*, 1129.

IMAMURA, T. and MOORE, G. E. (1968): Ability of human hematopoietic cell lines to form colonies in soft agar. *Proc. Soc. exp. Biol. Med. (N. Y.), 128*, 1179.

JENSEN, E. M., KOROL, W., DITTMAR, S. L. and MEDREK, T. J. (1967): Virus containing lymphocyte cultures from cancer patients. *J. nat. Cancer Inst., 39*, 745.

KAMEI, H. and MOORE, G. E. (1967): Production of phage-neutralizing activity *in vitro* by cells derived from Burkitt's lymphoma. *Nature (Lond.), 215*, 860.

LEVY, J. A., VIROLAINEN, M. and DEFENDI, V. (1968): Human lymphoblastoid lines from lymph node and spleen. *Cancer (Philad.), 22*, 517.

MATSUOKA, Y., MOORE, G. E., YAGI, Y. and PRESSMAN, D. (1967): Production of free light chains of immunoglobulin by a hematopoietic cell line derived from a patient with multiple myeloma. *Proc. Soc. exp. Biol. Med. (N. Y.), 125*, 1246.

MOORE, G. E. (1968): Biological ethics. The fantastic future. *Calif. Med., 109*, 494.

MOORE, G. E., FJELDE, A. and HUANG, C. C. (1969*a*): Established hyperdiploid hematopoietic cell line with a minute marker chromosome persisting both in culture and in the 'normal' donor. *Cytogenetics, 8*, 332.

MOORE, G. E. and GERNER, R. E. (1970): Cancer immunity. Hypothesis and clinical trial of lymphocytotherapy for malignant diseases. *Ann. Surg., 172*, 733.

MOORE, G. E., GERNER, R. E. and FRANKLIN, H. A. (1967): Culture of normal human leukocytes. *J. Amer. med. Ass., 199*, 519.

MOORE, G. E., GERNER, R. E. and MINOWADA, J. (1968*a*): Studies of normal and neoplastic human hematopoietic cells *in vitro*. In: *The Proliferation and Spread of Neoplastic Cells, M. D. Anderson Hospital and Tumor Institute Annual Symposium*, pp. 41–63. University of Texas, Houston.

MOORE, G. E. and KITAMURA, H. (1968): Cell line derived from patient with myeloma. *N. Y. St. J. Med., 68*, 2054.

MOORE, G. E., KITAMURA, H. and TOSHIMA S. (1968*b*): Morphology of cultured hematopoietic cells. *Cancer (Philad.), 22*, 245.

MOORE, G. E. and MINOWADA, J. (1969): Human hemopoietic cell lines: a progress report. In: *Hemic Cells in vitro. In vitro, 4*, 100.

MOORE, G. E., PORTER, I. H. and HUANG, C. C. (1969*b*): Lymphocytoid lines from persons with sex chromosome anomalies. *Science, 163*, 1453.

MOORE, G. E. and WOODS, L. K. (1972): Isoantigens of cultured human lymphoid cells. *Transplantation, 13*, 155.

POPE, J. H. (1967): Establishment of cell lines from peripheral leucocytes in infectious mononucleosis. *Nature (Lond.), 216*, 810.

PORTER, M. T., FLUHARTY, A. L. and KIHARA, H. (1971): Correction of abnormal cerebroside sulfate metabolism in cultured metachromatic leukodystrophy fibroblasts. *Science, 172*, 1263.

RABIN, E. R., BENYESH-MELNICK, M. and PIERRI BRUNSCHWIG, J. (1967): Studies on acute leukemia and infectious mononucleosis of childhood. II. Ultrastructure of cultured lymphoblastoid cells. *Exp. molec. Path., 7*, 196.

SINKOVICS, J. G., SYKES, J. A., SHULLENBERGER, C. C. and HOWE, C. D. (1967): Patterns of growth in cultures deriving from human leukemic sources. *Tex. Rep. Biol. Med., 25*, 446.

STOCKMAN, G. D., GALLAGHER, M. T., HEIM, L. R., SOUTH, M. A. and TRENTIN, J. J. (1971): Differential stimulation of mouse lymphoid cells by phytohemagglutinin and pokeweed mitogen. *Proc. Soc. exp. Biol. Med. (N. Y.), 136*, 980.

TANIGAKI, N., YAGI, Y., MOORE, G. E. and PRESSMAN, D. (1966): Immunoglobulin production in human leukemia cell lines. *J. Immunol., 97*, 634.

YAGI, Y. (1970): Production of immunoglobulin by cells of established human lymphocytoid cell lines. *Symp. int. Soc. Cell Biol., 9*, 121.

M. A. Ferguson-Smith

Departments of Genetics and Child Health, University of Glasgow, Glasgow, United Kingdom

Human chromosomes in meiosis

Early students of the human karyotype chose testis material principally because it contained large numbers of normal dividing cells which could be studied in standard histological sections. The technique presented formidable obstacles to the cytologist and it is truly remarkable how close those pioneers like De Winiwarter (1912) and Painter (1921, 1923) came to the correct description of the human karyotype. With the advent of short- and long-term tissue culture as a much better source of chromosome preparations in the 1950's, meiotic analysis from testicular material was largely abandoned. One important exception was the study by Ford and Hamerton (1956) which confirmed the diploid number as 46, discovered the same year in cell cultures of human embryos by Tjio and Levan (1956), and made the first reliable counts of the number of chiasmata at diakinesis and hence an estimate of the genetic length in man.

Recently there are signs that the interest of human cytogeneticists is returning once more to the study of meiotic chromosomes. This is because there are important problems in human cytogenetics which can be solved only by studying the behaviour of chromosomes in meiosis and, in particular, their synapsis and segregation. Also valuable techniques have been learnt in the detailed study and identification of mitotic chromosomes which when applied to meiosis yield information not revealed to those early pioneers. The techniques include the air-dried drop method of chromosome preparation (Evans *et al.*, 1964; Ferguson-Smith, 1964), the hypotonic pre-treatment using potassium chloride (Hungerford, 1971), the quinacrine-fluorescence technique (Caspersson *et al.*, 1970) and the centromeric heterochromatin staining technique (Arrighi and Hsu, 1971). Testicular material obtained by biopsy is still naturally the most important source of material, but methods devised principally by Edwards (1962), Yuncken (1968) and Jagiello *et al.* (1968) have also been developed for human ovarian tissue to allow meiotic analysis in the female.

The meiotic cycle is well described for a number of species much more suitable for cytology than man. It is, however, of interest to examine the various stages in the human meiotic cycle, if only to recognise the still formidable limitations of present-day technique.

Divisions in which 46 mitotic chromosomes can be identified are easily found in testicular material. It is possible that some of these are somatic mitoses derived from the supporting stroma of the testis, but many of them show a marked degree of major coiling which is seldom seen in somatic mitoses (Fig. 1). It is usually assumed that these are spermatogonial mitoses. In many of these mitoses the chromosomes are heavily condensed, and some investigators believe that spermatogonial mitoses can be classed into a condensed group and a non-condensed group and that this corresponds in some way to the two types of spermatogonia (types A and B). However, in our experience, all gradations between the two types can be seen and so it is still possible that these are simply extremes of the same pre-meiotic division. Some mitoses

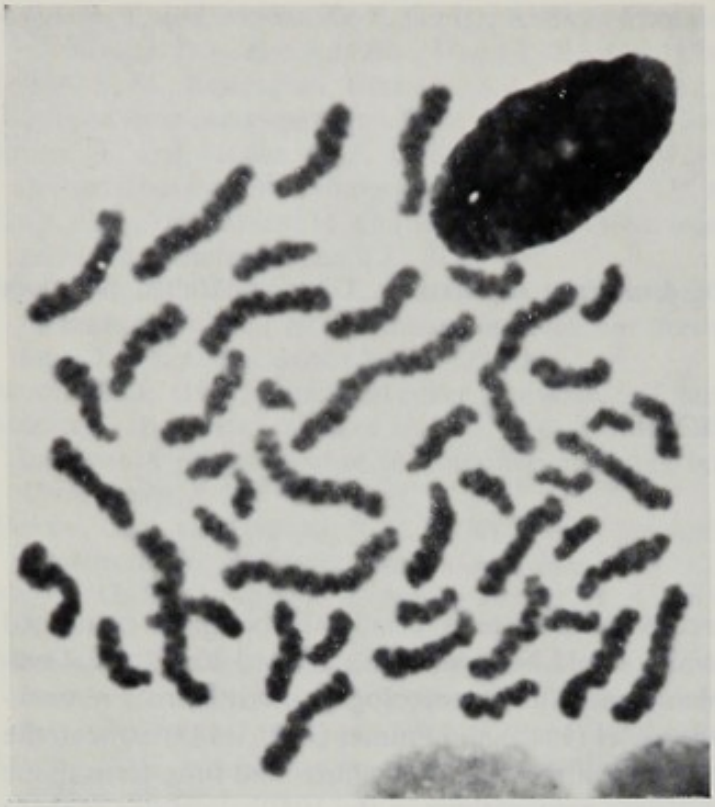


Fig. 1. Spermatogonial metaphase. Note marked degree of coiling.

show homologues lying close beside one another, but the question of true pre-meiotic pairing is still unresolved. In spermatogonial mitoses secondary constriction regions can sometimes show a failure of condensation so that the chromosome appears as two bodies joined by a tenuous thread stretched across the cell. In these cells it is easy to count more than the diploid number of chromosomes. This may in fact explain the counts of 47 and 48 obtained by our predecessors.

Primary spermatocytes in the early stages of the prophase of first meiosis are difficult to distinguish. Large prophase nuclei in which the chromosomes are slender and apparently single-stranded presumably represent leptotene (Fig. 2). Zygotene nuclei, in which the threads

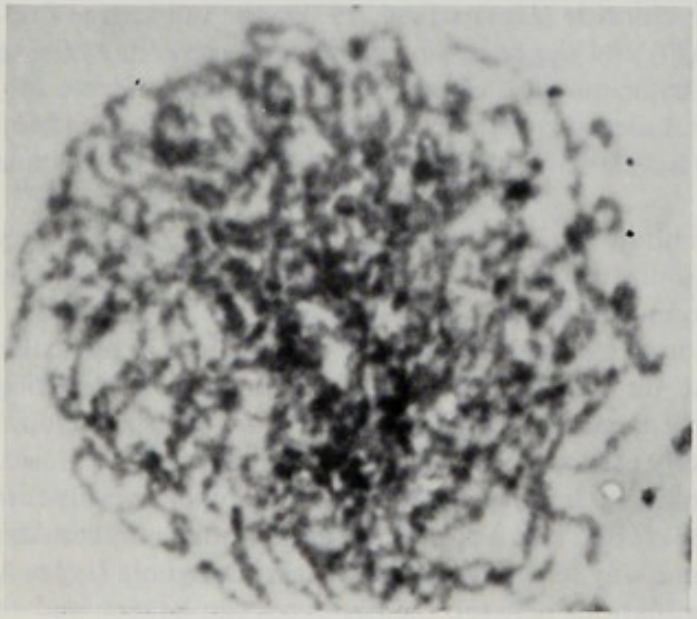


Fig. 2. Prophase of first meiosis; leptotene.

appear to be paired and in which there is no true sex vesicle can sometimes be identified; in some of these the chromosomes are polarised to one part of the nucleus producing the so-called 'bouquet' appearance (Fig. 3). There is no difficulty recognising pachytene, as cells in this stage are abundant in the preparations. In favourable cells, 23 bivalents can be made out, and sometimes these can be more closely identified by their length and centromere position (Fig. 4). The centromere appears as a prominent dense chromomere, almost always a single structure. The 5 acrocentric bivalents are relatively easily identified by their large terminal nucleolar chromomeres and their association with nucleoli. There are three large and two small acrocentric bivalents and these frequently associate by their nucleolar chromomeres.

The sex bivalent is easily recognised at pachytene because it is precociously condensed and appears usually as a block of dense chromatin in which one can sometimes imagine the outlines of the X and Y chromosomes tightly coiled together.

Bivalent number nine may also be recognised at pachytene by the extraordinary appearance of the paracentric constriction which is sometimes associated with a small nucleolus and a shower of minute paired bodies which lie close to the constriction (Hungerford, 1971). The significance of these structures remains unknown.

Fig. 3. A zygotene nucleus with a 'bouquet' appearance.

The appearance of the bivalents at pachytene is variable and highly sensitive to hypotonic treatment and fixation. In some cells, the chromosomes appear to have characteristic chromomere patterns, and in others only the centromeric heterochromatin is obvious. Among the latter one can sometimes distinguish a 'lampbrush' or diffuse stage which probably occurs just prior to the separation of the homologues at diplotene.

Good diplotene figures are rare in testicular preparations. This is unfortunate for this is the optimum stage for examining and counting chiasmata. For this purpose squash preparations are superior to air-dried preparations for they enable one to distinguish more easily between a chiasma and the overlapping of homologues produced by coiling. Many more cells are seen in diakinesis (Fig. 5), and because the bivalents are more condensed at this stage they are easy to count and analyse. Chiasma counts are usually made at diakinesis and Table 1 shows the mean chiasma counts in 23 normal subjects based on the analysis of 849 cells. The mean total chiasma count is 51.11 (S.D. 2.97) which is a little lower than that usually quoted and corresponds to a map length of 25.6 morgans. Table 2 shows similar estimates by eight other authors; some of these include data from infertile subjects.

As meiosis proceeds from diakinesis to first metaphase the bivalents condense further and there is a slight reduction in chiasma count. It is not clear whether this represents terminali-

sation of chiasmata or merely a greater difficulty in identification. There also seems to be a greater tendency for the sex chromosomes to be present as univalents – suggesting that precocious disjunction is a feature of the sex bivalent. Once again, this separation of the sex

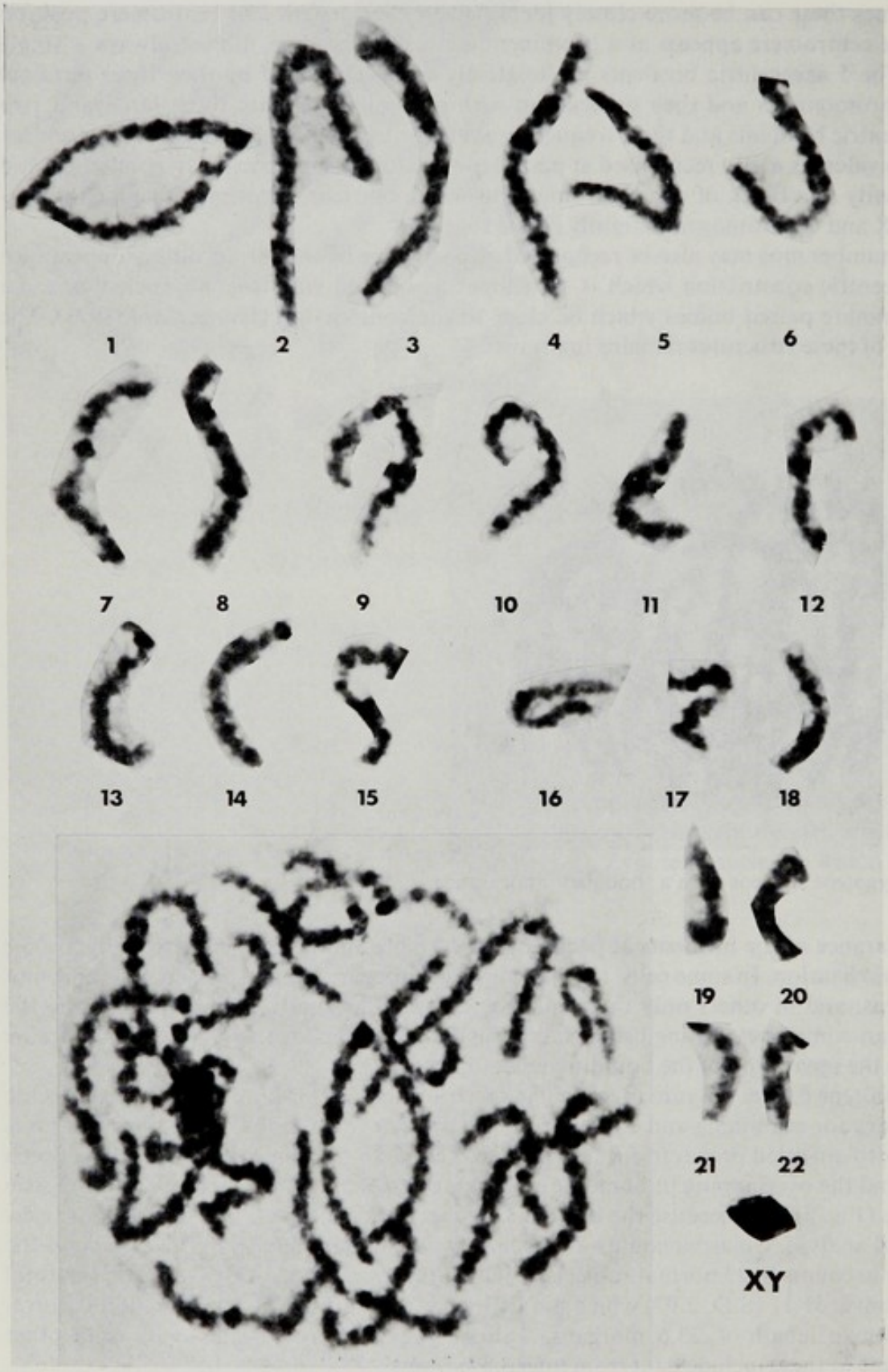


Fig. 4a. Pachytene. The sex bivalent appears as a dense mass of chromatin. Other bivalents can be arranged in order of decreasing length and centromere position into an approximate karyotype.

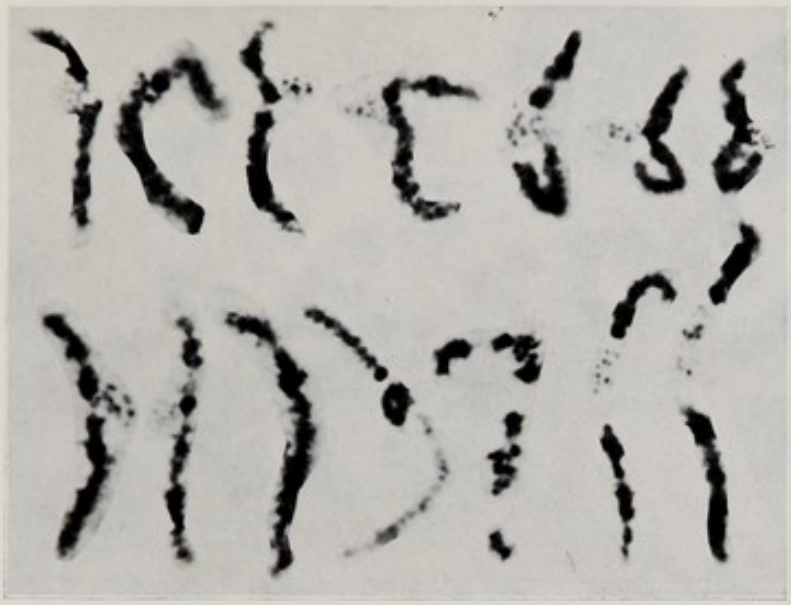


Fig. 4b. Pachytene. Bivalent 9 can be identified by its cluster of paracentric bodies.

TABLE 1 *Mean chiasma counts in human male meiosis. Control series from patients with apparently normal spermatogenesis*

Case	Age	Number of cells (total 849)	Mean total chiasma count	Standard deviation	% X,Y
1	25	12	50.50	5.39	16.67
2	27	25	45.28	2.82	8.00
3	27	26	55.12	3.22	15.38
4	28	11	53.09	4.77	9.09
5	29	36	52.61	4.03	13.89
6	30	12	50.08	3.63	41.67
7	30	36	49.00	4.98	25.00
8	31	22	51.23	4.34	18.18
9	31	14	49.22	6.46	33.33
10	32	16	51.44	3.85	25.00
11	33	118	48.47	3.37	1.60
12	34	63	53.97	3.92	13.85
13	34	48	53.50	4.26	4.17
14	37	18	51.28	4.57	27.78
15	39	15	54.53	5.77	0.00
16	39	8	55.25	4.99	20.00
17	49	12	51.50	5.00	16.67
18	49	13	53.23	6.17	15.38
19	50	44	50.18	5.33	25.71
20	53	57	54.82	3.57	29.82
21	65	20	49.15	4.68	45.00
22	67	13	44.62	2.66	46.15
23	71	12	47.42	3.58	50.00
Means	39.57	36.91	51.11 ± 2.97		21.84 ± 14.16

Data compiled by Brenda M. Page and M. A. Ferguson-Smith, Department of Genetics, University of Glasgow.

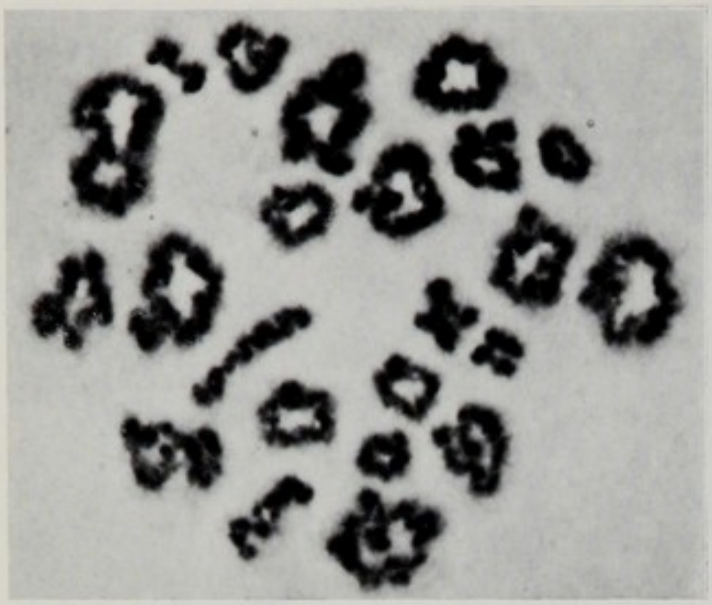


Fig. 5. Diakinesis.

TABLE 2 *Mean chiasma counts in human male meiosis*

Reference	Number of cells	Mean chiasma count
Ford and Hamerton, 1956	23	55.9
Sasaki and Makino, 1965	4	55.2
McIlree *et al.*, 1966*b*	96	53.4
Kjessler, 1966	692	52.4
Eberle, 1966	60	44.0
Falek and Chiarelli, 1968	8	52.3
Hultén and Lindsten, 1970	176	50.2
Luciani, 1970	88	47.5

Data from Hamerton (1971) and others.

chromosomes could have been misleading to those early workers in human meiosis who may have interpreted the 24 elements as 24 bivalents.

The sex bivalent deserves special comment. The short arms of the X and Y chromosome associate terminally, and there has been considerable speculation about the nature of this association and about which arm of the Y takes part. This latter question has been resolved by the quinacrine-fluorescence technique which shows clearly that the non-fluorescent short arm of the Y is invariably associated with the X (Pearson and Bobrow, 1970*a*). It is still not accepted by all investigators that there is true synapsis and crossing over between pairing segments of the X and Y. Despite the lack of evidence of partial sex linkage in man, I am persuaded that there are true pairing segments and that some associations at least have small terminal chiasmata. Supporting evidence of pairing has been claimed from the electron microscopy studies of Solari and Tres (1970).

Identification of the individual autosomal bivalents at diakinesis and first metaphase is made on the basis of size and centromere position. Secondary constrictions, which are frequently more exaggerated than in mitosis, are also helpful. Satellite regions tend to be heterochromatic. Caspersson and his colleagues (1971*a*) have recently applied the quinacrine-fluorescence technique and have shown that in favourable cells all bivalents can be recognised by their banding pattern.

The next characteristic stage to be identified in testicular preparations is the secondary

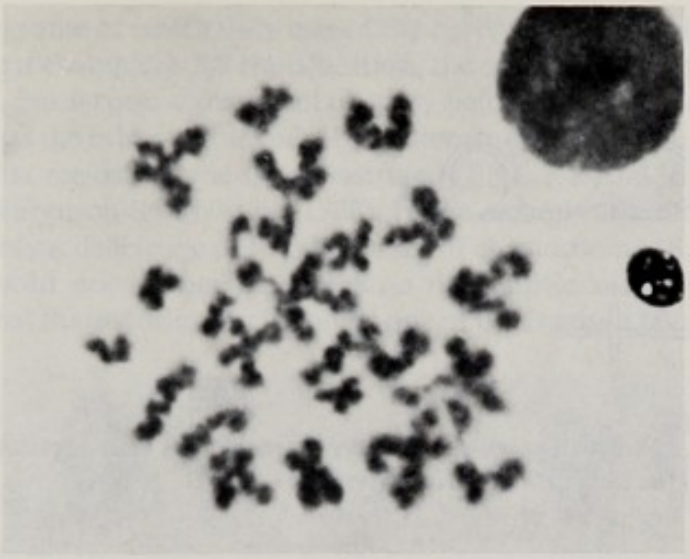


Fig. 6. Second metaphase. Note the heteropycnotic X (lower, centre) and chromosome 9 with its extended secondary constriction (lower, right).

spermatocyte in second metaphase (Fig. 6). Here the chromosomes more closely resemble mitotic chromosomes with the important difference that there are normally only 23 of them and also that they show relic coiling. The coiling and irregular condensation of the chromatids make karyotyping difficult. However, the more obvious chromosomes can often be distinguished, including the X or Y sex chromosome which is usually more heteropycnotic and less coiled than the autosomes. Chromosome 9 is frequently prominent because of its secondary constriction. After second metaphase there are, of course, no further divisions during the maturation of spermatids into sperm.

I hope the foregoing brief account of normal human meiosis in the male indicates at least the considerable limitations of the technique. Despite these limitations, certain facts emerge which are important for human cytogenetics:

1. The human chromosome map length is between 25 and 28 morgans.
2. The sex chromosomes pair by their short arms.
3. Certain individual bivalents can be identified throughout meiosis.

I would like to suggest that meiotic analysis can give important information for other current problems in human cytogenetics. Among these are:

I. The identification of aberrations undetectable by mitotic analysis.
II. The frequency of non-disjunctional gametes.
III. Meiotic defects as a cause of infertility.
IV. The nature of chromosomal polymorphism.
V. The non-randomness of chromosomal rearrangement.

I. IDENTIFICATION OF ABERRATIONS

Meiotic analysis can reveal some structural chromosome aberrations not detectable by conventional mitotic analysis. The most important of these are the reciprocal translocations. Only the most obvious translocations can be detected by mitotic analysis, that is those in which grossly unequal segments are exchanged. The reciprocal exchange of segments between non-homologous chromosomes is evident at first meiosis by the formation of a quadrivalent in place of two bivalents.

We have had the opportunity of studying meiosis in a number of reciprocal translocations

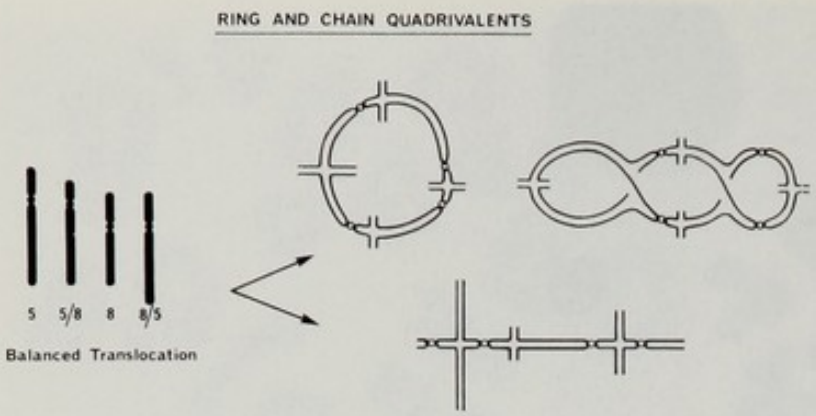


Fig. 7. Diagram indicating the types of quadrivalent expected from a reciprocal translocation between the short arm of chromosome 5 and the long arm of chromosome 8.

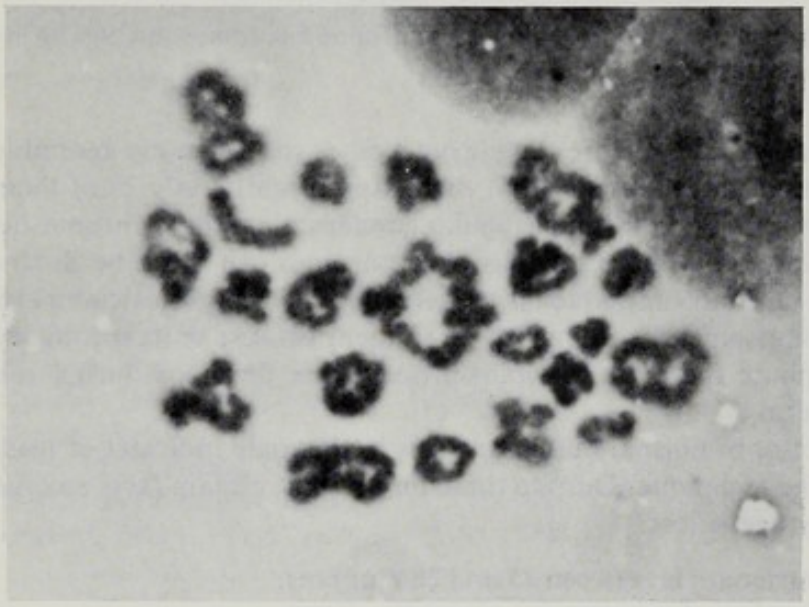


Fig. 8. Ring quadrivalent at diakinesis in a 5/8 translocation heterozygote.

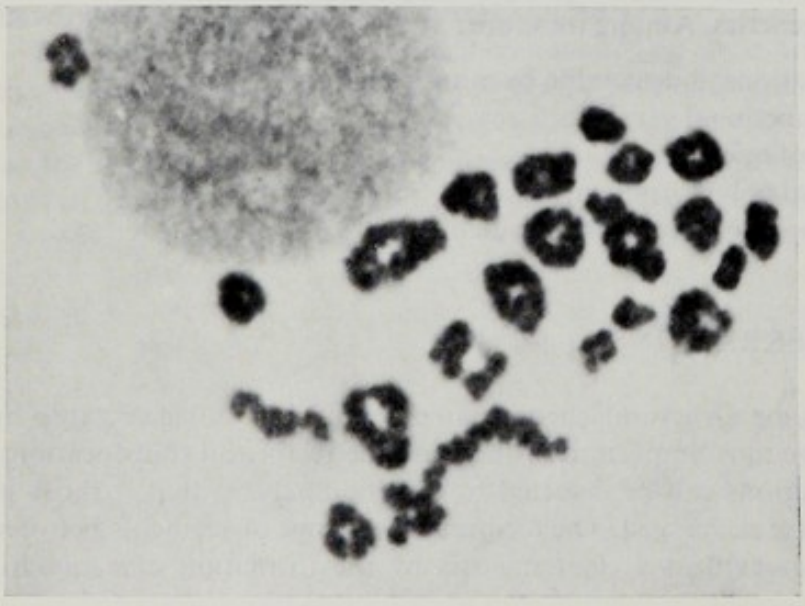


Fig. 9. Chain quadrivalent at first metaphase in a 5/8 translocation heterozygote.

in some of which only one of the chromosomes involved can be identified mitotically. In the first example, a 5/8 translocation, the exchange clearly involves the short arm of chromosome 5, but it took a great deal of study before we could show that the long arm of chromosome 8 was the other partner of the translocation*. One would expect ring and chain quadrivalents at first meiosis of the type illustrated (Figs. 7, 8 and 9) and indeed these were easily identified (Ferguson-Smith *et al.*, 1968). In the second case, the balanced translocation carrier showed only a deficiency of the short arm of chromosome 18, and the addition of this small segment could not be seen elsewhere on the mitotic karyotype. However, meiotic analysis showed that the quadrivalent involved one of the B group bivalents and the long rather than the short

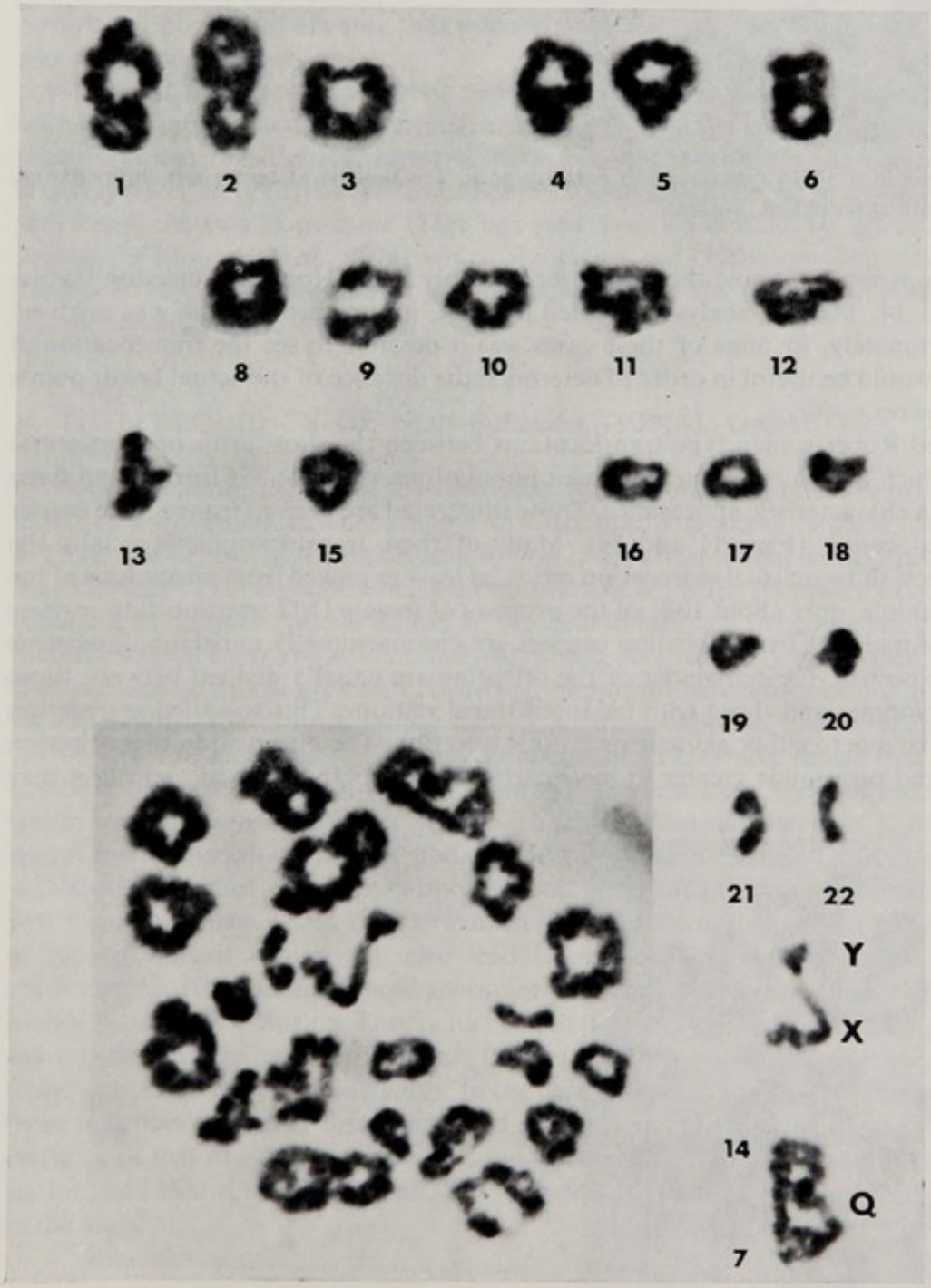


Fig. 10. Karyotype of a 7/14 reciprocal translocation at diakinesis.

* Since this paper was submitted, mitotic analysis by Giemsa banding shows that the chromosome involved is 10 and not 8.

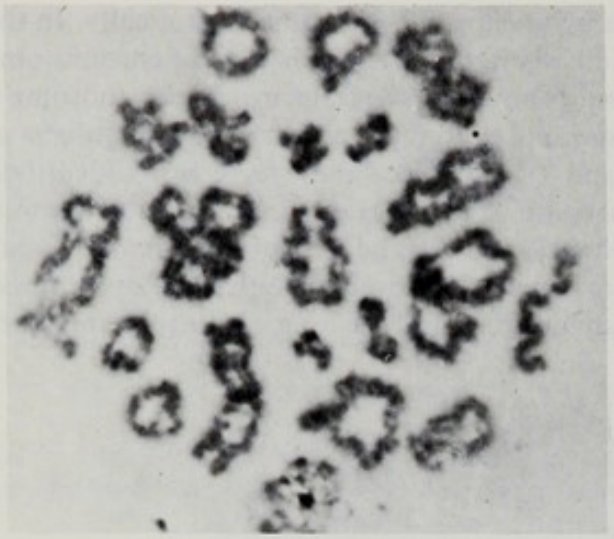


Fig. 11. Diakinesis in a 13/14 translocation heterozygote. Trivalent is at upper left, immediately above two apparently interlocked bivalents.

arm. A third case is similar in that the carrier showed only a small long arm deletion involving chromosome 14. Meiotic analysis revealed that a C group chromosome was involved (Fig. 10). Unfortunately, in none of these cases was it possible to see the translocation at pachytene. This would be useful in order to determine the distance of the actual break points from the centromeres.

In the so-called Robertsonian type translocations between the short arms of acrocentric chromosomes which are so common in human populations, a trivalent is formed and these again have quite a characteristic appearance. Those illustrated are derived from a male carrier of a 13/14 translocation (Figs. 11 and 12). Many of these translocations, especially the Robertsonian ones, show unusual segregation ratios, at least as judged from an analysis of the progeny. For example, only about 10% of the progeny of female D/G translocation carriers and about 4% of male D/G translocation carriers are chromosomally unbalanced, whereas 50% might be expected. The remainder of the offspring are equally divided between those with normal karyotypes and those with balanced translocations. This so-called segregation distortion could be due to either gametic or zygotic selection. The observation that selection against unbalanced progeny is greater in *male* carriers suggests that gametic selection may

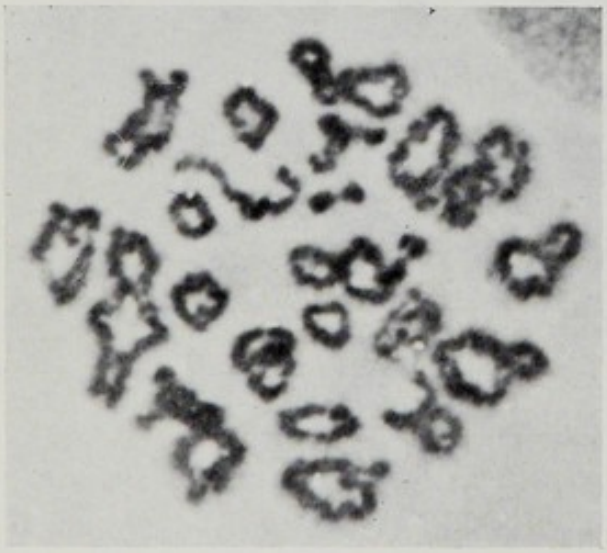


Fig. 12. Diakinesis in a 13/14 translocation heterozygote. Trivalent is at lower right, and shows the heteropycnotic short arms of chromosomes 13 and 14.

play some part. Evidence on this point could be obtained by determining in carriers the frequency of unbalanced secondary gametocytes at second metaphase. At present, the technical problems involved in obtaining a sufficient number of second metaphases make such an analysis difficult, but this should be possible in the near future.

Other types of chromosomal rearrangement should be detected and interpreted more exactly by meiotic than by mitotic analysis. The most obvious of these is the inversion, which produces a characteristic loop at pachytene. We have studied two families with obvious pericentric inversions in whom I have so far failed to persuade a male carrier to submit to testicular biopsy, and I know of no meiotic studies in other cases. Of course, in mitotic analysis in man there is no possibility of identifying inversions which do not cause a change in centromere position. I am sure that routine pachytene analysis will eventually turn up examples of interstitial inversions.

Meiotic analysis has also proved helpful in determining which G group chromosome is trisomic in mongolism. By pachytene analysis the two G bivalents can be distinguished by their chromomere patterns (Ferguson-Smith, 1964). One is clearly shorter than the other, and Hungerford *et al.* (1970) have shown that in an individual with mosaic Down's syndrome, the shorter of the two is trisomic. This has now been confirmed by quinacrine-fluorescence studies (O'Riordan *et al.*, 1971) which show that the G chromosome trisomic in Down's syndrome belongs to the shorter pair distinguished by the strongly fluorescent band in the proximal region of the long arm.

II. THE FREQUENCY OF NON-DISJUNCTIONAL GAMETES

Numerical abnormalities of the autosomes and sex chromosomes are probably among the most common genetically determined diseases and it is therefore particularly important to discover the factors which predispose to non-disjunction so that we may learn how to reduce the frequency of these conditions. One important factor is the frequency of occurrence of non-disjunctional gametes, and it should be possible to estimate the contribution of first meiotic non-disjunction by analysis of second metaphase. As previously mentioned, this type of analysis presents technical problems. However, important new information on the frequency of second meiotic non-disjunction has come from an unexpected source. Pearson and Bobrow (1970*b*), Barlow and Vosa (1970), and Sumner *et al.* (1971) have shown that Y-bearing sperm can be distinguished from X-bearing sperm by the presence of the fluorescent Y body in the sperm head. Moreover, Sumner and his colleagues showed that 1.26% of clearly haploid sperm carried two fluorescent Y bodies, and comparison of their DNA content with sperm with single Y bodies and sperm without Y bodies suggests that these exceptional sperm carry two Y chromosomes. These YY sperm must result from non-disjunction of the Y chromosome at second meiosis, which will also lead to an equal number of sperm carrying no sex chromosome. If these unbalanced sperm were successful in fertilisation, they would produce 47,XYY and 45,X offspring. The frequencies of the extra-Y syndrome and Turner's syndrome among the newborn are approximately 0.8 and 0.2 per thousand, respectively, which is much lower than expected on these figures. In the case of Turner's syndrome there is clear evidence from abortion studies of loss of affected embryos in early pregnancy, but there is no such evidence of loss of XYY embryos. It seems likely that YY sperm are less successful at fertilisation, and that the deficiency of XYY individuals is another example of genetic selection in the male.

III. MEIOTIC DEFECTS AS A CAUSE OF INFERTILITY

XXY Klinefelter's syndrome is invariably associated with sterility and it is well recognised

that about 11% of oligospermic males attending Infertility Clinics have this condition (Ferguson-Smith *et al.*, 1957). Mitotic studies of chromatin-negative infertile males have occasionally revealed other examples of aneuploidy, particularly XO/XY and other types of mosaicism. The meiotic studies of Kjessler (1966), McIlree *et al.* (1966*a*, *b*), Chandley (1970), Dutrillaux *et al.* (1971) and others including ourselves on infertile males also show that a small proportion of cases have significant abnormalities. The chromosomal abnormalities which have been found in association with male infertility may be summarised as follows:

1. Sex chromosome aneuploidy: XXY, X/XY and other mosaics, some cases of XYY.
2. Structural Y anomalies: Yq−, Y dic, etc.
3. Autosomal anomalies: 'supernumerary' chromosomes, trisomy, some translocations.
4. Defects in chiasma formation.
5. X-autosome translocations.

In our experience two main types of testicular pathology are associated with these genetic defects. X chromosome aneuploidy and structural abnormalities of the Y chromosome usually seem to be associated with failure of maturation and proliferation of foetal germ cells, so that the adult testis shows a striking and complete absence of germ cells. Failure of chiasma formation and some examples of autosomal trisomy, translocation and XYY tend to be associated with a maturation arrest of spermatogenesis. In this situation, spermatogenesis proceeds normally to a certain stage, usually to primary spermatocytes, where maturation seems to stop, so that very few later stages can be identified in the histological section. Meiotic analysis usually shows degeneration of cells in the late prophase of first meiosis with an absence or marked reduction of second metaphases.

One example of a long arm deletion of a Y chromosome (proved by the demonstration of normal Y chromosomes in first degree relatives) demonstrates the importance of the long arm of the Y for normal spermatogenesis. This patient showed the abnormal Y chromosome in all cells from lymphocyte and fibroblast cultures (Fig. 13). He is a normally developed male

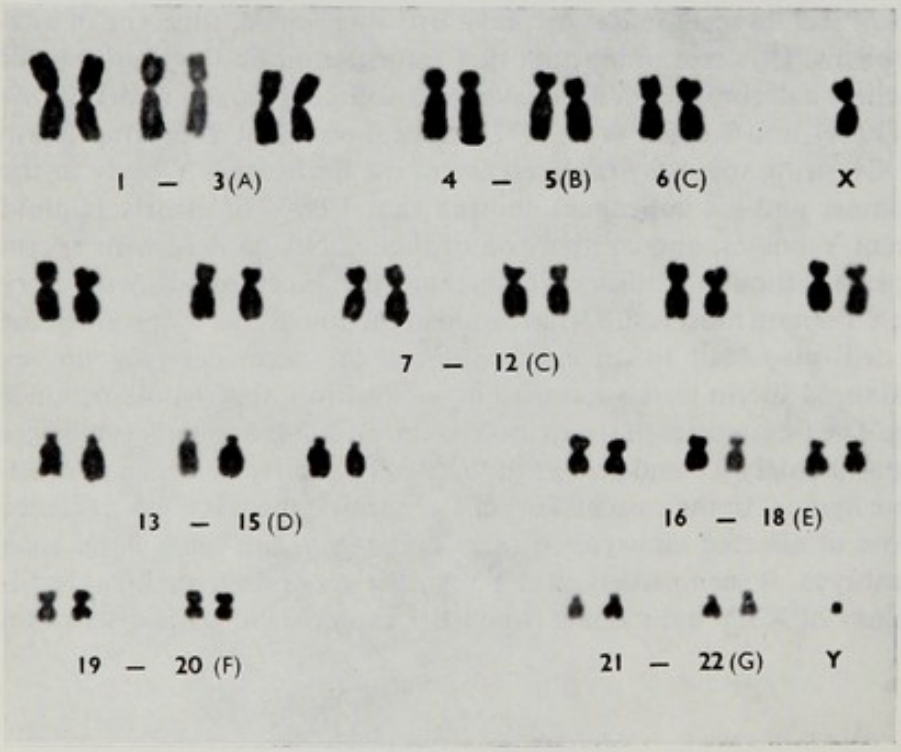


Fig. 13. Minute Y chromosome in a normally developed male whose testicular biopsy shows complete absence of germ cells. It is possible that long arm deletion of the Y is responsible for sterility, but translocation involving the Y cannot be excluded.

without stigmata of Turner's syndrome and testicular biopsy showed a complete absence of germ cells.

A number of oligospermic or azoospermic males have been described in whom supernumerary chromosomes have been found. Chandley (1970) describes meiotic studies in a case with typical maturation arrest, while the patient of Kjessler (1966) had absence of germ cells.

Pearson *et al.* (1970) and Hultén *et al.* (1970) have described cases of maturation arrest in which all stages up to diakinesis appear normal. At diakinesis there is a markedly reduced number of chiasmata and many bivalents show precocious disjunction as a result. Thereafter only a few cells proceed to maturation. In the patient of Pearson *et al.*, the failure of chiasma formation was associated with a defective DNA repair mechanism.

Most balanced translocations in human males do not present at infertility clinics and these individuals usually have normal sperm counts. However, McIlree *et al.* (1966*a*) describe two cases, one of whom had an obvious maturation arrest, in which quadrivalents were regularly found at diakinesis and first metaphase. Both cases had apparently normal karyotypes by mitotic analysis. It is not clear how the presence of these translocations caused the infertility. It is possible that one of the break points resulted in the deletion of a locus essential for spermatogenesis, or that the defect was due to a position effect. The cases seemed to differ in no other important respect to the majority of reciprocal translocations in whom infertility is not a problem, so it is difficult to postulate simply that the physical presence of a quadrivalent interfered with the segregation of chromosomes at meiosis to the extent of markedly reducing the number of viable gametes.

In mice balanced X-autosome translocations in the male are much more liable to be associated with complete sterility than autosomal translocations (Ford, 1970*b*). At least one example is known in humans in which males heterozygous for an X-autosome translocation are sterile (Buckton, *et al.*, 1971).

IV. THE NATURE OF CHROMOSOMAL POLYMORPHISMS

The extensive karyotyping of members of our species over the last few years has revealed some extraordinary examples of chromosomal polymorphism or normal variation. Some 5% of individuals show at least one example of chromosome variation in their karyotypes sufficiently striking to be used as a reliable chromosome marker in family studies. Elongation of the secondary constriction regions of chromosomes 9 and 16 and deletion of the short arm of a D group chromosome seem to be the most frequent of the polymorphisms revealed by routine microscopy. The quinacrine-fluorescence technique has revealed another class of variants, notably involving chromosomes 3, 4, 13 and 22, and although reliable figures are not yet available, these seem to be even more frequent than the others.

A number of claims have been made that the more striking of these variants are the result of reciprocal translocation (Court Brown *et al.*, 1966; Nusbacher and Hirschhorn, 1968). Meiotic analysis is therefore of interest in such cases. The two examples I wish to describe are extreme variants involving the secondary constriction regions of chromosomes 9 and 16.

In the case of the chromosome 9 polymorphism, some individuals show a marked elongation of the constriction site throughout meiosis (Page, unpublished data). At diakinesis this causes bivalent 9 to assume a strikingly asymmetrical appearance. At second metaphase cells with the secondary constriction in chromosome 9 in a normal, extended or asymmetrical appearance can be identified. It is presumed that the asymmetrical ones are the result of crossing-over between the polymorphic region and the centromere.

In the case of the chromosome 16 variant, there is also no evidence of translocation. The most striking feature is to be seen at pachytene where the centromeric block of heterochromatin is much enlarged (Fig. 14). This finding is consistent with duplication of part of the long arm.

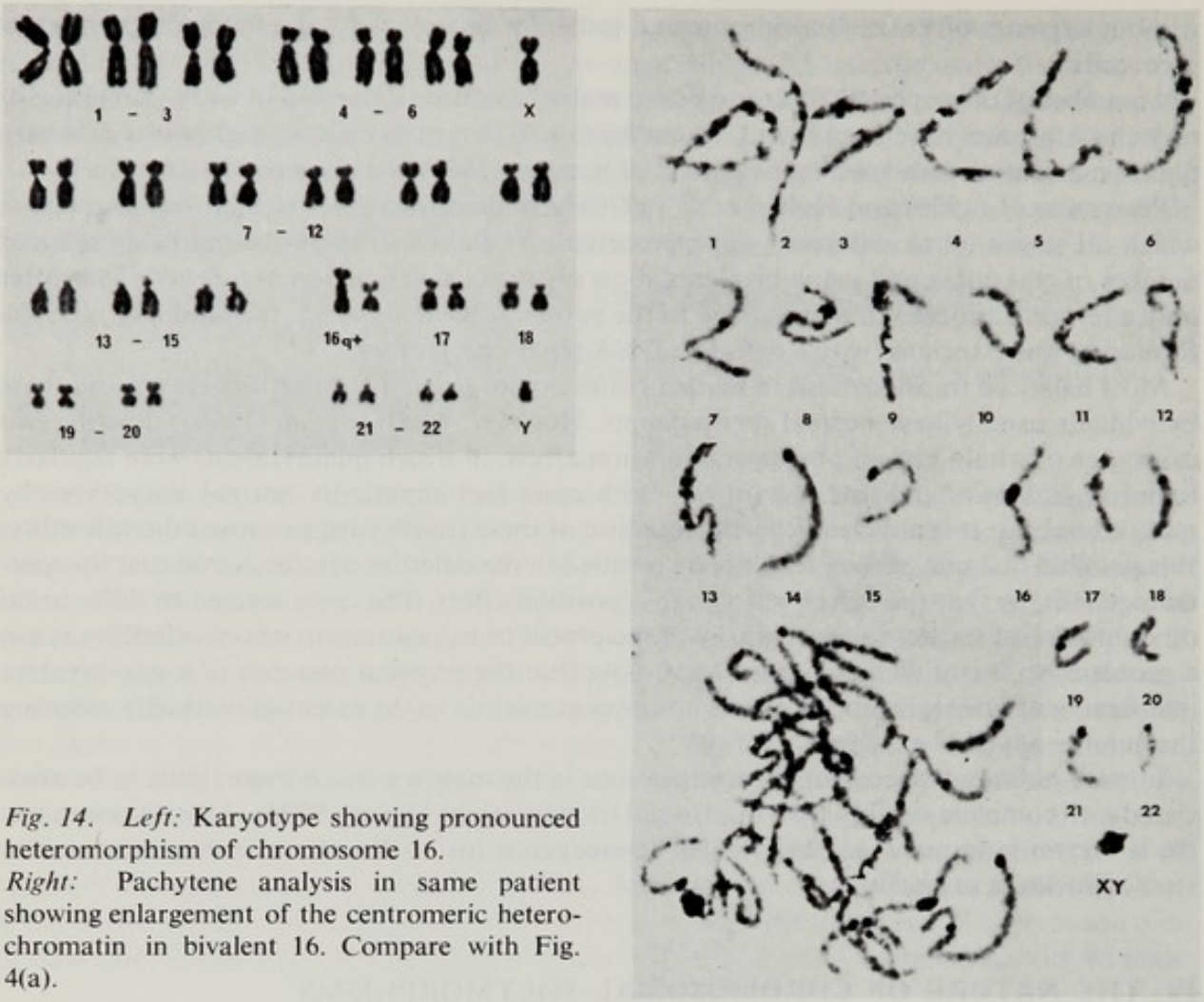


Fig. 14. Left: Karyotype showing pronounced heteromorphism of chromosome 16.
Right: Pachytene analysis in same patient showing enlargement of the centromeric heterochromatin in bivalent 16. Compare with Fig. 4(a).

V. THE NON-RANDOMNESS OF CHROMOSOMAL REARRANGEMENT

Those of us who have been interested in meiotic analysis cannot fail to be impressed by the frequency with which nucleolar bivalents seem to associate with one another during pachytene (Ferguson-Smith, 1964). It is also remarkable that these are the chromosomes which are most frequently involved in rearrangements, and which show the most marked polymorphism. Some of us have pondered on the possible significance of these observations and at least two hypotheses have been suggested to explain the relationship:

1. Nucleolar chromosomes associate with one another during mitosis and meiosis by *fusion* of their nucleoli. Nucleolar organiser sites may be more liable to breakage and reciprocal translocations are thus more likely to occur between chromosomes attached to a common nucleolus.

2. Nucleolus organiser sites are largely concerned with the mass production of ribosomal RNA, and are composed of repeated sequences which code for ribosomal precursors. Thus non-homologous chromosomes may have regions of homology at their nucleolus organiser sites. Non-homologous associations at meiosis could represent true synapsis between such regions, which then organise a common nucleolus. If true synapsis occurs, there is the possibility of rare accidental crossing-over between non-homologues which would lead to just the sort of rearrangement and polymorphism encountered (Ferguson-Smith, 1967).

I believe the accidental crossing-over hypothesis is worthy of serious consideration if only because it should be capable of being tested. There are two possibilities for the synapsis and accidental crossing-over between the short arms of two acrocentric chromosomes, depending

on whether the homologues pair 'side-by-side' or 'end-to-end' (Fig. 15). If the pairing is side-by-side, crossing-over could result in the transfer of a satellite variant from, for example, a D to G chromosome. Such an observation which has, as far as I am aware, not yet been made, would certainly be in favour of the hypothesis. Also, if the crossing-over were unequal, there could be duplication or deletion of material from either of the short arms. This, of course, would adequately explain the considerable polymorphism which is seen at these sites.

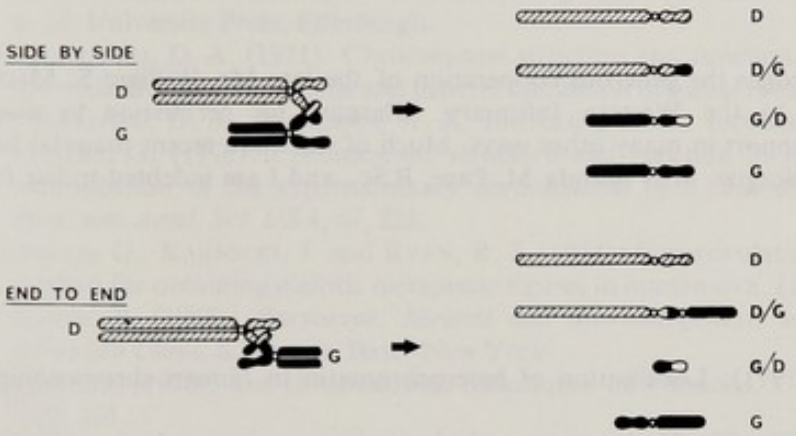


Fig. 15. The accidental crossing-over hypothesis to explain the marked polymorphism at satellite regions and the observation of double centromeres in some Robertsonian type translocations (see text). (From Ferguson-Smith, 1967.)

The situation is somewhat different if pairing between the short arms of acrocentric chromosomes occurs end-to-end, for crossing-over will produce dicentric and acentric recombinants. I believe that the strongest evidence in favour of this hypothesis is the observation that some of these translocations between the short arms of acrocentric chromosomes are dicentric. I have personally observed three obvious examples – two of them G/G translocations (Fig. 16) and the other a D/G translocation. I have also seen examples (although unrecognised) in the published illustrations of similar Robertsonian translocations. Of course, these dicentric translocations can only be recognised if there is a significant distance between the two centromeres, and I think it is of interest that in one D/G translocation reported by Barnicot *et al.* (1963), electron microscopy revealed a double centromere.

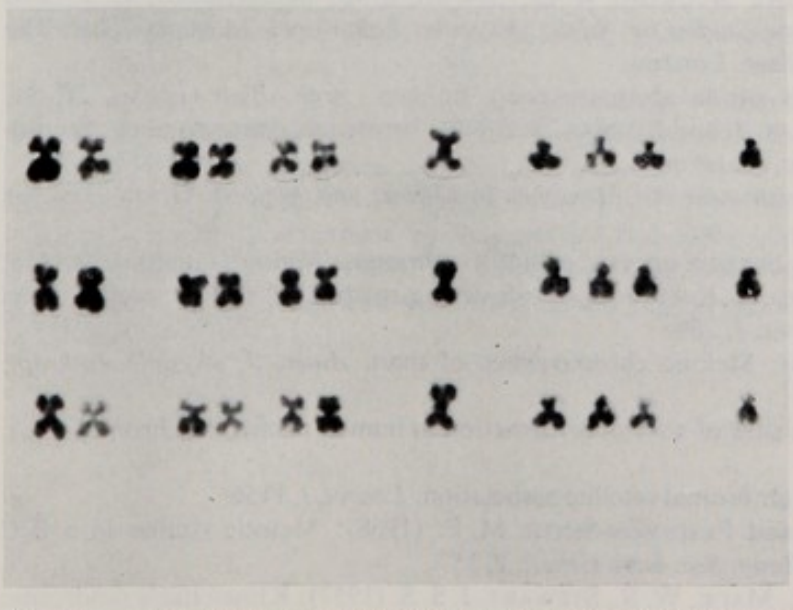


Fig. 16. An example of a dicentric G/G translocation in a case of Down's syndrome. Three partial karyotypes including only chromosome 16, and the F and G groups.

The accidental crossing-over hypothesis postulates that these polymorphic regions contain repeated sequences of DNA which are as similar between non-homologues as between homologues. In the case of the polymorphisms involving chromosomes 1, 9 and 16, one imagines that there might also be considerable opportunity for *unequal* crossing-over between homologues leading to visible duplication or deletion within the polymorphic region.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the generous cooperation of the late Mr. William S. Mack, F.R.C.S., Consultant Urologist to the Western Infirmary, Glasgow, for permission to study patients under his care and for support in many other ways. Much of the more recent material has been carefully prepared by my colleague, Miss Brenda M. Page, B.Sc., and I am indebted to her for a number of the illustrations.

REFERENCES

Arrighi, F. E. and Hsu, T. C. (1971): Localisation of heterochromatin in human chromosomes. *Cytogenetics*, *10*, 81.

Barlow, P. and Vosa, C. G. (1970): The Y chromosome in human spermatozoa. *Nature* (*Lond.*), *226*, 961.

Barnicot, N. A., Ellis, J. R. and Penrose, L. S. (1963): Translocation and trisomic mongol sibs. *Ann. hum. Genet.*, *26*, 279.

Buckton, K. E., Jacobs, P. A., Rae, L. A., Newton, M. S. and Sanger, R. (1971): An inherited X-autosome translocation in man. *Ann. hum. Genet.*, *35*, 171.

Caspersson, T., Hultén, M., Lindsten, J. and Zech, L. (1971*a*): Identification of chromosome bivalents in human male meiosis by quinacrine mustard fluorescence analysis. *Hereditas* (*Lund*), *67*, 147.

Caspersson, T., Lomakka, G. and Zech, L. (1971*b*): The 24 fluorescence patterns of the human metaphase chromosomes – distinguishing characters and variability. *Hereditas* (*Lund*), *67*, 89.

Caspersson, T., Zech, L., Johansson, C. and Modest, E. J. (1970): Identification of human chromosomes by DNA-binding fluorescent agents. *Chromosoma* (*Berl.*), *30*, 215.

Chandley, A. C. (1970): Chromosome studies on testicular cells of subfertile men In: *Advances in Experimental Medicine and Biology*: *X. Human Testis*, p. 151. Editors: E. Rosenberg and C. A. Paulsen. Plenum Press, New York, N. Y.

Court Brown, W. M., Jacobs, P. A., Buckton, K. E., Tough, I. M., Kuenssberg, E. V. and Knox, J. D. E. (1966): *Chromosome Studies on Adults. Eugenics Laboratory Memoirs XLII*. The Galton Laboratory, University College, London.

De Winiwarter, H. (1912): Etudes sur la spermatogenèse humain. *Arch. Biol.* (*Liège*), *27*, 91.

Dutrillaux, B., Le Lorier, G., Salat, J. and Rotman, J. (1971): Incidences des anomalies chromosomiques dans la stérilité masculine. *Presse méd.*, *79*, 1231.

Eberle, P. (1966): *Die Chromosomenstruktur des Menschen in Mitosis und Meiosis*. Gustav Fischer Verlag (Stuttgart), Göttingen.

Edwards, R. G. (1962): Meiosis in ovarian oocytes of adult mammals. *Nature* (*Lond.*), *196*, 446.

Evans, E. P., Breckon, G. and Ford, C. E. (1964): An air-drying method for meiotic preparations from mammalian testes. *Cytogenetics*, *3*, 289.

Falek, A. and Chiarelli, B. (1968): Meiotic chromosomes of man. *Amer. J. physiol. Anthrop.*, *28*, 352.

Ferguson-Smith, M. A. (1964): The sites of nucleolus formation in human pachytene chromosomes. *Cytogenetics*, *3*, 124.

Ferguson-Smith, M. A. (1967): Chromosomal satellite association. *Lancet*, *i*, 1156.

Ferguson-Smith, M. A., Boyd, E. and Ferguson-Smith, M. E. (1968): Meiotic studies in a B/C translocation heterozygote. *Bull. Europ. Soc. hum. Genet.*, *2*, 15.

Ferguson-Smith, M. A., Lennox, B., Mack, W. S., Stewart, J. S. S. (1957): Klinefelter's syndrome: frequency and testicular morphology in relation to nuclear sex. *Lancet*, *ii*, 167.

Ford, C. E. (1970*a*): Genetic activity of sex chromosomes in germinal cells. *Phil. Trans. B*, *259*, 53.

FORD, C. E. (1970*b*): The cytogenetics of the male germ cells and the testis in mammals. In: *The Human Testis*, p. 139. Plenum Press, New York, N.Y.

FORD, C. E. and HAMERTON, J. L. (1956): The chromosomes of man. *Nature* (*Lond.*), *178*, 1020.

HAMERTON, J. L. (1971): *Human Cytogenetics, Vol. I.* Academic Press, New York-London.

HULTÉN, M., ELIASSON, R. and TILLINGER, K. G. (1970): Low chiasma count and other meiotic irregularities in two infertile 46,XY men with spermatogenesis arrest. *Hereditas* (*Lund*), *65*, 285.

HULTÉN, M. and LINDSTEN, J. (1970): The behaviour of structural aberrations at male meiosis. Information from man. *Pfizer Medical Monographs 5, Human Population Cytogenetics, Edinburgh*, p. 24. University Press, Edinburgh.

HUNGERFORD, D. A. (1971): Chromosome structure and function in man. I. Pachytene mapping in the male, improved methods and general discussion of initial results. *Cytogenetics*, *10*, 23.

HUNGERFORD, D. A., MELLMAN, W. J., BACABAN, G. B., LABADIE, G. U., MESSATZZIA, L. R. and HALLER, G. (1970): Chromosome structure and function in man. III. Pachytene analysis and identification of the supernumerary chromosome in a case of Down's syndrome (mongolism). *Proc. nat. Acad. Sci. USA*, *67*, 221.

JAGIELLO, G., KARNICKI, J. and RYAN, R. J. (1968): Superovulation with pituitary gonadotrophins: method for obtaining meiotic metaphase figures in human ova. *Lancet*, *i*, 178.

KJESSLER, B. (1966): *Karyotype, Meiosis and Spermatogenesis in a Sample of Men attending an Infertility Clinic*. S. Karger, Basel-New York.

LUCIANI, J. (1970): Les chromosomes méiotiques de l'homme. I. La méiose normale. *Ann. Génét.*, *12/2*, 101.

MCILREE, M. E., PRICE, W. H., COURT BROWN, W. M., TULLOCH, W. S., NEWSAM, J. E. and MACLEAN, N. (1966*a*): Chromosome studies on testicular cells from 50 subfertile men. *Lancet*, *ii*, 69.

MCILREE, M. E., TULLOCH, W. S. and NEWSAM, J. E. (1966*b*): Studies on human meiotic chromosomes from testicular tissue. *Lancet*, *i*, 679.

NUSBACHER, J. and HIRSCHHORN, K. (1968): Autosomal anomalies in man. *Advanc. Teratol.*, *3*, 11.

O'RIORDAN, M. L., ROBINSON, J. A., BUCKTON, K. E. and EVANS, H. J. (1971): Distinguishing between the chromosomes involved in Down's syndrome (trisomy 21) and chronic myeloid leukaemia (Ph1) by fluorescence. *Nature* (*Lond.*), *230*, 167.

PAINTER, T. S. (1921): The Y chromosome in mammals. *Science*, *53*, 503.

PAINTER, T. S. (1923): Studies in mammalian spermatogenesis. II. The spermatogenesis in man. *J. exp. Zool.*, *37*, 291.

PEARSON, P. L. and BOBROW, M. (1970*a*): Definitive evidence for the short arm of the Y chromosome associating with the X chromosome during meiosis in the human male. *Nature* (*Lond.*), *226*, 959.

PEARSON, P. L. and BOBROW, M. (1970*b*): Fluorescence staining of the Y chromosome in meiotic stages of the human male. *J. Reprod. Fertil.*, *22*, 177.

PEARSON, P. L., ELLIS, J. D. and EVANS, H. J. (1970): A gross reduction in chiasma formation during meiotic prophase and a defective DNA repair mechanism associated with a case of human male infertility. *Cytogenetics*, *9*, 460.

SASAKI, M. and MAKINO, S. (1965): The meiotic chromosomes of man. *Chromosoma* (*Berl.*), *16*, 637.

SOLARI, A. J. and TRES, L. L. (1970): The three-dimensional reconstruction of the XY chromosomal pair in human spermatocytes. *J. Cell Biol.*, *45*, 43.

SUMNER, A. T., ROBINSON, J. A. and EVANS, H. J. (1971): Distinguishing between X,Y and YY bearing human spermatozoa by fluorescence and DNA-content. *Nature New Biol.*, *229*, 231.

TJIO, J. H. and LEVAN, A. (1956): The chromosome number of man. *Hereditas* (*Lund*). *42*, 1.

YUNCKEN, C. (1968): Meiosis in the human female. *Cytogenetics*, *7*, 234.

Kurt Benirschke

Department of Obstetrics and Gynecology, School of Medicine, University of California, San Diego, La Jolla, Calif., U. S. A.

Chimerism, mosaicism and hybrids*

This review is intended to highlight recent developments in our understanding of the complex process of mammalian chimerism and mosaicism, as well as aspects of interspecific hybridization. In general, only the spontaneously occurring events will be discussed in detail and only occasional mention made of the rapid advances made in our understanding of these phenomena through various *in vitro* techniques developed recently.

The terms chimerism and mosaicism are often used interchangeably, particularly in the older literature. For the purposes of this discussion I shall make a clear distinction, however, whenever possible between the two for, pathogenetically, the two conditions have presumably as different an etiology as they are genetically distinct phenomena. In accordance with the suggestion by Chu *et al.* (1964) the following definition is adopted and proposed for general future usage: A *mosaic* is an individual with cell populations of more than one genotype (*e.g.* karyotype) derived from a single zygotic genotype through mutational or zygotic events (*e.g.* somatic mutation, somatic crossing over, mitotic nondisjunction, etc.). A *chimera* is an individual with cell populations of more than one genotype arising through a mixture of different zygotic genotypes (*e.g.* transplantation, chorionic vascular anastomosis, double fertilization and subsequent participation of both fertilized meiotic products into one developing embryo, etc.). Russell and Woodiel (1966) suggest that true chimerism may be defined as the condition in which two separately derived *genomes* coexist in the same individual.

It will be apparent to anyone who has worked with this problem that often the distinction is difficult to make. Nevertheless, if possible it is recommended that attempts to differentiate the conditions be undertaken and that cases be properly designated as chimeras, mosaics, or as admixtures of unknown origin to clarify what is now a confusing field. The subject has been reviewed in greater detail recently (Benirschke, 1970).

So far as hybrids are concerned, the term connotes originally the offspring of a tame sow and wild boar (Oxford) but is now generally applied to the offspring of two species. Its definition hence rests on the acceptance of species designation, a most controversial field (Mayr, 1963). Nevertheless, for our purposes here, where generally indisputable species will be considered, no further definition may be necessary. More detailed considerations will be found in some recent reviews (Benirschke, 1969).

CHIMERISM

Spontaneous mammalian chimerism was first described by Owen (1945) in his discussion of the 'Immunogenetic consequences of vascular anastomoses between bovine twins', the

* Support by a grant (RF 70029) from the Rockefeller Foundation is gratefully acknowledged.

freemartin problem. Unfortunately, he termed the condition mosaicism, which should not detract from this fundamental discovery of permanent antigenic tolerance among fraternal cattle twins on which much of modern immunology is based.

It is useful to consider three types of chimerism separately: (1) blood chimerism, as in fraternal twins and due to placental anastomoses and perhaps extending to germ cells; (2) transplacental chimerism; and (3) what I like to call 'whole-body chimerism', an event of totally different morphogenetic consequences.

A. BLOOD CHIMERISM

When fraternal twins or higher multiple births develop anastomoses between their placental circulations their bone-marrow-like nucleated circulatory cells pass from one to the other, 'home' for the marrow spaces, and induce permanent blood chimerism. Presumably because the event involves nucleated cells and takes place before immunologic competence develops in the fetus, a state of permanent immunologic tolerance ensues. Blood chimerism was first detected and is best known in cattle twins, but it also occurs regularly in South American marmoset monkeys, less commonly in sheep, goat and swine, and uncommonly in man. This biologic phenomenon has attracted great interest not only because of the immunologic consequences but also because of the freemartin effect observed in cattle, sheep, goat and swine. In man and in marmosets, no sterilization or sexual modification ever takes place under what appear to be identical embryologic events when compared with those in ruminants. It cannot be emphasized too much that any explanation of the freemartin effect must ultimately also be capable of being reconcilable with this absence of sexual modification in primates. None has been forthcoming to date. The original hypothesis of a purely circulatory endocrine cause of the modified female genitals has been countered by failure to reduce Müllerian ducts by exogenous testosterone (Jost *et al.*, 1963; Jainudeen and Hafez, 1965). The very attractive hypothesis of Witschi (1965) and Jost (1970) of a separate medullary antagonist to emanate from the fetal testis, *i.e.* a dual system of endocrine control substances, also fails to explain why such an effect is absent in primate chimeras. It was next hypothesized that the chimerism *per se*, *i.e.* the XY cellular admixture in the female, caused the sex modification. It was never succinctly stated just how this mechanism might be operative nor whether gonadal chimerism was a prerequisite (Herschler and Fechheimer, 1967; Goodfellow *et al.*, 1965). Suffice it to say, XY cells have not been identified regularly in freemartin gonads nor in any other of their solid tissues. Furthermore, this hypothesis again does not take into account how human and marmoset chimeras should be exempt from the action of chimeric Y chromosomes. It has also been suggested that chorionic fusion occurred perhaps later in primates than in ruminants and that primate fetuses might thus escape the deleterious action of chimerism during their genital formative period. It has now been shown, however, that marmoset chorionic fusion occurs prior to angiogenesis (Benirschke and Layton, 1969) and at the time of gonadal differentiation well established interplacental anastomoses were depicted by Wislocki (1939). A temporal effect is thus not the base of this important difference between these two orders.

My personal bias is still in favor of a strictly hormonal mechanism. I envisage fetal endocrine relations and the 'feto-placental unit' to be quite different in primates as opposed to ruminants. As a first step to further elucidate the mechanism we have recently determined the blood testosterone content of a large number of cattle fetuses and plan to analyze their androstenedione content next. A significantly higher testosterone level was found in male fetuses from early gestation on. Near term the female and male testosterone levels showed little difference (Kim *et al.*, 1971). It is obvious that these results are not a satisfactory explanation for freemartinism as yet; the levels of primate fetuses might first be explored in a similar manner. However, I feel that these data are ultimately more important than results

TABLE 1 *Human blood group chimerae*

No.	Author	Sex	Proportion of		Age	Saliva	Lymphocyte culture
			RBC %	GPS			
1.	Dunsford *et al.* (1952)	F	61	O		No A	
			39	A	25		
		M	?			?	
2.	Booth *et al.* (1957)	F	99	O	21	H	
			1	A			
		M	14	O			
			86	A		A, H	
3.	Nicholas *et al.* (1957)	F	49	O		H, trace Le^a	
			51	A	29		
		M	39	O		No A, H	
			61	A		Strong Le^a	
4.	Ueno *et al.* (1959)	F	68	O		A, H	
			32	A	12		
		M	100	A		A, H (weak)	
5.	Turpin *et al.* (1959)	F	100	O N_s N_s			
		F	95	O MNS	Infants	Temporary chimerism	
			5	O N_s N_s			
6.	Velez-Orozco (1961)	F	5	A	Adult	?	
			95	B			
		M	?				
7.	Hartemann *et al.* (1963)	F	10	O	Newborns	?	
			90	A			
		F	20	O			
			80	A			
		F	85	O			
			15	A			

TABLE 1 *(Continued)*

8.	Chown *et al.* (1963)	F	85	O	Newborns	O	70% XY
			15	A			30% XY
		M	85	O		A	78% XX
			15	A			22% XY
9.	Van de Hart and Van Loghem (1967)	M	99.8	O	18	H	37 XY
			0.2	B			1 XX
		F	20	O		B, H	32 XY
			80	B			9 XX
10.		F	99	O	17	A, H	
			1	A			
		F	99	O			
			1	A		H	
11.		M	99.7	A	20	?	
			0.3	B			
12.	Bias and Migeon (1967)	M	95	A	Newborns	?	153 XY Tri 21
			5	B			11 XY
		M	100	B			94 XY
13.	Massimo *et al.* (1966)	M		OS	Infants	Temporary	XY Tri 21
		M		OS		chimerism	XY Tri 21/XY
14.	Crookston *et al.* (1970)	F	50	A	35	H	50% XX
			50	O			50% XY
		M	99	A		H, A	99% XY
			1	O			1% XX

from exogenously administered androgens whose concentration in the fetus remains unknown to us at the time of sex differentiation. As 60 years ago, freemartinism, nature's 'experiment of surpassing interest' (Lillie, 1916), eludes us with respect to its precise pathogenesis.

Several other aspects of blood chimerism have been of recent interest. Thus, it has been observed that the admixture ratio in the chimeric twins changes with advancing age, both in man (Dunsford and Stacey, 1957) and in cattle (Stone *et al.*, 1964). While it was tempting to suggest an immunologic mechanism for this phenomenon, it must be borne in mind that equally possible is the elimination of grafted clones with advancing age due to irregular fat replacement of the marrow space. More detailed work in this area is required before immunologic factors can be held responsible for the shifts in admixture rates.

Ohno and colleagues (1962) showed first that newborn chimeric male testes of cattle contained a few XX cells and presumed these to be germ cells since no marrow elements reside in the testes. Similar XX cells can be found in adult marmoset testes (Benirschke and Brownhill, 1963), the only other species studied so far. Embryologic support for the occasional intravascular dissemination of germ cells in mammals, an event regularly found in birds, has been published (Gropp and Ohno, 1966). Naturally, this type of chimerism has caused considerable interest since the fate of such cells is unknown. Would they mature into female cotwin-derived spermatozoa or, if similar germ cells come to the ovary from a male twin, could these develop into ova? Positive findings have not been made for either assumption. In part, the difficulty has been to identify positively the chimeric germ cells to their final destination. In breeding experiments only once has a change in sex ratio been found in the offspring from bulls that were twins to freemartins (Lojda and Černý, 1970) and the female genotypes have not yet been detected in the offspring (Ohno, 1969). So far, however, it must be admitted that the negative findings have not been conclusive. One would like to identify by genetic markers or by chromosome morphology the haploid derivatives of such chimeric germ cells. Unfortunately, no fluorescent Y chromosome markers are found in cattle or marmoset Y chromosomes. One may hope that chromosome banding techniques recently described may enable further study of meiotic chromosomes in the future. At least it would be a worthwhile study to ascertain positively whether heterosexual mammalian germ cells can be directed by their environment *in vivo* as suggested by the *in vitro* experiments in mice of Turner and Asakawa (1964). A first step in this direction has been the study of first meiotic metaphases in such chimeric testes. Unequivocal XX mitoses are regularly found and complete cells lacking an XY bivalent (hence interpreted as XX cells) are occasionally encountered in marmosets (Benirschke and Brownhill, 1963) and bulls (Teplitz *et al.*, 1967). These are few and represent only indirect evidence. XY germ cells in marmoset ovaries have not been looked for and no XY cells of any type have been found in freemartin gonads. Weiss and Hoffmann (1969) report a gradual elimination of such cells from the testes of chimeric bulls which further reduces the chances of functional germ cell chimerism.

Studies of the blood chimerism in marmosets have supported the experience from cattle and man that the tolerance of blood cells extends to other tissues, as skin. Switchgrafts of skin in five fraternal marmoset twins was tolerated over one year in Porter's (1968) experience. In these species multiple births with chimerism are apparently the rule and make blood grouping difficult, as nonchimeric individuals have not yet been identified. In 261 pregnancies observed by Gengozian and Merritt (1970) 77% were twins, 5% triplets and 18% singletons. The singletons, however, were often chimeric as well, and fetal death of one twin has been observed to account for this phenomenon. Often such dead fetuses are resorbed. Therefore, apparent singletons may still be chimeric, an important observation to be borne in mind when other chimeras are observed. Of final interest is that attempts to induce uniparity by unilateral maternal oophorectomy have not been successful in the hands of these investigators.

To date only fourteen sets of human blood chimeras (Table 1) have been reported in which the data are acceptable and whose origin must be inferred to be a placental communication with a twin. In two instances the chimerism was only temporary. As is readily seen, there is no

consistent admixture ratio and not all twins show a preponderance of one cell line from one of the twins as has been suggested for other chimeras. Happenstance size of chorionic anastomoses and their type presumably determine these results. It is surprising that no such placentas have yet been described. While Cameron (1968) demonstrated anastomoses in two sets of dichorial twins in his Birmingham twin survey, these were ultimately shown to be monozygous in origin. Nylander and Osunkoya (1970) reported a similar unusual placenta without, however, demonstrating chimerism in the twins. A more detailed study of fraternal twins is thus warranted in the future. Clearly, isosexual chimeras must exist as frequently but only one such instance has been recorded (Table 1). Finally, why it is that some species have chorionic fusion so regularly as marmosets and cattle, others often, as sheep, and some only rarely, as swine and goat, is completely unknown. Original proximity of blastocysts at implantation is hardly the ultimate cause, since most human multiple births with fused placentas share no vessels, and 'stuffing' the uterus of mice with blastocysts, as undertaken by McLaren and Michie (1959) yielded no certain fusion of vessels.

B. TRANSPLACENTAL CHIMERISM

Transfer of fetal red cells to the mother occurs regularly during pregnancy at various times and in small quantities. Larger, occasionally fatal hemorrhages including maternal transfusion reaction have been reported rarely (Benirschke, 1970). Therefore, fetal lymphocytes also must reach the mother and Walknowska *et al.* (1969) have identified XY cells in maternal lymphocyte cultures, maximally 1.5% as early as during the 14th week of pregnancy. One can confidently predict that the quinacrine method will soon be widely used on similar material for prenatal sex diagnosis. Such transferred fetal lymphocytes are presumably only temporary passengers, to be rejected by the mother ultimately; at least permanent chimerism is not currently expected. Other fetal cells, such as neuroblastoma metastases, may reach the placenta but they do not induce a state of chimerism by metastasizing into the mother (Strauss and Driscoll, 1964), except for the well documented chorionic placental tumors. Choriocarcinoma may metastasize to mother and fetus simultaneously, and it represents of course in the mother a chimeric graft.

Of maternal tumors only the melanoma is known to become a chimeric transplant to the fetus, other metastases being retained by the placenta, with the possible exception of a single case of lymphosarcoma (Potter and Schoeneman, 1970). Many investigators have searched for maternal blood elements in human and rodent infants; their findings are controversial and not uniformly accepted. Leukemia cells have not unequivocally been found in the newborn circulation despite often impressive intervillous deposits. Certainly, no newborn with congenital leukemia due to transplacental chimerism has been observed. On the other hand, various reports have suggested the presence of maternal lymphocytes grafted onto the fetus transplacentally. The possibility has not only theoretical but practical importance. Kadowaki *et al.* (1965) described in the lymphocyte cultures of a male infant with thymic alymphoplasia XX cells and a clinical picture which is consistent with graft (XX) *vs.* host (XY) reaction. Only lymphocytes were chimeric, the marrow and skin were purely XY, a finding that rules out a missed twin gestation or whole-body chimerism. Indeed, maternal lymphocyte transfer seems the only tenable explanation for this case. It must be emphasized that recognition of this chimera was possible only because of the fortuitous male sex of the infant. In other deficiency cases with possible chimerism the graft-*versus*-host reaction may be inexplicable when female infants are involved. This is discussed by Githens *et al.* (1969) who describe another lymphoid chimera with thymic alymphoplasia in whom the graft appeared to be immunologically inert.

It is not surprising then that direct studies by culture have been undertaken in male newborns. We found a few presumed XX cells in three of four males in blood aspirated from placental surface vessels (Benirschke and Sullivan, 1968). At six weeks of age none were de-

TABLE 2 *Human whole-body chimerae*

	Author	Clinical conditition	Lymphocyte culture	Fibroblast culture	Gonadal culture	Other evidence of chimerism	Other unusual findings
1.	Gartler *et al.* (1962)	Ovary; ovotestis; enlarged clitoris	XX/XY	XX/XY	Ovary XX Ovotestis XX/XY Clitoris XX/XY	2 populations of red cells	Heterochromia of iris
2.	De Grouchy *et al.* (1964)	Ovary; ovotestis; enlarged clitoris	XX/XY	XX/XY	–	? Haptoglobin mixture	–
3.	Corey *et al.* (1967)	Hypospadias; bifid scrotum; ovary; descended ? testis	XX/XY	XX/XY	Ovary XX	2 populations of red cells; ? haptoglobin mixture; phospho-glucomutase mixture	Pigment mottling with striking laterality
4.	Zuelzer *et al.* (1964)	Normal blood donor; normal testes; gynecomastia	XX/XY Many tetraploids	XX/XY Light skin only XY Many tetraploids	–	2 populations of red cells; including sick-ling; Lewis secretor genotype mixture	Pigment mottling; predominantly male cells. XX clone assumed to derive
5.	Myhre *et al.* (1965)	Hypospadias; congenital heart disease; abnormal testes	XX/XY	Inferred XX clone from buccal smear	–	2 populations of red cells	Maternal family history of 2 dizygous twin pairs

TABLE 2 *(Continued)*

6. Lejeune *et al.* (1967)	Hypospadias; 'triploid anomaly'; testes	2nXX/3nXXY	XX/XXY in skin, liver, fascia	Testis XX/XXY	2 populations of red cells	Triploid line smaller; heterochromia of iris; macerated twin with separate placenta
7. Race and Sanger (1968)	Normal (Durban case)	XX			2 populations of red cells	Extensive skin mottling
8. Race and Sanger (1968)	Bil. ovotestes; hypospadias (Glasgow case)	XX/XY	XX		2 populations of red cells	
9. Klinger *et al.* (1970)	Hypospadias; gynecomastia	XX/XY			2 populations of red cells	
10. Park *et al.* (1970)	Ambiguous genitalia; ovary; ovotestis	XX/XY			2 populations of red cells; haptoglobin mixture	
11. Present case	Clitoral enlargement; ovary; ovotestis	XX/XY		Ovary XX/XY	2 populations of red cells	

tected in the same infants. Conversely, Sharpe (1970) and Olding (1971) in very careful studies of 6 and 14 cases, respectively, with 3000 and 1772 cells analyzed, found no female cells. The difference in these results may be that our blood was collected from the delivered placenta, the cord having been clamped; Olding (1971) on the other hand collected from the cord before placental delivery. It is possible that maternal cells are forced into the fetal placenta vessels, perhaps into the fetus only terminally during delivery. At least permanent transplacental chimerism of the fetus must be exceptional in man and the observations of El-Alfi and Hathout (1969) support this view. In their newborn 22 XX and 28 XY lymphocytes were found; at age five months only XY cells were left. Similarly, Eimer and Weiland (1969) find maternal erythrocytes by differential agglutination in newborns after labor, but not in primary cesarian section delivered infants.

It was thus all the more surprising when Tuffrey *et al.* (1969*a*) reported transplacental chimerism up to 33% in transplanted mouse blastocytes and later (1969*b*) also in normally derived embryos, although here in much smaller proportions. Subsequent investigators, employing superior techniques, have refuted these findings and it can now no longer be assumed either that (*a*) a substantial number of cells are transferred normally from mother to fetus or that (*b*) permanent chimerism is established in normal infants or rodent fetuses (Billington *et al.*, 1969; Seller, 1970). In passing it may be mentioned that the increasingly frequent practice of intrauterine transfusion has led to blood chimerism up to at least two years (Hutchinson *et al.*, 1971). Nevertheless, with the possible exception of one case (Naiman *et al.*, 1969) no graft-*versus*-host reaction seems to have occurred due to the presence of prenatally transfused lymphocytes for prolonged periods in newborns.

C. WHOLE-BODY CHIMERISM

This anomaly is easily the most interesting type of chimerism, but also it is the most difficult to separate succinctly from mosaicism. Indeed, in most cases reported as mosaics no such attempts at differentiation were undertaken and the nature of admixture of cell lines with different chromosome constitution remains usually in doubt.

True chimerism has been identified among many mammals and of course is well described in other forms, for instance in Hymenoptera (Cooper, 1959). Almost invariably this admixture is recognized because of sexual abnormalities such as true hermaphroditism or because of an unusual phenotype, as in tricolored male cats, or hemihypertrophy in man. Consequently, most often heterosexual admixtures are found in karyological analysis. Only once, in a mouse, has isosexual chimerism been identified because this XX/XX chimera produced unexpected genotypes in her litters (Russell and Woodiel, 1966). It is expected that many more isosexual chimeras exist whose recognition presents a challenge to human geneticists, primarily to individuals engaged in sophisticated blood group studies. In such chimeras the admixture of cells extends potentially to all organs, although the actual admixture ratio varies widely from tissue to tissue. A pertinent case came to our attention recently:

Case: Miss O.S. was identified as having abnormal genital development by the immigration examiner and was referred at age 20. She had normal menses, markedly enlarged clitoris, underdeveloped breasts, and some excessive hair growth. Buccal smears contained 12-18% Barr bodies and lymphocyte culture showed normal female karyotypes (5 karyotypes). Clitorectomy and removal of a right ovotestis was undertaken, the left gonad appearing to be a normal ovary. Interestingly, adjacent to her infertile testis was normal ovarian tissue with an active corpus luteum. Her hypertrichosis diminished and she came to our attention three years later. At that time lymphocyte cultures showed an admixture of XX and XY cells. The availability of atabrin fluorescence microscopy at that time much lightened the task of enumeration. In several hundred cells analyzed there were 15% Y-positive lymphocyte nuclei, 12% positive lymphocyte metaphases, 0% positive buccal cells, 15% positive hair follicle cells and, from a left ovarian biopsy culture, 3% positive cells were identified. Unfortunately, we did

not succeed in discriminating between XY and other cells in the formalin fixed and paraffin embedded testis material. Blood grouping was kindly undertaken by Dr. F. H. Allen (New York) who found a 50% admixture of red cells differing as Fy (b+) and Fy (a+b+). The patient was not a twin nor did she have any skin or iris color markings that might have provided a clue to her chimeric state.

There are now eleven individuals on record whose studies clearly indicate that they were whole-body chimeras (Table 2). That is to say, these persons had two lines of cells (XX/XY), some sexual developmental error, *and* genetic evidence of two lines of cells, such as two blood groups, haptoglobin types, etc. Of course, this is a highly arbitrary separation from the other four or five cases of XX/XY admixture in whom despite search no evidence of genetic chimerism was found. There may merely not have been enough deviant parental markers (present or studied) to enable one to make the differential diagnosis. Similarly, the individuals with diploid/triploid admixture whose genesis is almost certainly similar, cannot be accepted as chimeras with certainty, although a different background for their state of admixture is difficult to envisage.

Several exhaustive reviews of true hermaphroditism have shown that a majority of patients have an XX chromosome constitution, some are XY, others are admixtures of various types. The cumulative evidence indicates, however, that because of the often very minor population of one line or another, and variably distributed through the body, caution must be exercised when interpreting the data. The fluorescent technique, as used in the present case, should aid significantly in the future study of such patients. Nevertheless, just as XX men exist with certainty (Boczkowski *et al.*, 1969) such chromosome constitution may appertain to true hermaphrodites and is surely the karyotype in the three siblings described by Rosenberg *et al.* (1963).

With more widespread chromosome studies of diverse mammals, chimerism has been recognized often, and again most frequently in sexually abnormal animals. Among cats the phenotype tortoiseshell (black and yellow) demands two X chromosomes and is therefore most commonly seen in females (Jones, 1969). Rarely it occurs in infertile males and it is of historic interest that Sprague and Stormont (1956) suggested 'mosaicism' as the most likely cause of this unusual condition. Of the 19 male calico cats studied since chromosome morphology became possible 16 proved to be chromosomal admixtures; only 3 had an XXY constitution (Table 3). Of interest is further the frequency of 2n/3n cats of which five have been reported. One fertile male tricolored cat is presumably an XY/XY chimera according to Ramberg *et al.* (1969). The latter would be the only presumptive true chimera since coat color in all the others could be accounted for by the XX line. Nevertheless, the difficulty of explaining XX/XY mosaicism, as opposed to the case of accounting for this admixture assuming chimerism, leads me to assume the latter condition. Of further interest is that most of these cats had testes, not ovotestes as is the case in similar chromosomal conditions in man or the single well studied 2nXX/3nXXY true hermaphrodite mink reported by Nes (1966). A most interesting intersex horse has also recently been described by Basrur *et al.* (1969) in whom only one kryptorchid testis was present. The complex XO/XY/XXY/XXY mosaicism was initially explained by a complicated mitotic nondisjunctional sequence; subsequent culture and blood grouping studies (Basrur *et al.*, 1970), however, clearly show this animal to be an XX/XY chimera and indicate not only the value of these accessory studies but also the perhaps greater likelihood of chimerism than complex mitotic nondisjunction.

True hermaphroditism and other intersex states have been described in many other species, pig, rhesus, dog, goat, mouse, etc., and if chromosome studies have been carried out they were usually performed only on leukocyte cultures. When an admixture was found, blood chimerism of the freemartin type was not differentiated from the whole-body type, a necessity for future studies if greater insight is to be gained on the frequency of chimerism in mammals. Finally, it is important to note that the condition has been described in at least three bovines. Dunn *et al.* (1968) presented an XX/XY true hermaphrodite calf with male phenotype and an

TABLE 3 *Male tortoiseshell cats*

No. Author	Chromosomes	Chimeric ratio
1. Thuline and Norby (1961)	39 chromosomes	presumed XXY
2. Thuline and Norby (1961)	39 chromosomes	presumed XXY
3. Chu *et al.* (1964)	38 XX/57 XXY	62/209
4. Biggers and McFeeley (1966)	38 XX/39 XXY	2/13
5. Malouf *et al.* (1967)	38 XX/XY Some spermatogenesis	37/59
6. Thuline (1964)	38 XX/XY	
7. Jones (1969)	38 XX/XY/39 XXY/40 XXYY	
8. Jones (1969)	38 XY/39 XXY/40 XXYY	
9. Loughman *et al.* (1970)	38 XY/39 XXY	22/83
10. Loughman *et al.* (1970)	38 XY/39 XXY	13/25
11. Loughman *et al.* (1970)	38 XY/34 XXY	20/28
12. Ramberg *et al.* (1969)	38 XY – ? 2 populations of XY – probably fertile	
13. Thuline (1964)	38 XX/XY True hermaphrodite - not calico, iris heterochromia	
14. Gregson and Ishmael (1971)	38 XX/57 XXY True hermaphrodite	101/3
15. Gregson and Ishmael (1971)	38 XX/57 XXY	46/21
16. Gregson and Ishmael (1971)	38 XY/57 XXY Spermatogenesis – probably fertile	93/2
17. Benirschke (unpublished)	39 XXY	100
18. Benirschke (unpublished)	38 XX/57 XXY	variable
19. Benirschke (unpublished)	38 XX/XY	50/50

overall admixture ratio of 88 XX/12 XY in nearly 500 cells examined with proportions varying from 2% XY in muscle to 29% XY cells in kidney. In 1970 Dunn *et al.* described a diploid XX/triploid XXY bovine true hermaphrodite with a very small percentage of triploid cells. They could show a significantly higher percentage of Y-bearing cells in the testis as compared with other tissues and point out that only because of their perseverance was a second line ever detected in this animal. Only after scoring 200 cells was the first triploid metaphase discovered; eventually 2352 cells were examined with a 2n/3n ratio of 87/13. An intersex male with XXY/XX/XY admixture was discussed by Rieck *et al.* (1969) that was not a twin but in which tissue studies were not carried out. All these reports contradict the view of Goodfellow *et al.* (1965) who purport similarities in phenotype between these intersexes and the freemartins and view gonadal differentiation to be influenced by the gonadal content of XX or XY germ cells.

These cases raise two principal problems which demand explanation in the future. The fact that in a bovine true hermaphrodite with testis and ovary, uterine structures may be present, and that viable ova are found in the ovary, while in the merely blood chimeric freemartin the latter structures are usually completely reduced, is presently inexplicable. Dunn *et al.* (1968) reason that the ovotestis was poorly vascularized and could merely have exerted a local effect by diffusion of secretions. However, intuitively this does not explain the discrepancy away. To be sure, not in all freemartins is the uterus completely atrophied (Laster *et al.*, 1971), but the two experiments of nature are not easily reconciled by current hypotheses. The variability of gonadal structures in XX/XY chimeras is enormous and, superficially at least, significant species differences exist that may offer a clue for understanding. It is striking that almost all XX/XY chimeric cats are male, have testes and lack uteri. It does not seem reasonable then to reason from the relative rarity of true hermaphroditism observed in experimental mouse chimeras (Mystkowska and Tarkowski, 1968; Mintz, 1968) that their situation is necessarily

typical for all mammals. It seems most acceptable at this date to assume that the percentage of Y-bearing somatic cells of the gonadal anlage determines its differentiation into a testis (Jacobs, 1969), resp. ovotestis in most species, while it is recognized at the same time that XX males and animals with a similar chromosomal constitution pose a real problem in explaining sex differentiation.

The other aspect of interest is the mechanics of how chimeric individuals come about. Most papers and reviews dealing with this subject have enumerated the possibilities at length and it may thus be forgiven if I do not review polyandry, polygyny, etc. Cotterman (1958) raised the question whether this accident occurs only in uniparous animals, the multiparous species having better safeguards against its occurrence. This assumption can now be refuted from the accumulated evidence cited above. Indeed, in man, a number of true chimeras were of twin-bearing families, or twins themselves. Aside from the common assumptions of double fertilization, etc., the possibility of early fusion of zygotes, so readily accomplished experimentally in mice, must be kept in mind. Rarely can sufficient markers be studied to discriminate these events.

It is striking, finally, to witness how frequently 2n/3n chimeras have been observed, particularly in the cat. To these observations must be added the diploid/triploid admixtures in pig embryos (McFeely, 1967), in rabbit embryos (Hansen-Melander and Melander, 1971) and the haploid/triploid, diploid/tetraploid, etc. embryos discovered by Miller *et al.* (1971) among chick embryos. All of these data suggest that abnormal fertilization and some degree of development is a frequent event among a wide variety of species. Perhaps only the most favorable types of chimeras survive into adulthood; nevertheless, it may be assumed that the phenomenon is more widely represented in our population and that it is too infrequently recognized at present.

MOSAICISM

The frequency of chromosomal mosaicism is apparently great and case reports, particularly of sex chromosomal mosaics, are legion. Up to four good stemlines are reported and cases with lines of different autosomal trisomies are on record. Regrettably, in most cases chimerism has not been ruled out. Nevertheless, in occasional reports such attempts have been unsuccessful and one has to assume that postzygotic chromosomal nondisjunction occurs relatively frequently. This is further supported by the finding of the interesting group of 11 heterokaryotic monozygotic twins (Benirschke, 1970). It is only surprising that among the spontaneous abortions in man mosaicism has been so rarely reported (Boué and Boué, 1969) and, therefore, it is of interest to consider the possibility of placental *vs.* fetal mosaicism. Almost all abortion studies are carried out on placental tissue, the fetus usually having died before. Perhaps the placenta does not always reflect the fetal karyotype since its cells are set aside in the first few days after conception. Mosaicism, *i.e.* postzygotic nondisjunction, may well occur later and affect only embryonic tissue.

In this respect an anomaly should be of interest to human geneticists that is rarely seen except by obstetricians. The acardiac monster is surely the worst type of abnormal development imaginable. It exists in many variants, but characteristic is its defective or absent cardiac development. Consequently, the circulation is accomplished by a cotwin through vascular anastomoses in the placenta. Regularly an artery-to-artery and vein-to-vein anastomosis are present, and circulation is accomplished in a reversed manner by the cotwin who is normal. In man, the anomaly occurs always in monozygous twins (monochorionic placenta, always like sex) and the defect is occasionally found in other animals, notably ruminants. Dunn *et al.* (1967) reported a bovine anomaly of this type with XX chromosomes whose blood and cotwin are XY, and were thus able to settle the long disputed hypothesis that vascular reversal is the primary agent in the genesis of this anomaly.

In several human acardiacs, as well as in the single bovine specimen, mosaic clones have been cultured, the normal cotwin usually having normal karyotypes (Benirschke, 1970). In view of the reversed circulation of these monsters, their poor vascularization, and their supply by only venous blood, one may consider the possibility that such disadvantageous intrauterine conditions may lead to the development of nondisjunctional events with ensuing mosaicism. Further study of these anomalies may be useful for the further understanding of the origin of mosaicism.

HYBRIDS

It may be asked why the subject of interspecific hybridism is to be discussed at a Congress of Human Genetics. To date several serious attempts at artificial hybridization of female chimpanzees, the animal with a most similar karyotype, and human semen have been unsuccessful, for unknown reasons. Other aspects of advances in knowledge in interspecific hybrids have led to much fascinating insight into reproductive and chromosomal behavior, however, and they justify a consideration of this topic. Three topics will be reviewed briefly: pig hybrids, sheep × goat hybrids, and mules.

A. CHROMOSOMES IN PIGS

We assume in general that a good species has a uniform chromosome number and that deviations from this diploid number characterize mutations or nondisjunctional events. When a group of animals that are now considered to be a good species shows numerical chromosomal polymorphism, it is less clear whether these diverse members should indeed be considered separate species. Many arguments have been made to view Robertsonian mechanisms as part of an evolutionary change and fusion is the more readily understood event. Whether fission exists at all in nature remains to be shown definitively.

I have thus taken the view that with progressive evolution in a group of related animals the finding of a lower number of chromosomes indicates a more recently evolved species (Benirschke and Malouf, 1967). It was a surprise then to learn that domestic pig has a diploid number of $2n = 38$, while boars and boar hybrids in Tennessee have $2n = 36$ and $2n = 37$; the change being clearly the result of a Robertsonian fusion between two acrocentrics (McFee *et al.*, 1966). From this observation it was inferred that the German wild boar, imported in 1912, had a diploid number of $2n = 36$, a value since confirmed by Gropp *et al.* (1969). One might also have made the assumption that the domestic pig has a higher diploid number than its wild ancestor, and that chromosomal fission was the mechanism. More likely is the explanation that the German wild boar is a chromosomal variant of the Asiatic pig whose diploid value is $2n = 38$ in two distant locations and from which the domestic species differentiated (Muramoto *et al.*, 1965; Zivkovic *et al.*, 1970). In this respect then it differs little from the polymorphic population of Swedish cattle reported by Gustavsson (1969).

It is remarkable that in various crosses of these three karyotypic variants McFee and Banner (1969) find the expected ratios with only a slight reduction in litter size and no unbalanced offspring. There seems thus no grossly obvious barrier in this cross.

B. SHEEP × GOAT HYBRIDS

Sheep have 54 chromosomes, goats have 60 chromosomes, the difference readily accounted for by a Robertsonian change with constant nombre fondamental (NF). An extensive literature exists that indicates relatively frequent hybridization among these species (McGovern,

1969). However, with the exception of reports from Bratanov's laboratory, experimental hybridization in both directions has not yielded live offspring. While embyronic growth proceeds for about 60 days, fetal death invariably occurs, even after egg transplantation or in association with isologous twins. The hybrid embryos have the anticipated intermediate chromosome number of 57 and appear normally formed. Two suggestions have been made to explain the phenomena: (1) the hybrid placenta fails to establish normal contact with the uterus or becomes necrotic (Hancock *et al.*, 1970), or (2) an immunologic reaction takes place, since hemolysins are regularly observed shortly after implantation (Alexander *et al.*, 1967). At this time neither phenomenon is clearly established as the responsible agent nor are the contradictory results from Bulgaria explicable. Suffice it to say, cytologic factors are not involved in the hybrid development, rather, reproductive processes have seemingly diverged sufficiently in the evolution from the animals with 2n = 60 to those with 2n = 54 that they serve as efficient barriers. In line with this assumption is the finding that goat × Barbary sheep hybrids (2n = 60 × 2n = 58) are more capable of being achieved than Barbary sheep × × sheep hybrids (Steklenev, 1966). Moreover, Nadler *et al.* (1971) have presented evidence that chromosomal polymorphism (2n = 54–58) exists in wild sheep which necessitates future chromosomal study of the animals employed by Bratanov and Dikov (1962).

C. EQUINE HYBRIDS

Chromosome numbers in the Equidae vary between 2n = 66 for the Przewalski horse and 2n = 32 for the South African mountain zebra (Benirschke and Malouf, 1967). Hybrids between most of these species have been recorded (Gray, 1954). The difference in chromosome number does not appear to be a barrier in hybridization and should also not be considered as the most likely barrier in Bos × Bubalus crosses, as was suggested by Fischer (1971). Most of the equine interspecific hybrids are sterile with the notable exception of *E. przewalskii* × *E. caballus* (Koulischer and Frechkop, 1966).

There are two interesting current aspects to these hybrids: (1) the reason for their sterility, and (2) the controversy over the randomness of inactivation of one or the other X in female hybrids.

1. Mules or the reciprocal crosses between horse and donkey, the hinny, are notoriously infertile. We have assumed that sterility is secondary to synaptic failure at first meiosis of their 63 chromosomes. On karyotype analysis of donkey and horse there are profound structural differences between these species, the donkey having many more metacentrics and, unlike the difference between domestic and Przewalski's horse, the numerical change cannot be accounted for by a simple Robertsonian mechanism. Similar differences exist between the other good species. Consonant with this observation then is the finding in sections and direct squash preparations of now numerous adult hybrid testes that mitoses and early stages of first meiosis are abundant but diakinesis and spermatozoa have been invariably absent. Our findings differ from the reports by Bratanov *et al.* (1964) and Trujillo *et al.* (1969) who found occasional spermatozoa in mules and one hinny, respectively. While the reason for the discrepancy is difficult to understand, the paucity of spermatozoa recorded and their immobility would surely be consistent with the reported sterility of the male hybrids.

More controversial still is the veracity of isolated reports of female mule fertility (Bonadonna, 1966; Savory, 1970). All reports rest on the hearsay diagnosis 'mule', however authoritative it may be. In three such cases studied by us the alleged mule was, chromosomally, a donkey. It is of interest, however, that female mules have periods of heat, develop corpora lutea and cystic follicles and isolated tubal ova have been recorded in two of three mules by Bielanski and Zapletal (1968). Regrettably, chromosome studies and ovarian histology of these animals was not reported by these authors. In our own studies of chromosomally verified mules, in only one section of 47 pairs of ovaries were we able to find a primordial follicle, despite the fact that some ovaries were serially sectioned (Benirschke and Sullivan, 1966).

Control donkeys had adequate numbers of readily identifiable ova which precludes mistaken histologic observations. It would thus appear that adult mules lack a normal complement of ova, perhaps they die in asynapsis in embryonic life in a manner possibly similar to that assumed for the XO female (Singh and Carr, 1966). Dr. Roger V. Short and colleagues have pursued this aspect further and permitted me to relate some of their findings. Fetal mules were noted to have a large number of germ cells, while in the neonate the number is remarkably reduced when compared with nonhybrid equines. It would thus appear feasible that an occasional female mule may bear young, although the cytologic mechanism for segregation of chromosomes in these hybrids is difficult to imagine and at this time wholly speculative. Direct cytologic verification of such events would be most valuable in the future.

2. The X chromosomes of donkey and horse are readily distinguished cytologically, that of the donkey being nearly acrocentric. Inasmuch as both are of approximately the same size and no obvious deficiencies exist in female mules or hinnies that could be blamed on a difference in the respective X, it was reasonable to assume that random inactivation of X in such hybrids should take place according to Lyon's hypothesis (Lyon, 1962). This hypothesis might be tested in this cross by (*a*) a study of the proportion of 'late-replicating X chromosomes' of the two types, (*b*) an analysis of the X-linked G6PD which has differential mobility in the two species, and (*c*) by evaluating similar studies performed on single cell clones derived from cultures of these clones.

Mukherjee and Sinha (1964) initially found in lymphocyte cultures of a female mule indeed a 50% late replicating X of each variety. This contrasts sharply with the results of Hamerton *et al.* (1969) who found 88–100% late replicating donkey (paternal) X chromosomes in lymphocytes and fibroblasts of three mules and in one animal only weak representation of donkey G6PD in erythrocytes and fibroblast cultures. These findings prompted Mukherjee *et al.* (1970) to repeat their study; again a nearly 1:1 ratio of late X's was found in fibroblasts. Next, Cohen and Rattazzi (1971) have addressed themselves to this problem. Fibroblast cultures from three female mules and lymphocytes from another were examined. Late replication of donkey X was found between 62 and 91% (mean 77%) in these four cultures and this corresponded approximately to the amount of horse G6PD in cell lysates. Their findings thus confirm in a general way those of Hamerton *et al.* (1969) and suggest that late replication is indicative of genetic inactivity of the affected X. Hook and Brustman (1970) had preferential horse G6PD activity in extracts of 37 mule tissues. This was true for blood, pancreas, brain, spinal cord, kidney and parotid, while in spleen and nodes the horse activity predominated but was not as unequal; in thyroid and lungs both activities were equal, while in liver lysate the donkey G6PD probably predominated. It would thus appear that sampling factors may seriously affect this type of study, although the trend toward a preferential paternal X inactivation in these hybrids is apparent. Most recently, Hamerton and colleagues (1971) have studied the problem in much greater detail and also encompass the reciprocal cross, two hinnies whose paternal ancestor is the horse. One female mule and two hinnies were used to establish fibroblast cultures. The mule and one hinny had strong horse G6PD bands, while the other hinny developed a stronger donkey G6PD representation. Thus, variation in the originally proposed preferential paternal X inactivation has been observed by the group proposing this concept originally. More importantly, these investigators have proceeded to clone these cells and provide good evidence for (*a*) the veracity of the Lyon hypothesis, (*b*) the fact that maternal cytoplasm (derived from the ovum) is not important in determining which X is inactivated, and (*c*) that strong selective forces exist *in vitro* that favor selection of cells with active horse X in this particular system.

Much of these inferences of course may be speculative since the horse and donkey X are not homologous, as pointed out by Hamerton and Giannelli (1970). Nevertheless, the study of these hybrids has been useful to further support the Lyon hypothesis and these animals may ultimately be of value in gaining an understanding of control mechanisms and cytoplasmic factors in embryogenesis.

CONCLUSIONS

In summary then, the study of chimeras has greatly enhanced our knowledge of immunologic aspects of development and one can hope that it will eventually lead to a concise understanding of sex differentiation. Of the three types, intertwin blood chimerism, the transplacental variety, and whole-body chimerism, the latter type is not only the most common but also the most interesting. Its frequency is unknown, but there is reason to believe that it is more common than currently recognized. At least so far only one isosexual chimera has been described and they present an interesting challenge for the future. Diploid/triploid chimeras, often of the XX/XXY variety, raise the possibility that all four maternal gametes of a single germ cell may be participants, an event that might be specifically searched for. The desirability of differentiating chimerism from mosaicism should be apparent from this presentation.

So far as interspecific hybrids are concerned, their genetics are easily as challenging as their *in vitro* counterparts. They have only recently been studied in detail, but provide aspects that should make attractive investigations in the future.

REFERENCES

ALEXANDER, G., WILLIAM, D. and BAILEY, K. (1967): Natural immunization in pregnant goats against red blood cells of their sheep × goat hybrid foetuses. *Aust. J. biol. Sci.*, *20*, 1217.

BASRUR, P. K., KANAGAWA, H. and GILMAN, J. P. W. (1969): An equine intersex with unilateral gonadal agenesis. *Canad. J. comp. Med.*, *33*, 297.

BASRUR, P. K., KANAGAWA, H. and PODLIACHOUK, L. (1970): Further studies on the cell populations of an intersex horse. *Canad. J. comp. Med.*, *34*, 294.

BENIRSCHKE, K. (Ed.) (1969): *Comparative Mammalian Cytogenetics*. Springer-Verlag, New York, N.Y.

BENIRSCHKE, K. (1970): Spontaneous chimerism in mammals: a critical review. *Curr. Top. Path.*, *51*, 1.

BENIRSCHKE, K. and BROWNHILL, L. E. (1963): Heterosexual cells in testes of chimeric marmoset monkeys. *Cytogenetics*, *2*, 331.

BENIRSCHKE, K. and LAYTON, W. (1969): An early twin blastocyst of the golden lion marmoset, *Leontocebus rosalia*. *Folia primatol.*, *10*, 131.

BENIRSCHKE, K. and MALOUF, N. (1967): Chromosome studies of equidae. *Equus*, *1/2*, 253.

BENIRSCHKE, K. and SULLIVAN, M. M. (1966): Corpora lutea in proven mules. *Fertil. Steril.*, *17*, 24.

BENIRSCHKE, K. and SULLIVAN, M. M. (1968): The human placenta in relationship to the development of chimerism. In: *The Foeto-Placental Unit*. Editors: A. Pecile and C. Finzi. ICS 183, Excerpta Medica, Amsterdam.

BIAS, W. B. and MIGEON, B. R. (1967): Blood-group chimaerism with Down's syndrome. *Lancet*, *2*, 257.

BIELANSKI, W. and ZAPLETAL, Z. (1968): Ovulation in she-mules: a report of two cases. In: *Proceedings, VI International Congress on Animal Reproduction and Artificial Insemination, Vol. II*, p. 1555.

BIGGERS, J. D. and MCFEELY, R. A. (1966): Intersexuality in domestic mammals. In: *Advances in Reproductive Physiology*. Editor: A. McLaren. Logos Press, London.

BILLINGTON, W. D., KIRBY, D. R. S., OWEN, J. J. T., RITTER, M. A., BURTONSHAW, M. D., EVANS, E. P., FORD, C. E., GOULD, I. K. and MCLAREN, A. (1969): Placental barrier to maternal cells. *Nature (Lond.)*, *224*, 1969.

BOCZKOWSKI, K., JANCZEWSKI, Z., PHILIP, J. and MIKKELSEN, M. (1969): A clinical and cytogenetical study of XX male. *Hereditas (Lund)*, *62*, 285.

BONADONNA, T. (1966): Le croisement interspécifique réciproque cheval-âne et la fécondité des hybrides obtenus. *Wld Rev. Anim. Prod.*, *July*, 46.

BOOTH, P. B., PLAUT, G., JAMES, J. D., IKIN, E. W., MOORES, P., SANGER, R. and RACE, R. R. (1957): Blood chimerism in a pair of twins. *Brit. med. J.*, *1*, 1456.

BOUÉ, J. G. and BOUÉ, A. (1969): Fréquence des aberrations chromosomiques dans les avortements spontanés humains. *C. R. Acad. Sci. (Paris)*, *269*, 283.

BRATANOV, K. and DIKOV, V. (1961): Fécondation entre les espèces brebis et chèvres et obtention

d'hybride inter-espèces. In: *Proceedings, IV International Congress on Animal Reproduction, Vol. IV* (Section AI), p. 744.

BRATANOV, K., DIKOV, V. and DOKOV, V. K. (1964): Recherches sur l'infécondité chez l'hybride mâle. In: *Proceedings, V International Congress on Animal Reproduction and Artificial Insemination, Vol. III*, Session II, pp. 103, 560.

CAMERON, A. H. (1968): The Birmingham twin survey. *Proc. roy. Soc. Med., 61*, 229.

CHOWN, B., LEWIS, M. and BOWMAN, J. M. (1963): A pair of newborn human blood chimeric twins. *Transfusion (Philad.), 3*, 494.

CHU, E. H. Y., THULINE, H. C. and NORBY, D. E. (1964): Triploid-diploid chimerism in a male tortoiseshell cat. *Cytogenetics, 3*, 1.

COHEN, M. M. and RATTAZZI, M. C. (1971): Cytological and biochemical correlation of late X-chromosome replication and gene inactivation in the mule. *Proc. nat. Acad. Sci. USA, 68*, 544.

COOPER, K. W. (1959): A bilaterally gynandromorphic Hypodynerus, and a summary of cytologic origins of such mosaic Hymenoptera. Biology of eumenine wasps. VI. *Bull. Fla State Mus. biol. Sci., 5/2*, 25.

COREY, M. J., MILLER, J. R., MACLEAN, J. R. and CHOWN, B. (1967): A case of XX/XY mosaicism. *Amer. J. hum. Genet., 19*, 378.

COTTERMAN, C. W. (1958): Erythrocyte antigen mosaicism. *J. cell. comp. Physiol., 52*, 69.

CROOKSTON, M. C., TILLEY, C. A. and CROOKSTON, J. H. (1970): Human blood chimaera with seeming breakdown of immune tolerance. *Lancet, 2*, 1110.

DE GROUCHY, J., MOULLEC, J., SALMON, CH., JOSSO, N., FREZAL, J. and LAMY, M. (1964): Hermaphroditisme avec caryotype XX/XY. Etude génétique d'un cas. *Ann. Génét., 7*, 25.

DUNN, H. O., KENNEY, R. M. and LEIN, D. H. (1968): XX/XY chimerism in a bovine true hermaphrodite: an insight into the understanding of freemartinism. *Cytogenetics, 7*, 390.

DUNN, H. O., LEIN, D. H. and KENNEY, R. M. (1967): The cytological sex of a bovine anidian (amorphous) twin monster. *Cytogenetics, 6*, 412.

DUNN, H. O., MCENTEE, K. and HANSEL, W. (1970): Diploid-triploid chimerism in a bovine true hermaphrodite. *Cytogenetics, 9*, 245.

DUNSFORD, I., BOWLEY, C. C., HUTCHINSON, A. M., THOMPSON, J. S., SANGER, R. and RACE, R. R. (1952): A human blood group chimera. *Brit. med. J., 2*, 81.

DUNSFORD, I. and STACEY, S. M. (1957): Partial breakdown of acquired tolerance to the A antigen. *Vox Sang. (Basel), 2*, 414.

EIMER, H. and WEILAND, A. (1969): Untersuchungen über die Placentapassage mütterlicher Erythrocyten. *Arch. Gynäk., 208*, 113.

EL-ALFI, O. S. and HATHOUT, H. (1969): Maternofetal transfusion: immunologic and cytogenetic evidence. *Amer. J. Obstet. Gynec., 103*, 599.

FISCHER, H. (1971): Kann man Wasserbüffel und Rinder erfolgreich kreuzen? *Dtsch. tierärztl. Wschr., 78*, 327.

GARTLER, S. M., WAXMAN, S. H. and GIBLETT, E. (1962): An XX/XY human hermaphrodite resulting from double fertilization. *Proc. nat. Acad. Sci. USA, 48*, 332.

GENGOZIAN, N. and MERRITT, C. B. (1970): Effect of unilateral ovariectomy on twinning frequency in the marmoset. *J. Reprod. Fertil., 23*, 504.

GITHENS, J. H., MUSCHENHEIM, F., FULGIUITI, V. A., ROBINSON, A. and KAY, H. E. M. (1969): Thymic alymphoplasia with XX/XY lymphoid chimerism secondary to probable maternal-fetal transfusion. *J. Pediat., 75*, 87.

GOODFELLOW, S. A., STRONG, S. J. and STEWART, J. S. S. (1965). Bovine freemartins and true hermaphroditism. *Lancet, 1*, 1040.

GRAY, A. P. (1954): *Mammalian Hybrids. A Check-list with Bibliography*. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks.

GREGSON, N. M. and ISHMAEL, J. (1971): Diploid-triploid chimerism in 3 tortoiseshell cats. *Res. vet. Sci., 12*, 275.

GROPP, A., GIERS, D. and TETTENBORN, U. (1969): Das Chromosomenkomplement des Wildschweins (*Sus scrofa*). *Experientia (Basel) 25*, 778.

GROPP, A. and OHNO, S. (1966): The presence of a common embryonic blastema for ovarian and testicular parenchymal (follicular, interstitial and tubular) cells in cattle, *Bos taurus*. *Z. Zellforsch., 74*, 505.

GUSTAVSSON, I. (1969): Cytogenetics, distribution and phenotypic effects of a translocation in Swedish cattle. *Hereditas (Lund), 63*, 68.

HAMERTON, J. L. and GIANNELLI, F. (1970): Non-random inactivation of the X chromosome in the female mule. *Nature (Lond.), 228*, 1322.

HAMERTON, J. L., GIANNELLI, F., COLLINS, F., HALLETT, J., FRYER, A. and MCGUIRE, V. M. (1969): Non-random X-inactivation in the female mule. *Nature (Lond.), 222*, 1277.

HAMERTON, J. L., RICHARDSON, B. J., GEE, P. A., ALLEN, W. R. and SHORT, R. V. (1971): Non-random X-chromosome expression in female mules and hinnies. *Nature (Lond.), 232*, 312.

HANCOCK, J. L., MCGOVERN, P. T. and STAMP, J. T. (1970): Placentae of goat × sheep hybrids. *J. Anat. (Lond.), 2*, 413.

HANSEN-MELANDER, E. and MELANDER, Y. (1971): A case of spontaneous haploidy and notes on triploid-diploid mosaics in rabbit embryos. *Hereditas (Lund), 67*, 83.

HARTEMANN, J., PETERS, A., DELLESTABLE, P., DUPREZ, A. F. and TOUATI, E. (1963): Les transfusions foeto-foetales lors des grossesses biovulaires existent-elles? *Gynéc. Obstét., 62*, 663.

HERSCHLER, M. S. and FECHHEIMER, N. S. (1967): The role of sex chromosome chimerism in altering sexual development of mammals. *Cytogenetics, 6*, 204.

HOOK, E. and BRUSTMAN, L. D. (1970): Preferential expression of alleles of an X-linked locus (G6PD) in organs of the female mule. *Genetics, 64/2, Suppl. 2*, abstr. s30.

HUTCHINSON, D. L., TURNER, J. H. and SCHLESINGER, E. R. (1971): Persistence of donor cells in neonates after fetal and exchange transfusions. *Amer. J. Obstet. Gynec., 109*, 281.

JACOBS, P. A. (1969): The chromosome basis of some types of intersexuality in man. *J. Reprod. Fertil., Suppl. 7*, 73.

JAINUDEEN, M. R. and HAFEZ, E. S. E. (1965): Attempts to induce bovine freemartinism experimentally. *J. Reprod. Fertil., 10*, 281.

JONES, T. C. (1969): Anomalies of sex chromosomes in tortoiseshell male cats. In: *Comparative Mammalian Cytogenetics*. Editor: K. Benirschke. Springer-Verlag, New York, N.Y.

JOST, A. (1970): Hormonal factors in the sex differentiation of the mammalian foetus. *Phil. Trans. B, 259*, 119.

JOST, A., CHODKIEWICZ, M. and MAULÉON, P. (1963): Intersexualité du foetus de veau produite par des androgènes. Comparaison entre l'hormone foetale responsable du free-martinisme et l'hormone testiculaire adulte. *C. R. Acad. Sci. (Paris), 256*, 274.

KADOWAKI, J. I., THOMPSON, R. I., ZUELZER, W. W., WOOLLEY, P. V., BROUGH, A. J. and GRUBER, D. (1965): XX/XY lymphoid chimaerism in congenital immunological deficiency syndrome with thymic alymphoplasia. *Lancet, 2*, 1152.

KIM, C. K., YEN, S. S. C. and BENIRSCHKE, K. (1971): Serum testosterone in fetal cattle. *Gen. comp. Endocr., 18*, 404.

KLINGER, R., CARBONARA, A. O., MIGGIANO, V. C. and SANGER, R. (1970): Studio dei gruppi sanguini, su di un caso di chimerismo XX/XY umano. *Atti Ass. Genet. ital., 15*, 100.

KOULISCHER, L. and FRECHKOP, S. (1966): Chromosome complement: a fertile hybrid between *Equus prjewalskii* and *Equus caballus*. *Science, 151*, 93.

LASTER, D. B., TURMAN, E. J., JOHNSON, B. H., STEPHENS, D. F. and RENBARGER, R. E. (1971): Effect of sex ratio *in utero* on degree of transformation and chimaerism in neonatal bovine freemartins. *J. Reprod. Fertil., 24*, 361.

LEJEUNE, J., SALMON, CH., BERGER, R., RETHORE, M. O., ROSSIER, A. and JOB, J. C. (1967): Chimère 46 XX/69 XXY. *Ann. Génét., 10*, 88.

LILLIE, F. R. (1916): The theory of the freemartin. *Science, 43*, 611.

LOJDA, L. and ČERNÝ, L. (1970): Chromosome chimerism and skin grafts in bovine twins and a shift in the sex ratio of their progeny. *Acta Genet. med. (Roma), 19*, 231.

LOUGHMAN, W. D., FRYE, F. L. and CONDON, T. B. (1970): XY/XXY bone marrow mosaicism in three male tricolor cats. *Amer. J. vet. Res., 31*, 307.

LYON, M. I. (1962): Sex chromatin and gene action in the mammalian X-chromosome. *Amer. J. hum. Genet., 14*, 135.

MALOUF, N., BENIRSCHKE, K. and HOEFNAGEL, D. (1967): XX/XY chimerism in a tricolored male cat. *Cytogenetics, 6*, 228.

MASSIMO, L., GEMME, G.. VIANELLO, M. G. and VERRI, B. (1966): Studio di una coppia di gemelli dizigotici di cui uno mongolide con trisomia 21, e l'altro normale con chimerismo transitorio di cellule con trisomia 21. *Acta Genet. med. (Roma), 15*, 208.

MAYR, E. (1963): *Animal Species and Evolution*. Belknap Press, Cambridge.

MCFEE, A. F. and BANNER, M. W. (1969): Inheritance of chromosome number in pigs. *J. Reprod. Fertil., 18*, 9.

McFee, A. F., Banner, M. W. and Rary, J. M. (1966): Variation in chromosome number among European wild pigs. *Cytogenetics, 5*, 75.

McFeely, R. A. (1967): Chromosome abnormalities in early embryos of the pig. *J. Reprod. Fertil., 13*, 579.

McGovern, P. T. (1969): Goat and sheep hybrids. *Anim. Breed. Abstr., 37*, 1.

McLaren, A. and Michie, D. (1959): Experimental studies on placental fusion in mice. *J. exp. Zool., 141*, 47.

Miller, R. C., Fechheimer, N. S. and Japp, R. G. (1971): Chromosome abnormalities in 16-18-hour chick embryos. *Cytogenetics, 10*, 121.

Mintz, B. (1968): Hermaphroditism, sex chromosomal mosaicism and germ cell selection in allophenic mice. *J. Anim. Sci., 27, Suppl. I*, 51.

Mukherjee, B. B., Mukherjee, A. B. and Mukherjee, A. B. (1970): Is inactivation of the X-chromosome in the female mule non-random? *Nature (Lond.), 228*, 1321.

Mukherjee, B. B. and Sinha, A. K. (1964): Single-active-X hypothesis: cytological evidence for random inactivation of X-chromosomes in a female mule complement. *Proc. nat. Acad. Sci. USA, 51*, 252.

Muramoto, J. I., Makino, S., Ishikawa, T. and Kanagawa, H. (1965). On the chromosomes of the wild boar and the boar-pig hybrids. *Proc. Jap. Acad., 41*, 236.

Myhre, A., Meyer, T., Opitz, J. M., Race, R. R., Sanger, R. and Greenwalt, T. J. (1965): Two populations of erythrocytes associated with XX/XY mosaicism. *Transfusion (Philad.), 5*, 501.

Mystkowska, E. T. and Tarkowsi, A. K. (1968): Observations on CBA-p/CBA-T_6T_6 mouse chimeras. *J. Embryol. exp. Morph., 20*, 33.

Nadler, C. C., Lay, D. M. and Hassinger, J. D. (1971): Cytogenetic analyses of wild sheep populations in northern Iran. *Cytogenetics, 10*, 137.

Naiman, J. L., Punnett, H. H., Lischner, H. W., Destiné, M. L. and Arey, J. B. (1969): Possible graft-*versus*-host reaction after intrauterine transfusion for Rh erythroblastosis fetalis. *New Engl. J. Med., 281*, 647.

Nes, N. (1966): Diploid-triploid chimerism in a true hermaphrodite mink *(Mustela vison). Hereditas (Lund), 56*, 159.

Nicholas, J. W., Jenkins, W. J. and Marsh, W. L. (1957): Human blood chimeras: a study of surviving twins. *Brit. med. J., 1*, 1458.

Nylander, P. P. S. and Osunkoya, B. O. (1970): Unusual monochorionic placentation with heterosexual twins. *Obstet. Gynec., 36*, 621.

Ohno, S. (1969): The problem of the bovine freemartin. *J. Reprod. Fertil., Suppl. 7*, 53.

Ohno, S., Trujillo, J. M., Stenius, C., Christian, L. C. and Teplitz, R. L. (1962): Possible germ cell chimeras among newborn dizygotic twin calves *(Bos taurus). Cytogenetics, 1*, 258.

Olding, L. (1971): The possibility of materno-foetal transfer of lymphocytes in man. *Acta paediat. scand., 61*, 73.

Owen, R. D. (1945): Immunogenetic consequences of vascular anastomoses between bovine twins. *Science, 102*, 400.

Park, I. J., Jones, H. W. and Bias, W. B. (1970): True hermaphroditism with 46,XX/46,XY chromosome complement. Report of a case. *Obstet. Gynec., 36*, 377.

Porter, P. (1968): Chimerism and immunologic tolerance: a consequence of fraternal twinning in the marmoset. *Diss. Abstr., 29, 479B*, 601.

Potter, J. F. and Schoeneman, M. (1970): Metastasis of maternal cancer to the placenta and fetus. *Cancer (Philad.), 25*, 380.

Race, R. R. and Sanger, R. (1968): *Blood Groups in Man, 5th ed.* F. A. Davis Co., Philadelphia, Pa.

Ramberg, R. E., Norby, D. E. and Thuline, H. C. (1969): Chromosome mosaicism in male calico cats. *Northw. Sci., 43/1*, 42.

Rieck, G. W., Höhn, H. and Herzog, A. (1969): Hypogonadismus, intermittierender Kryptorchismus und segmentäre Aplasie der Ductus Wolffii bei einem männlichen Rind mit XXY-Gonosomen-Konstellation bzw. XXY/XX/XY-Gonosomen-Mosaik. *Dtsch. tierärztl., Wschr., 76*, 133.

Rosenberg, H. S., Clayton, G. W. and Hsu, T. C. (1963): Familial true hermaphroditism. *J. clin. Endocr., 23*, 203.

Russell, L. B. and Woodiel, F. N. (1966): A spontaneous mouse chimera formed from separate fertilization of two meiotic products of oogenesis. *Cytogenetics, 5*, 106.

SAVORY, T. H. (1970): The mule. *Scientif. Amer.*, *223*, 102.
SELLER, M. J. (1970): Lack of porosity of the mouse placenta to maternal cells. *Nature (Lond.)*, *225*, 1254.
SHARPE, H. B. A. (1970): Human foetal maternal barrier. *Nature (Lond.)*, *226*, 453.
SINGH, R. P. and CARR, D. H. (1966): The anatomy and histology of XO human embryos and fetuses. *Anat. Rec.*, *155*, 369.
SPRAGUE, L. M. and STORMONT, C. (1956): A reanalysis of the problem of the male tortoiseshell cat. *J. Hered.*, *47*, 237
STEKLENEV, E. P. (1966): Hybridisation of the Barbary ram (*Ammotragus lerria* Pallas) with domestic sheep and goats. *Ovtsevodstvo (Kiev)*, *2*, 24.
STONE, W. H., FRIEDMAN, J. and AUDREY, F. (1964). Possible somatic cell mating in twin cattle with erythrocyte mosaicism. *Proc. nat. Acad. Sci. USA*, *51*, 1036.
STRAUSS, L. and DRISCOLL, S. G. (1964): Congenital neuroblastoma involving the placenta. *Pediatrics*, *34*, 23.
TEPLITZ, R. L., MOON, Y. S. and BASRUR, P. K. (1967): Further studies of chimerism in heterosexual cattle twins. *Chromosoma (Berl.)*, *22*, 202.
THULINE, H. C. (1964): Male tortoiseshells, chimerism, and true hermaphroditism. *J. Cat Genet.*, *4*, 2.
THULINE, H. C. and NORBY, D. E. (1961): Spontaneous occurrence of chromosome abnormality in cats. *Science*, *134*, 554.
TRUJILLO, J. M., OHNO, S., JARDINE, J. H. and ATKINS, N. B. (1969): Spermatogenesis in a male hinny: histological and cytological studies. *J. Hered.*, *60*, 79.
TUFFREY, M., BISHUN, N. P. and BARNES, R. D. (1969*a*): Porosity of the mouse placenta to maternal cells. *Nature (Lond.)*, *221*, 1029.
TUFFREY, M., BISHUN, N. P. and BARNES, R. D. (1969*b*): Porosity of the placenta to maternal cells in normally derived mice. *Nature (Lond.)*, *224*, 701.
TURNER, C. D. and ASAKAWA, H. (1964): Experimental reversal of germ cells in ovaries of fetal mice. *Science*, *143*, 1344.
TURPIN, R., SALMON, CH. and CRUVEILLER, J. (1959): A propos des chimères humaines. Présence temporaire d'une double population d'hématies chez l'un des jumeaux d'un couple dizygote. *Rev. franç. Etud. clin. biol.*, *4*, 809.
UENO, S., ZUZUKI, K. and YAMAZAWA, K. (1959): Human chimerism in one of a pair of twins. *Acta genet. (Basel)*, *9*, 47.
VAN DE HART, M. and VAN LOGHEM, J. J. (1967): Blood group chimerism. *Vox Sang. (Basel)*, *12*, 161.
VELEZ-OROZCO, A. C. (1961): Estudio de una quimera. *Bol. Inst. Estud. méd. biol. (Méx.)*, *19*, 61.
WALKNOWSKA, J., CONTE, F. A. and GRUMBACH, M. M. (1969): Practical and theoretical implications of fetal/maternal lymphocyte transfer. *Lancet*, *1*, 1119.
WEISS, E. and HOFFMANN, R. (1969): Eliminierung der XX-Zellen im Hoden heterosexueller Rinderzwillinge mit XX/XY Chimerismus. *Cytogenetics*, *8*, 68.
WISLOCKI, G. B. (1939): Observations on twinning in marmosets. *Amer. J. Anat.*, *64*, 445.
WITSCHI, E. (1965): Hormones and embryonic induction. *Arch. Anat. micr. Morph. exp.*, *54*, 601.
ZIVKOVIC, S., ISAKOVIC, I., JORANOVIC, V. and MILOSEVIC, M. (1970): Chromosome complement of the European wild pig (*Sus scrofa* L.). *Experientia (Basel)*, *27*, 224.
ZUELZER, W. W., BEATTIE, K. M. and REISMAN, L. E. (1964): Generalized unbalanced mosaicism attributable to dispermy and probable fertilization of a polar body. *Amer. J. hum. Genet.*, *16*, 38.

Patricia A. Jacobs

Medical Research Council, Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh, United Kingdom

Human population cytogenetics

At the time of the Third International Congress of Human Genetics a substantial amount was known about the frequencies and effects of abnormalities of number and structure of the X chromosome in both males and females. This was due to the ease with which large numbers of people could be screened using the buccal smear technique. The frequency and effects of an additional autosome 21 were known, and we had some idea of the proportion of individuals where this was associated, not with a simple trisomy, but with an aneuploid structural rearrangement. Also results were available on the frequency with which chromosome aberrations were found in spontaneous abortions. However, very little was known about the frequency of individuals with abnormalities of the Y chromosome, or of the effects of such abnormalities on the phenotype. Estimates of the frequency of structural rearrangements and of autosome trisomics, other than that for chromosome 21, were based on little more than inspired guesswork; similarly segregation ratios of structural rearrangements were estimated from a small amount of data obtained from interesting, but often atypical, families.

In the five years between the Third International Congress and the present one significant advances have been made in human population cytogenetics, advances best summarised by the words perspective and precision. Perspective has come gradually and unspectacularly with the accumulation of substantial bodies of data on the incidence and effects of chromosome abnormalities in particular groups of individuals, both those considered as representative of the population at large and those defined by an abnormal pattern of behaviour or by a diseased state. Precision on the other hand has come suddenly and spectacularly with our ability to unambiguously identify all the human chromosomes. Techniques for this involve banding patterns produced with a fluorescent dye (Caspersson *et al.*, 1971) or by one of a variety of Giemsa staining techniques (Sumner *et al.*, 1971). As such techniques, as applied to human chromosomes, are scarcely a year old we are only just beginning to exploit the advantages made available by this increase in precision.

In the present communication I will review briefly data on the incidence of chromosome abnormalities in man, and deal in more detail with one class of aberration – namely structural rearrangements of the autosomes. Data on the origin, mutation rate and segregation of such rearrangements will be described and the way in which chromosome identification may help to resolve some of the unsolved problems associated with this common class of abnormality will be discussed.

FREQUENCIES OF CHROMOSOME ABERRATIONS IN MAN

There are two main sources of data from which we can attempt to determine the frequency of chromosome aberrations in man – cytogenetic surveys of spontaneous abortions and

cytogenetic surveys of the live born neonatal population. However, estimates of the incidence of chromosome abnormalities obtained from these two sources must be underestimates for a number of reasons. In somatic cells we can only detect those abnormalities which result either in alterations of number, or alterations of structure associated with chromosomes with an abnormal morphology. We are unable to detect any abnormality of structure, such as paracentric inversions or pericentric inversions and other exchanges that involve equal amounts of chromosome material and which do not result in morphologically abnormal chromosomes. Furthermore, we can only examine those foetuses which survive long enough to give rise to a clinically recognisable pregnancy, and we have no information on conceptuses which are lost early in pregnancy. Finally, there is always the suspicion that in any estimate based on the results of successful tissue culture there may be a bias in favour of the normal, as the abnormal may well be overrepresented among the failures. For these reasons the frequencies of chromosome aberrations obtained from abortion and neonatal data refer only to those abnormalities detectable in mitotic cells and which are compatible with a recognisable pregnancy, and must therefore be regarded as minimal estimates.

DATA FROM SPONTANEOUS ABORTIONS

There have been a number of reports of chromosome studies of spontaneous abortions. The results obtained from the two largest surveys (Carr, 1967; Boué and Boué, 1970) have been combined and are summarised in Table 1. From these data, obtained from a total of 747 successfully cultured abortions, it appears that some 42% of all spontaneous abortions are associated with a detectable chromosome aberration. Furthermore, the earlier in gestation the aborted material is obtained the greater the probability that there will be an associated chromosome abnormality (Carr, 1970). About one half of the chromosomally abnormal abortuses have an additional autosome, one quarter are either triploid or tetraploid, while the majority of the remainder have an XO constitution. As approximately 15% of all recognised pregnancies terminate as spontaneous abortions it can be inferred from these data alone that at least 6% of all recognised conceptions have an abnormal chromosome constitution.

DATA FROM SURVEYS OF LIVE BORN NEONATAL POPULATIONS

Surveys of the chromosome constitution of consecutive live born hospital births have been undertaken in at least five different laboratories. The chromosomes of peripheral blood leucocytes from a total of 18,911 babies have been examined and 105 found to have an abnormal chromosome constitution (Table 2).

When the sex chromosome abnormalities are considered it can be seen that 0.29% of the males and 0.16% of the females were abnormal. Among the males an additional Y chromosome was the most frequent anomaly being found in approximately 1 in 580 live born males, while an additional X chromosome was present in approximately 1 in 810 males. Among the females 1 in 670 was found to have an additional X chromosome, while only one female with an XO sex chromosome constitution was found in the 6,723 female babies examined.

The frequency of autosomal trisomics in the newborn population was 0.13%. Of the 25 babies with an additional autosome, 21 had an additional chromosome 21, 3 an additional E group chromosome and one an additional D group chromosome.

A total of 33 babies (0.17%) were found to have a structural abnormality of the autosomes. Twenty-seven of these had a euploid or balanced rearrangement, 11 having a Robertsonian translocation involving two D group chromosomes, 3 a Robertsonian translocation involving a D group and a G group chromosome and 12 having a reciprocal translocation. Only 7 babies were found to have an aneuploid or unbalanced rearrangement. Two of these had

TABLE 1 *Chromosome abnormalities – spontaneous abortions*

Total	Sex chromosome ab. (number)				Autosome ab. (number)		Autosome ab. (structure)		Polyploids		Mosaics and others	Total
	XO	XYY	XXY	XXX	Single trisomy	Double trisomy	Euploid	Aneuploid	Triploid	Tetraploid		
747	53	–	1	–	171	9	–	8	60	13	2	317
Spontaneous abortions (%)	7.10	–	0.13	–	22.89	1.20	–	1.07	8.03	1.74	0.27	42.44
All recognised conceptions (%)	1.06	–	0.02	–	3.43	0.18	–	0.16	1.20	0.26	0.04	6.36

Data from Carr (1967); Boué and Boué (1970).

TABLE 2 *Newborn surveys (consecutive hospital births)*

Total	Males	Females	Sex chromosome ab.					Autosome trisomics			Autosome structural (euploid)			Autosome structural (aneuploid)				Total
			XO	XYY	XXY	XXX	Other	D+	E+	G+	D/D	D/G	Rec.T	D/D +D	D/G +G	Rec.T	Others	
Boston U. S. A. 1,384	1,384	–	–	3*	2	–	–	–	–	1	2	–	–	–	–	–	–	8
Edinburgh U.K. 7,989	5,980	2,009	–	11**	7	3	1	–	2	13	2	2	6	1	–	–	4**	52
New Haven U. S. A. 4,353	2,176	2,177	1	3	4	3	–	1	1	3	2	1	3	–	–	–	–	22
London Canada 2,081	1,066	1,015	–	4	1	–	–	–	–	2	1	–	–	1	–	–	1	10
Winnipeg Canada 3,104	1,582	1,522	–	–	–	4**	–	–	–	2	4	–	3	–	–	–	–	13
18,911	12,188	6,723	1	21	14	10	1	1	3	21	11	3	12	2	–	–	5	105

* = includes 1 mosaic. ** = includes 2 mosaics.

Data from Gerald (personal communication); Ratcliffe *et al.* (1970 and personal communication); Lubs and Ruddle (1970); Sergovich *et al.* (1969); Hamerton (personal communication).

a Robertsonian translocation involving two D group chromosomes and had in addition an extra D group chromosome, one had a short arm deletion of a chromosome in group B, two had an additional structurally abnormal chromosome, one had additional material translocated on to the long arms of a chromosome 16, and the remaining one had a dicentric chromosome replacing two chromosomes in group D.

TABLE 3 *Chromosome abnormalities (% live born population)*

Sex chromosome ab.	Autosome trisomics	Autosome structural ab.		Total
		Euploid	Aneuploid	
0.22	0.13	0.14	0.04	0.53

Therefore, from the neonatal data we can say that 0.53% of all conceptions which result in a live born child are associated with a chromosome abnormality (Table 3). As approximately 85% of all recognised conceptions result in the birth of a live born child we can infer from the newborn data alone that at least 0.45% of all recognised conceptions are associated with a chromosome abnormality. When data obtained from the newborn are combined with that obtained from spontaneous abortions we can conclude that some 7% of all recognised conceptions in man are associated with a chromosome abnormality. Furthermore, for the reasons given above this figure is likely to be a serious underestimate of the true frequency of chromosomally abnormal conceptions in man.

ORIGIN OF CHROMOSOME ABNORMALITIES

The majority of congenital chromosome abnormalities arise *de novo* in each generation and the reasons for their occurrence are still completely obscure. With the exception of the triploids and tetraploids, which probably originated in the main as the result of errors of fertilization, the majority must arise as the result of an error occurring either during the formation of the male or female gametes or at an early cell division of the zygote. The only factor which has clearly been shown to be of importance in the aetiology of the chromosomally abnormal individual is that of increasing maternal age. This is associated with the live born autosome trisomics and additional X chromosomes in both males and females (Court Brown *et al.*, 1969). In these classes of abnormality it is usually assumed that the ageing ovary is more prone to producing gametes with an additional, but apparently not a missing, chromosome. The reasons why the ageing ovary is at an increased risk are not as yet understood.

There is, however, one important group of aberrations which do not all arise *de novo* at each generation, namely the structural rearrangements of the autosomes. The origin of this class of rearrangement will now be considered in more detail.

ORIGIN OF EUPLOID STRUCTURAL REARRANGEMENTS OF THE AUTOSOMES

A total of 26 babies ascertained in the neonatal surveys was found to have a euploid rearrangement of the autosomes, and the chromosomes of both parents of 18 of these babies have been examined. Both parents of five of the babies, one with a Robertsonian translocation involving two D group chromosomes and four with reciprocal translocations, had a normal chromosome constitution. The rearrangement was found to be familial in 14 cases. In six of these, four with a Robertsonian translocation and two with a reciprocal translocation, the father carried the rearrangement and in the remaining seven, three Robertsonian and four reciprocal translocations, the mother carried the rearrangement.

These somewhat meagre data therefore suggest that some 25% of euploid rearrangements are the result of a new mutation, while the remaining 75% are inherited in approximately equal numbers from the male and female parent. However, when considering the proportion of euploid autosome rearrangements which are due to mutations we can use information from rearrangements ascertained from other populations, in addition to the data from neonatal surveys. During the course of a variety of cytogenetic surveys in Edinburgh we have found 73 independently ascertained euploid rearrangements of the autosomes. Ten of these rearrangements were found during the newborn survey and are included with the neonatal data discussed above. The chromosomes of both parents of 24 of the remaining 63 probands have been examined. In four instances both parents were normal, in nine cases there was an affected father and in 11 an affected mother. When these data are combined with those obtained from all the newborn surveys there is a total of 42 probands where the chromosomes of both parents have been examined (Table 4). Nine appeared to be new mutants, 15 had an affected father and 18 an affected mother. From these data it appears that 21% of euploid autosome rearrangements are new mutants and 79% are familial, half of the familial rearrangements being inherited from the mother and from the father.

TABLE 4 *Origin of euploid rearrangements (all data)*

Rearrangement	Total	Both parents examined	Both parents normal	Affected parent	
				Father	Mother
D/D	32	12	1	5	6
D/G	9	6	1	2	3
Reciprocal T.	41	20	7	6	7
Inv.	7	4	0	2	2
Total	89	42	9	15	18

As the frequency of euploid rearrangements in the newborn is 0.137% this gives a mutation rate of 1.4×10^{-4} per gamete per generation for all balanced rearrangements of the autosomes. This mutation rate is a realistic one providing there is no differential selection operating against either gametes or early conceptuses with a euploid structural autosome rearrangement. However, the mutation rate for different types of rearrangements may well be different. For example consideration of the data in Table 4 suggests that reciprocal translocations may well have a higher mutation rate than Robertsonian translocations.

ORIGIN OF ANEUPLOID STRUCTURAL REARRANGEMENTS OF THE AUTOSOMES

Aneuploid structural rearrangements of the autosomes are one of the most infrequent types of chromosome rearrangement found in the newborn population. Only seven were found among the 18,911 babies examined. Two of these were mosaics with a normal cell line and therefore had a high probability of arising as the result of a post-fertilization error in a normal zygote. The chromosomes of both parents had been examined in only two of the remaining five babies and both were found to have a mother with a balanced form of the rearrangement. These data are too few to permit of any conclusions on the origin of aneuploid chromosome rearrangements.

However, we can again consider data available from other sources. In Edinburgh we have found 52 non-mosaic individuals with an aneuploid chromosome rearrangement, all of whom were ascertained through a single affected proband. Data on the origin of the rearrangement in these 52 cases have been combined with those available from the five non-mosaic babies and are shown in Table 5. There are 38 probands where the chromosomes of both parents have

been examined. Of these, 25 appear to be new mutations, while the remaining 13 have an affected parent. In one case the father is affected and in the remaining 12 the mother is the affected parent. From these data it therefore appears that 66% of non-mosaic aneuploid structural rearrangements are the result of a new mutation and the remaining 34% have an affected parent. Of the 34% with an affected parent the mother is some 10 times more likely to have an abnormal chromosome constitution than the father.

TABLE 5 *Origin of aneuploid rearrangements (all data)*

Rearrangement	Total	Both parents examined	Both parents normal	Affected parent	
				Father	Mother
D+, D/D	3	2	1	0	1
G+, D/G	11	10	5	1	4
G+, G/G	3	2	2	0	0
Deletions	12	9	9	0	0
Duplications	8	7	4	0	3
Rings and dicentrics	3	2	2	0	0
Additional abnormal chromosomes	17	6	2	0	4
Total	57	38	25	1	12

The incidence of non-mosaic aneuploid rearrangements among the newborn is 0.026% and as 66% are mutants the mutation rate for all aneuploid structure rearrangements is 0.845×10^{-4} per gamete per generation. This mutation rate is only realistic if there is no selection operating against either aneuploid gametes or aneuploid conceptuses. However, it is reasonable to assume that such differential selection does operate and therefore that the mutation rate for aneuploid rearrangements may well be higher than the one given here. Furthermore, the mutation rate for various kinds of rearrangement may well be very different. However, the data presented here are too few to permit a detailed analysis.

SEGREGATION OF STRUCTURAL REARRANGEMENTS OF THE AUTOSOMES

BALANCED STRUCTURAL REARRANGEMENTS OF THE AUTOSOMES

Approximately 80% of all structural rearrangements ascertained through a balanced carrier are familial and they are equally likely to be paternally or maternally inherited. The segregation of balanced rearrangements among the relatives of probands with a segregating balanced rearrangement has been considered for both Robertsonian and reciprocal translocations (Jacobs *et al.*, 1970) and for inversions (Jacobs *et al.*, 1967). For all the cases of euploid structural rearrangements it has been found firstly that half the live born offspring of carrier parents, irrespective of whether they are male or female, have the balanced rearrangement and half have a normal chromosome constitution. Secondly, there has been no reported case of an aneuploid segregant, that is an individual with an unbalanced form of the rearrangement, reported in families ascertained in this way, in spite of the substantial numbers of individuals studied. Furthermore, we have not been able to detect any obvious reduction in the reproductive fitness of the carriers of balanced rearrangements by comparison with their sibs who have a normal chromosome constitution. The number of live born children in the two groups is the same and there does not appear to be any significant difference in the number of foetal or infant deaths.

ANEUPLOID STRUCTURAL REARRANGEMENTS OF THE AUTOSOMES

When a familial structural rearrangement is ascertained through an aneuploid individual the situation is different. First, among the phenotypically normal individuals half are found to have the balanced rearrangement and half to have a normal chromosome constitution, irrespective of the sex of the carrier parent. Secondly, there is a risk, probably of the order of 10 to 20% among carrier mothers, of having a child with an aneuploid form of the rearrangement. Thirdly, there is a risk which is considered less, probably of the order of 2–5% among carrier fathers, of having a child with an aneuploid rearrangement (Lejeune *et al.*, 1970; Hamerton, 1970). There are as yet insufficient accurate data available to know whether there is an increased number of foetal deaths among the conceptions of either males or females with a rearrangement ascertained through an aneuploid proband, or whether there is a reduction in the total number of their live born offspring by comparison with their sibs with a normal chromosome constitution.

It therefore appears from a consideration of all the available information on the segregation of rearrangements that among the phenotypically normal offspring of carrier parents of either sex, half have the balanced rearrangement and half have a normal chromosome constitution. However, when the rearrangement is ascertained through a balanced proband there is no evidence for the production of aneuploid offspring, whereas when the rearrangement is ascertained through an aneuploid proband there is a risk of production of aneuploid individuals. While this risk is not very high it is considerably greater in female carriers than in male carriers.

There are a number of possible explanations for these observations. The one which seems most plausible is that each rearrangement has its own probability of producing aneuploid gametes, depending on the chromosomes involved, the position of the break-points and the meiotic configuration. Furthermore, the probability that any aneuploid gametes produced will give rise to a recognisable conceptus will depend on (1) whether there is any differential selection operating against aneuploid gametes and (2) whether aneuploid conceptuses survive long enough to give rise to recognisable pregnancies.

It seems reasonable to postulate that structural rearrangements, while they all carry their own unique risk, fall into one of three general classes. First, a class in which the rearranged chromosomes always undergo regular meiotic pairing and in which exchange and disjunction occur in such a way that only balanced gametes are produced in both males and females. This category of rearrangement would not give rise to aneuploid gametes.

The second class of rearrangement gives rise to aneuploid gametes either by irregular pairing or because of the type of exchange and disjunction which occurs at meiosis. The control of these events and therefore the production of aneuploid gametes might well be different in males and females. It is postulated that the resulting aneuploid zygotes in this class are lethal before they give rise to a recognisable pregnancy and therefore that this category of rearrangement would not be associated with an increased number of recognisable abortions or aneuploid offspring. If there was complete reproductive compensation this class would be indistinguishable from the previous one. However, if there was not effective reproductive compensation this class of rearrangement might well be associated with subfertility in either male carriers or female carriers or both.

The third class of rearrangement also gives rise to aneuploid gametes and zygotes, but in this category the aneuploid zygotes survive long enough to be recognised either as abortions or aneuploid offspring. Therefore, this class is the one which is always identified when ascertainment of a familial rearrangement is made through an aneuploid child.

In order to explain why familial rearrangements ascertained in the general population do not seem to be associated with any evidence of aneuploid offspring or reduction in reproductive fitness we must assume that the majority of segregating structural rearrangements in man, detectable with conventional techniques, must belong to the first class or, alternatively, if

they belong to the second class, that there is effective reproductive compensation. Rearrangements falling into the third class must be in the minority, and it may well be a very small minority. However, it is this class which is likely to come to the attention of the clinician by virtue of the aneuploid child and, because of the comparative ease with which this class can be ascertained, it appears overrepresented in the data available at this time.

IDENTIFICATION OF CHROMOSOMES INVOLVED IN STRUCTURAL REARRANGEMENTS

Until recently it was not possible to know on anything but empirical grounds whether a rearrangement fell into a high, low or absent risk class, or even whether such distinct classes existed. However, the advent of simple reliable techniques for the recognition of every chromosome has made possible a new order of precision and their application over the next few years must surely shed much light on the problem of the behaviour and significance of structural rearrangements. The way in which these techniques have already helped us can be illustrated by reference to one rearrangement – namely Robertsonian translocations involving a group D and a group G chromosome.

The majority of such translocations have been ascertained through a proband with Down's syndrome. Therefore, it is reasonable to assume that the G group chromosome involved was a 21. Familial translocations ascertained in this way are associated with a risk of producing aneuploid offspring with Down's syndrome of approximately 10–15% in female carriers and about 3–5% in male carriers (Hamerton, 1970).

However, there are a number of familial Robertsonian translocations involving a D and a G group chromosome which have been ascertained independently of an individual with Down's syndrome. No individual with Down's syndrome has been reported in a family ascertained in this way. The reasons for this could be that all, or a large proportion, of the randomly ascertained D/G translocations do not involve a chromosome 21; or alternatively that the translocations do involve a chromosome 21, but the failure to find aneuploid individuals in the randomly ascertained group is a chance one due to the comparatively small number of families studied.

In Edinburgh we have ascertained 16 D/G translocations where it has been possible to identify the chromosomes involved and the results are shown in Table 6. As expected all the translocations ascertained through mongol probands involved a chromosome 21, six of them were between a chromosome 14 and a 21, one involved a 13 and a 21 and one a 15 and a 21. These results confirm those of others who had previously identified such translocations using autoradiography and had also reported an excess of 14/21 translocations (Cohen, 1971). However, among the 8 translocations ascertained randomly 5 also involved a 14 and a 21, while one involved a 14 and a 22 and two a 15 and a 22. If our results on randomly ascertained D/G translocations are found to be generally true it would appear that the failure to find

TABLE 6 *D/G translocations – identification of chromosomes*

Ascertainment	Total	Chromosomes involved					
		13/21	14/21	15/21	13/22	14/22	15/22
Mongol	8	1	6**	1	–	–	–
Random	8	0	5	0	0	1	2*
Total	16	1	11	1	0	1	2

* Includes 1 mutant. ** Includes 3 mutants.

mongol offspring in such families is only in part due to the fact that chromosome 21 is not involved. If one half have the same translocation, namely a 14/21, as that most frequently found to be associated with Down's syndrome, it must mean that the risk of aneuploid offspring to the carriers of such translocations is considerably lower than that previously estimated. However, it may be that, even within each individual type of Robertsonian translocation, there are two distinct classes depending on the centromere and short arm material which is present in the translocated chromosome. A 14/21 translocation where the centromere and short arm material present is that of a chromosome 21 may be associated with a different risk of producing aneuploid offspring to that of a 14/21 translocation where the centromere and short arm material is that of a chromosome 14. If two such classes exist within each Robertsonian translocation it may well be possible to distinguish between them. The centromere regions of many of the chromosomes, and especially the acrocentrics, are associated with distinctive features which are polymorphic and distinguishable using either the fluorescence or one of the various Giemsa banding techniques (Evans *et al.*, 1971). Judicious use of these techniques should enable us, at least in favourable situations, to determine the centromere which is present in such translocations.

CONCLUSIONS

It is clear that gross abnormalities of both number and structure of the chromosomes occur with an extremely high frequency in man. If we consider also those polymorphisms which are detectable either by the use of conventional techniques, or by the use of fluorescent or annealing techniques, we now have available under the light microscope direct evidence of an enormous wealth of genetic variation. The origin and significance of this variation in terms of population structure, reproductive fitness or disease are still obscure. This is perhaps not surprising when we remember that it was only 15 years ago that we discovered that our species had 46 chromosomes, and less than one year ago that we were able to tell each of these chromosomes apart. The last five years have seen an enormous increase in our knowledge of the variation which is present, the next five years must surely answer at least some of our questions as to why this variation exists and how it is maintained in the population.

REFERENCES

Boué, J. G. and Boué, A. (1970): Les aberrations chromosomiques dans les avortements spontanés humains. *Presse méd.*, *78*, 635.

Carr, D. H. (1967): Chromosome anomalies as a cause of spontaneous abortion. *Amer. J. Obstet. Gynec.*, *97*, 283.

Carr, D. H. (1970): Chromosome abnormalities and spontaneous abortions. In: *Human Population Cytogenetics, Pfizer Medical Monographs No. 5*, pp. 103–118. Editors: P. A. Jacobs, W. H. Price and P. Law. University of Edinburgh Press, Edinburgh.

Caspersson, T., Lomakka, G. and Zech, L. (1971): The 24 fluorescence patterns of the human metaphase chromosomes – distinguishing characters and variability. *Hereditas (Lund)*, *67*, 89.

Cohen, M. M. (1971): The chromosome constitution of 165 human translocations involving D group chromosomes identified by autoradiography. *Ann. Génét.*, *14*, 87.

Court Brown, W. M., Law, P. and Smith, P. G. (1969): Sex chromosome aneuploidy and parental age. *Ann. hum. Genet.*, *33*, 1.

Evans, H. J., Buckton, K. E. and Sumner, A. T. (1971): Cytological mapping of human chromosomes: results obtained with quinacrine fluorescence and the acetic-saline-Giemsa techniques. *Chromosoma (Berl.)*, *35*, 310.

Gerald, P. S. (1971): Personal communication.

Hamerton, J. L. (1970): Robertsonian translocations. In: *Human Population Cytogenetics, Pfizer*

Medical Monographs No. 5, pp. 63–75. Editors: P. A. Jacobs, W. H. Price and P. Law. University of Edinburgh Press, Edinburgh.

HAMERTON, J. L. (1971): Personal communication.

JACOBS, P. A., AITKEN, J., FRACKIEWICZ, A., LAW, P., NEWTON, M. S. and SMITH, P. G. (1970): The inheritance of translocations in man: data from families ascertained through a balanced heterozygote. *Ann. hum. Genet.*, *34*, 119.

JACOBS, P. A., CRUICKSHANK, G., FAED, M. J. W., FRACKIEWICZ, A., ROBSON, E. B., HARRIS, H. and SUTHERLAND, I. (1967): Pericentric inversion of a group C autosome: a study of three families. *Ann. hum. Genet.*, *31*, 219.

LEJEUNE, J., DUTRILLAUX, B. and DE GROUCHY, J. (1970): Reciprocal translocations in human populations. In: *Human Population Cytogenetics, Pfizer Medical Monographs No. 5*, pp. 81–87. Editors: P. A. Jacobs, W. H. Price and P. Law. University of Edinburgh Press, Edinburgh.

LUBS, H. A. and RUDDLE, F. H. (1970): Applications of quantitative karyotypy to chromosome variation in 4,400 consecutive newborns. In: *Human Population Cytogenetics, Pfizer Medical Monographs No. 5*, pp. 119–142. Editors: P. A. Jacobs, W. H. Price and P. Law. University of Edinburgh Press, Edinburgh.

RATCLIFFE, S. G., STEWART, A. L., MELVILLE, M. M., JACOBS, P. A. and KEAY, A. J. (1970): Chromosome studies on 3,500 newborn male infants. *Lancet*, *i*, 121.

SERGOVICH, F., VALENTINE, G. H., CHEN, A. T. L., KINCH, R. A. H. and SMOUT, M. S. (1969): Chromosome aberrations in 2159 consecutive newborn babies. *New Engl. J. Med.*, *280*, 851.

SUMNER, A. T., EVANS, H. J. and BUCKLAND, R. A. (1971): New technique for distinguishing between human chromosomes. *Nature new Biol.*, *232*, 31.

Chapter V Mental deficiency

George A. Jervis

New York State Institute for Basic Research in Mental Retardation, Staten Island, N. Y., U.S.A.

Introductory remarks

Genetic problems of mental retardation are as old as human genetics itself. During the past decade or so we have witnessed a truly extraordinary development of our knowledge of genetic factors in mental retardation following the discovery of chromosomal abnormalities and the identification of an increasing number of types of mental retardation caused by genetically determined metabolic errors. This new scientific knowledge, however, concerns only a rather small group of retardates. The larger group comprising some 75% of the retarded population is still the object of much controversy as to the relevance of genetic factors.

This symposium was organized with the purpose of clarifying some aspects of this problem. Dr. Myrianthopoulos will present data on the mental development of 450 twins. This large material is derived from a comprehensive study on physical and mental development of some 50,000 children who were followed from prenatal life to 7 and 12 years of age using an array of biological and psychological tests.

Dr. Scally will present his data on offspring of mentally retarded individuals. His material is smaller but has the advantage of covering the whole defective population of a limited geographic area, Northern Ireland. Dr. Jensen will discuss his recent data on 'primary' and secondary' mental retardation and their significance to genetics. His work is well known in America where it has become somewhat controversial and at times even explosive in our presently tense social and political climate. I trust that in the more rational atmosphere of France his findings and hypotheses will find a more objective evaluation. As a clinician who for some 40 years has examined thousands of mentally retarded children, I have found his work most interesting and helpful.

Ntinos C. Myrianthopoulos[1], *Paul L. Nichols*[2], *Sarah H. Broman*[1] *and V. Elving Anderson*[2]

[1] National Institutes of Health, Bethesda, Md. and [2] Dight Institute, University of Minnesota, Minneapolis, Minn., U.S.A.

Intellectual development of a prospectively studied population of twins and comparison with singletons

This investigation is a part of a larger, more comprehensive and still continuing study of intellectual performance of twin and sibling pairs who were born to mothers registered in the Collaborative Study of Cerebral Palsy, Mental Retardation and Other Neurological and Sensory Disorders of Infancy and Childhood*. This Study is a cooperative effort on the part of 12 institutions throughout the United States and the National Institute of Neurological Diseases and Stroke of the National Institutes of Health to observe and study events which affect the parents before and during pregnancy and to relate them to the outcome of pregnancy. To this end more than 50,000 pregnant women have been followed from the first months of their pregnancy through labor and delivery, and the children born to Study mothers are being followed to the seventh year of life. The Study population is about 45% white, 47% Negro, 7% Puerto Rican, and the rest a variety of other ethnic groups. The collection of information, medical examinations and laboratory tests are done in uniform fashion and according to pre-established protocol.

Several thousand women entered the Study more than once, thus the total population of children contains a large number from repeat pregnancies, most of them second, a smaller number third and a few fourth and even fifth. It was originally our intention to include in this presentation an extensive investigation of sibling pairs from the repeat pregnancies, but as the twin data grew in volume and, we hope, importance we decided to devote the whole paper to the twin material and present the findings on sibling pairs in detail separately.

MATERIALS AND METHODOLOGY

In all, 615 pairs of twins were born among 56,249 pregnancies with known outcome, or 1 in 91.5 births (Table 1). Among whites the twin birth incidence was 1 in 100.3, among Negroes

* The Collaborative Study of Cerebral Palsy, Mental Retardation, and Other Neurological and Sensory Disorders of Infancy and Childhood is supported by the National Institute of Neurological Diseases and Stroke. The following institutions participate: Boston Lying-In Hospital; Brown University; Charity Hospital, New Orleans; Children's Hospital of Buffalo; Children's Hospital of Philadelphia; Children's Medical Center, Boston; Columbia University; Johns Hopkins University; Medical College of Virginia; New York Medical College; Pennsylvania Hospital; University of Minnesota; University of Oregon; University of Tennessee and the Perinatal Research Branch, NINDS.

TABLE 1 *Twin births in the Collaborative Study*

	White	Negro	Other	Total
Twin pairs	259	331	25	615
All cases with known outcome	25,991	26,080	4,178	56,249
Twin birth incidence	1/100.3	1/78.8	1/167.1	1/91.5

1 in 78.8 and among an 'other' group, consisting mostly of Puerto Ricans, 1 in 167.1. The zygosity of 508 pairs was established by comparison of sex, blood types using nine systems (ABO, MNS, Rh, P, Kell, Lewis, Lutheran, Duffy and Kidd), and gross and microscopic examination of the placenta. In most of the remaining pairs one or both of the twins died early, before zygosity determination could be made, and placental examinations were not available or conclusive. A few pairs were lost to the Study either because they were delivered outside a Study hospital or because their families withdrew their cooperation.

It is well known that the mortality of twins is much higher than that of singletons and that most deaths occur during the first four weeks of life. In our material 17.3% of the twins died *in utero* or during the neonatal period, leaving 487 intact pairs (Myrianthopoulos, 1970). In this investigation we have included white and Negro twin pairs whose members were both alive and were both given psychological tests at one or more of the Study milestones, *i.e.* at 8 months, 4 years and 7 years. The sample of 25 pairs among the 'other' ethnic group has been excluded as being too small to make a meaningful contribution. For one special analysis we used 44 twin individuals whose co-twins died at birth or soon after.

Most analyses will be presented by race since about half the population of the Collaborative Study is Negro and it is well known that there are significant IQ differences between whites and Negroes in the United States. The causes of these differences, however, are not the objective of this study and will not be discussed. The distribution of the individual twins by zygosity, sex, birth order and race is given in the tables displaying the results of each test. In all, there were 666 twins with 8-month mental scores, 662 with 8-month motor scores, 592 with 4-year IQs and 396 with 7-year IQs. A large number of singletons from the population of the Collaborative Study, also tested with the same tests as the twins at 8 months, and 4 and 7 years, is available for a variety of comparisons. These comparisons have the advantage that both twins and singletons came from the same prospectively ascertained population of mothers. There were over 31,000 singletons with 8-month mental and motor scores and over 26,000 with 4-year IQs. A much smaller number, 3,400, with 7-year IQs is available because only about half the Study children have come through the 7-year milestone and only a small portion of these data have been processed and computerized.

The socioeconomic background of the families in which the twins were born has been assessed by means of a socioeconomic index that was specially developed to describe the population of the Collaborative Study. This index follows the methodology of the U.S. Bureau of the Census and combines scores for education, occupation and family income in a composite numerical score which runs from a low of 0 to a high of 99 (or 9.9) (Myrianthopoulos and French, 1968). Figure 1 shows the distribution of twins by socioeconomic index, compared with that of the Study population. The fit is very good except at the extreme right, where there is an increase of twins in the 6.0–9.9 socioeconomic range (Myrianthopoulos, 1970). This increase is independent of maternal age, race or zygosity and seems to imply that mothers in higher socioeconomic categories have more twins.

At 8 months the twins were tested with a research version of the Bayley Scales of Mental and Motor Development, very similar to that described in the manual by Bayley (1969). The mental scale contains 105 pass-fail items standardized from one to 15 months of age; the motor scale has 43 pass-fail items standardized from one to 12 months of age. For both

Bayley tests the raw score which is the number of passes between basal and ceiling levels is reported. At 4 years they were tested with the 1960 revision of the Stanford-Binet Intelligence Test, short form, which is described in detail in the manual by Terman and Merrill (1960). At 7 years they were given the Wechsler Intelligence Test for Children (WISC). The seven scales used are Information, Comprehension, Vocabulary and Digit Span (Verbal); and Picture Arrangement, Block Design and Coding (Performance). The test is fully described by Wechsler (1952).

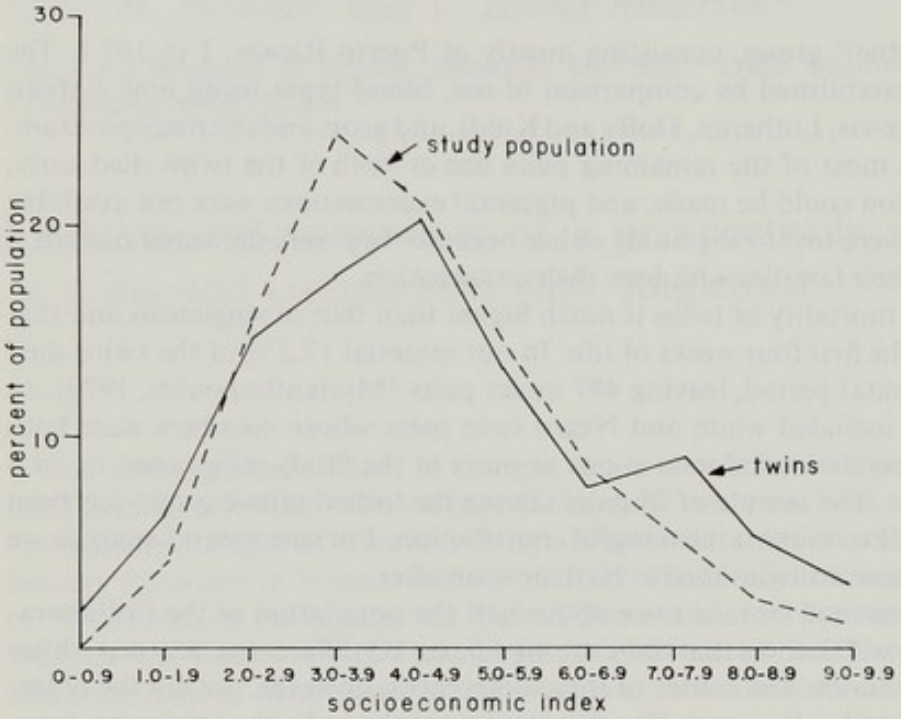


Fig. 1. Distribution of socioeconomic index scores of twins compared with that of the Study population.

RESULTS OF THE 8-MONTH, 4-YEAR AND 7-YEAR TESTS

Table 2 shows the distribution of means and standard deviations of the 8-month Bayley mental scores of twins and singletons. The most impressive feature here is the fairly large difference between white and Negro twins, especially in the face of very little difference between white and Negro singletons. The greater variability that is apparent in the twin scores, especially those of the Negro twins, is due largely to the excess of very low scores. If, for example, the frequency of Negro twins with scores below 65 is adjusted to equal that of singletons, the mean score for the twins becomes 77.3 and the standard deviation 6.2.

A factorial analysis of variance was performed to determine the magnitude and significance of the race, sex and twin effects on the scores. Least squares adjustments were used for disproportionality in sample sizes. In singletons, the main race effect is 0.7 points in favor of the whites, a small but statistically significant increase due to the large sample. There is no sex effect. White twins average about 3 points lower than white singletons while Negro twins average about 5 points lower than Negro singletons. In other words the detrimental effects on the Bayley mental scale of being a twin are greater in Negroes than in whites. Among Negroes the LSDZ twins average approximately 4 points higher than the MZ and USDZ twins.

The results of the Bayley motor examination, shown in Table 3, are very similar to those of the mental examination, although the difference in variability between the twin and singleton scores is less pronounced. The analysis of variance shows that white twins score about 2

TABLE 2 *Means and standard deviations of 8-month Bayley mental scores of twins and singletons*

	White			Negro		
	N	Mean	SD	N	Mean	SD
Twins						
Monozygotic	90	76.2	8.4	138	73.7	10.0
Like-sexed dizygotic	80	78.1	6.4	90	75.9	10.2
Unlike-sexed dizygotic	120	77.8	7.1	148	73.3	11.5
Male	142	77.3	7.0	172	73.5	11.6
Female	148	76.9	7.7	204	74.5	9.8
First born	145	77.2	7.9	188	74.1	10.9
Second born	145	77.0	6.9	188	74.0	10.5
All twins	290	77.1	7.4	376	74.0	10.7
Singletons						
Male	7699	79.5	5.2	8247	78.7	6.3
Female	7339	79.6	5.1	8365	79.0	5.6

TABLE 3 *Means and standard deviations of 8-month Bayley motor scores of twins and singletons*

	White			Negro		
	N	Mean	SD	N	Mean	SD
Twins						
Monozygotic	88	30.7	5.9	138	29.7	6.0
Like-sexed dizygotic	80	30.8	4.8	90	29.6	6.2
Unlike-sexed dizygotic	120	30.0	5.3	146	29.0	6.7
Male	140	30.5	5.3	171	28.6	6.7
Female	148	30.3	5.4	203	30.0	5.9
First born	144	30.5	5.3	187	29.5	6.1
Second born	144	30.3	5.5	187	29.3	6.5
All twins	288	30.4	5.4	374	29.4	6.3
Singletons						
Male	7699	32.8	4.8	8247	32.8	4.7
Female	7339	33.1	4.5	8365	33.3	4.4

TABLE 4 *Means and standard deviations of 4-year Stanford-Binet IQs of twins and singletons*

	White			Negro		
	N	Mean	SD	N	Mean	SD
Twins						
Monozygotic	80	95.6	15.2	126	83.2	14.2
Like-sexed dizygotic	66	93.4	14.2	82	85.8	14.8
Unlike-sexed dizygotic	102	96.5	17.3	136	82.9	14.5
Male	123	94.2	15.4	150	83.0	14.6
Female	125	96.6	16.3	194	84.3	14.4
First born	124	95.7	16.0	172	84.0	14.8
Second born	124	95.1	15.8	172	83.5	14.2
All twins	248	95.4	15.9	344	83.7	14.5
Singletons						
Male	6175	102.8	16.5	7068	90.2	13.9
Female	5738	106.5	16.7	7134	92.5	13.9

points below singletons while in Negro twins the decrement is nearly 4 points. There is a small but statistically significant sex effect: among whites, females average 0.3 points higher than males while among Negroes the female advantage is 0.5 points. Thus, a race × sex interaction indicates that the sex effect is slightly greater in Negroes than in whites.

Table 4 shows the distribution of 4-year IQs in twins and singletons. The most apparent difference between these results and the results on the 8-month examination is the large race effect. The analysis of variance indicates that this effect in singletons is approximately 13 points and it is highly significant. A sex effect in favor of females of 4 points in whites and 2 points in Negroes was noted; and the race × sex interaction shows that the sex effect here is greater in whites than in Negroes. In general, twins average about 8 points lower on the 4-year examination than singletons.

The results at 8 months are consistent with the observation that tests designed to measure cognitive development during the first two years of life usually fail to detect any systematic racial differences. Some investigators contend that Negroes perform even better than whites on the Bayley motor scale (Bayley, 1965; Jensen, 1969). It is clear, however, that the kinds of performances sampled at 8 months and 4 years are quite different. This difference is well emphasized by the fact that the Bayley scores correlate weakly with the Stanford-Binet at 4 years (0.22–0.24) and are, thus, poor predictors of IQ. Nevertheless, of approximately 100 variables examined in the Collaborative Study, only the socioeconomic index is a better predictor of the 4-year IQ than the Bayley scales (Broman and Nichols, unpublished observation). Interestingly, the relationship between test performance and socioeconomic index that is observed at 4 years is absent at 8 months.

The Swiss psychologist Piaget believes that cognitive development occurs in several distinct stages, the first of which is the sensory-motor period (0–2 years). In terms of Piaget's scheme, it appears that the Negro-white differences in intellectual performance exist only during the subsequent stages that involve operation of classes, relations, numbers, and later, symbols. These differences and their causes, however, are not the concern of this study.

TABLE 5 *Means and standard deviations of 7-year WISC IQs of twins and singletons*

	White			Negro		
	N	Mean	SD	N	Mean	SD
Twins						
Monozygotic	42	95.7	14.5	88	83.8	13.4
Like-sexed dizygotic	42	98.5	13.6	42	84.7	13.4
Unlike-sexed dizygotic	80	96.8	15.5	102	85.2	14.8
Male	80	99.6	15.3	95	84.3	14.7
Female	84	94.4	13.9	137	84.8	13.6
First born	82	96.2	15.8	116	83.7	14.4
Second born	82	97.7	13.7	116	85.4	13.6
All twins	164	96.9	14.8	232	84.6	14.0
Singletons						
Male Female	1940	101.9	13.5	1461	91.3	12.6

Table 5 shows the distribution of means and standard deviations of the 7-year IQs. These data are based on smaller samples of both twins and singletons but are still large enough to make meaningful comparisons. Preliminary analysis of these data suggests that the sex, race and twin effects are all smaller on the 7-year WISC than on the 4-year Stanford-Binet. The sex effect is, in fact, reversed, with the males scoring higher than the females, especially in the

whites. The race effect is approximately 10 points in favor of the whites. The scores of the twins average about 6 points lower than singletons, and a race × zygosity interaction is again evident. On all the tests, the scores of Negro twins are lower than would be expected from the observed combined Negro decrement and twin decrement.

CHANGES FROM 4 TO 7 YEARS

It would be instructive to study the change in IQ from 4 years to 7 years which is shown in Figure 2. Since the WISC has a lower ceiling than the Stanford-Binet it is more difficult to get very high scores on this test. It is not surprising, therefore, to find that the mean IQ of the Collaborative Study children is lower at 7 years than at 4 years. This decline is mainly due to the lower mean score of white singletons (2.6 IQ points) while that of the Negro singletons

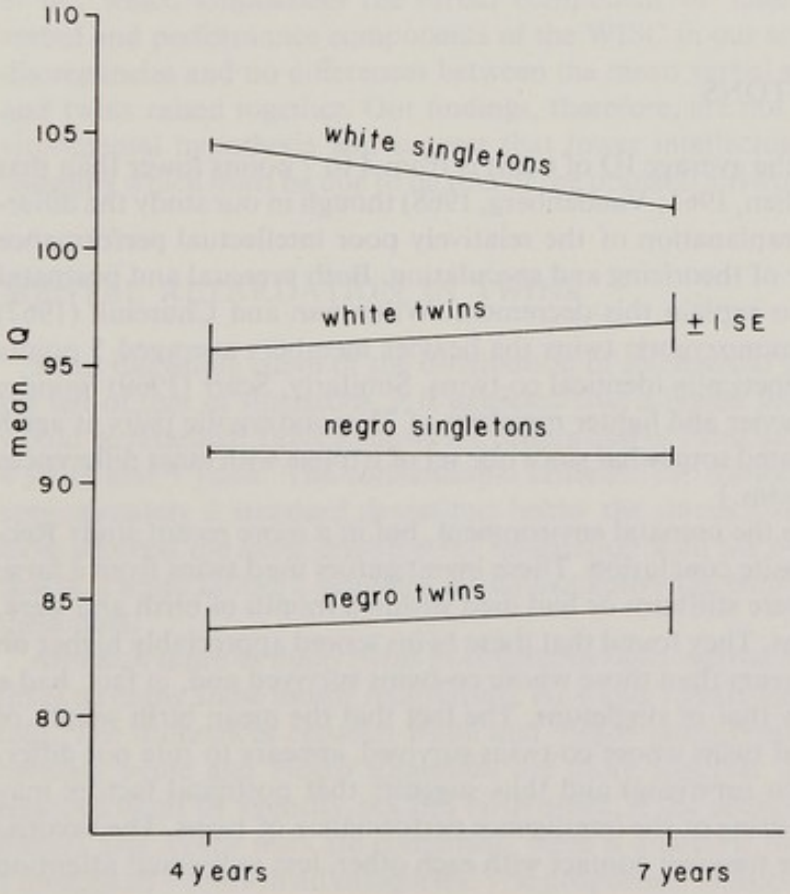


Fig. 2. Change in mean IQ from 4 to 7 years in twins and singletons.

remains essentially the same. Both white and Negro twins, however, show a slight increase in IQ from 4 to 7 years which supports the contention that twins tend to 'catch up' with time. The increase appears to be mostly due to higher scores on the performance scale. Table 6 shows that the twin-singleton difference is smaller on the performance than on the verbal scale and much smaller in whites than in Negroes. It is not appropriate to extrapolate the mental development of the twins back to the 8-month Bayley scales because these tests measure different abilities from those measured by the Stanford-Binet and the WISC. In any case, it is possible that the factors in the environment which affect IQ tend to improve for the twins, relative to singletons, so that during the 3-year period from 4 to 7 years the discrepancy between twins and singletons decreases in both whites and Negroes.

TABLE 6 *Difference in means of verbal, performance and full-scale 7-year IQs of twins and singletons by race*

	Twins		Singletons		Difference in means
	Mean	SD	Mean	SD	
White					
Verbal scale	96.0	14.3	100.0	13.7	−4.0
Performance scale	101.5	13.4	103.8	14.4	−2.3
Full-scale IQ	96.9	14.8	101.9	13.5	−5.0
Negro					
Verbal scale	84.9	12.1	90.7	12.4	−5.8
Performance scale	88.7	14.6	93.7	13.3	−5.0
Full-scale IQ	84.6	14.0	91.3	12.6	−6.7

TWINS RAISED AS SINGLETONS

It is now well-established that the average IQ of twins is from 4 to 7 points lower than that of singletons (Sandon, 1957; Drillien, 1961; Vandenberg, 1968) though in our study the difference extends to 10 points. The explanation of the relatively poor intellectual performance of the twins is at present a matter of theorizing and speculation. Both prenatal and postnatal deprivation have been invoked to explain this decrement. Willerman and Churchill (1967) showed that among 27 pairs of monozygotic twins the heavier members averaged 5 points higher on the WISC than their genetically identical co-twins. Similarly, Scarr (1969) found a 9 point difference between the heavier and lighter members of 25 monozygotic pairs at age 8 years. (This value is probably inflated somewhat since one set of triplets with large differences in IQs was counted as 3 pairs of twins.)

These studies seem to implicate the prenatal environment, but in a more recent study Record *et al.* (1970) reached the opposite conclusion. These investigators used twins from a large English sample whose co-twins were stillborn or had died within a month of birth and were, therefore, brought up as singletons. They found that these twins scored appreciably higher on a verbal reasoning test at age 11 years than those whose co-twins survived and, in fact, had a mean score of only 1 point below that of singletons. The fact that the mean birth weight of these twins was lower than that of twins whose co-twins survived, appears to rule out differential selection (the healthier twin surviving) and thus suggests that postnatal factors may also be important in causing a lowering of the intelligence performance of twins. The favorite postnatal theory is that twins have frequent contact with each other, less individual attention

TABLE 7 *Mental test performance of twins raised as singletons and raised together*

	Mean 4-year IQ	Mean 7-year IQ	Mean SE index score
Twins raised as singletons			
White	95.4 (N = 22)	97.0 (N = 18)	51.2
Negro	83.1 (N = 22)	85.3 (N = 15)	37.3
Twins raised together			
White	95.8	97.2	56.9
Negro	84.1	84.0	38.7

from their parents, a much restricted circle of friends, and reduced opportunities for verbal communication with adults and other sibs; while singletons and singleton sibs tend to be exposed to each other's friends and to have a much wider variety of associations (Koch, 1966; Record *et al.*, 1970).

The findings of Record *et al.*, however, are not confirmed in our material. Four-year IQs were available on 44 twins, 22 white and 22 Negro, whose co-twins died (42), or were separated (2) at birth or soon after. Seven-year IQs were available on 33 of these. As is seen in Table 7, the mean 4-year and 7-year IQs of twins raised as singletons are practically identical with the IQs of twins raised together. This finding is true of white as well as Negro twins. Though our sample of 44 twins raised as singletons is smaller than the 148 of Record *et al.*, it should be adequate to show a trend, if such existed. The socioeconomic background of white and Negro twins raised as singletons is not significantly lower than that of twins raised together and could not explain the findings. The Stanford-Binet and the WISC, with which our twins have been tested, are more comprehensive intelligence tests than that used by Record *et al.*, which emphasizes the verbal component of intelligence. An examination of the verbal and performance components of the WISC in our small sample shows no unexpected discrepancies and no differences between the mean verbal score of twins raised as singletons and twins raised together. Our findings, therefore, are not consistent with the postnatal environmental hypothesis and suggest that lower intellectual performance is an attribute of twinning which must be due to factors of the prenatal environment.

MENTAL RETARDATION IN TWINS

Since the whole curve of the distribution of intellectual performance of twins is shifted to the left of that of singletons, it is expected that a higher proportion of twins than singletons would be retarded. Table 8 shows the proportion of twins and singletons retarded at 8 months, 4 years and 7 years. The conventional criterion for mental retardation, an IQ below 70, is approximately 2 standard deviations below the standardized population mean of 100. We have adopted the same criterion for retardation with the 8-month mental and motor scales, that is, a score falling 2 standard deviations below the empirical mean of our population on these tests.

Almost 6 times as many twins as singletons show mental retardation at 8 months and over 7 times as many show motor retardation. At 4 and 7 years this difference drops to about threefold. The differences between white and Negro twins remain consistently great while those between white and Negro singletons are more variable. Table 9 shows the proportion of retarded twins by zygosity, sex and birth order. If the proportions of retarded on the 8-month mental and motor tests are combined, there is a decline in the proportion of retarded from 8 months to 4 years in all categories. The proportion of like-sexed dizygotic twins is the lowest among all twins. Relatively fewer females are retarded at 8 months but the situation is surprisingly reversed at 4 and 7 years when relatively fewer males are retarded.

The retarded twins at 4 or 7 years or at both ages were distributed among 72 pairs, 22 pairs with both retarded and 50 pairs with one retarded. It should be pointed out that here we are dealing mostly with high grade, undifferentiated mental retardation. The mean IQ of the group with both twins retarded is 60.4 ± 7.3, and only three twins from this group have an IQ below 50; the mean IQ of retarded twins from the group with one retarded is 60.3 ± 8.5, with six twins having an IQ below 50. There is obviously no difference in the magnitude of mental retardation between the two groups. A first step in approaching the question of the relative contribution of heredity in such a trait as mental retardation should be to examine the distribution of retarded twins in monozygotic and dizygotic pairs.

The distribution of pairs with retarded twins by zygosity and race is shown in Table 10. Concordance for retardation among monozygotic pairs is obviously higher than among dizygo-

tic pairs and the difference is significant on a conventional 2×2 test with correction for continuity. It is surprising that very few examples of this type of approach to mental retardation exist in the literature. Allen and Kallmann (1962) in a study of institutionalized twins found 100% concordance among monozygotic twins with high grade, undifferentiated mental retardation, but also found a high concordance among dizygotic twins which could be partly explained on the basis of differences in institutionalization of like-sexed and unlike-sexed pairs. The concordance rate of our monozygotic twins is actually underestimated, for in a large proportion of monozygotic pairs that were considered discordant, the non-retarded twin

TABLE 8 *Per cent twins and singletons retarded at 8 months, 4 years and 7 years**

	8-month mental	8-month motor	4-year IQ	7-year IQ
All twins	14.0	18.3	13.0	8.9
White	6.6	12.8	7.2	4.3
Negro	19.7	22.5	17.2	12.0
All singletons	2.5	2.5	4.6	2.4
White	1.6	2.4	2.0	0.9
Negro	3.3	2.6	6.3	3.9

* Scoring 2 standard deviations below the mean (70 on the 8-month Bayley mental scale, the Stanford-Binet and the WISC, and 25 on the 8-month Bayley motor scale).

TABLE 9 *Per cent twins retarded at 8 months, 4 years and 7 years by zygosity, sex and birth order*

	8-month mental	8-month motor	4-year IQ	7-year IQ
Twins				
Monozygotic	14.5	18.6	14.6	9.2
Like-sexed dizygotic	8.9	13.0	10.2	4.8
Unlike-sexed dizygotic	16.8	21.4	13.5	10.4
Male	15.3	21.2	12.8	7.9
Female	12.8	15.6	13.1	9.6
First born	14.8	16.9	14.2	9.6
Second born	13.2	19.6	11.8	8.1

TABLE 10 *Twin pairs with one or both twins retarded by zygosity and race*

	One twin retarded	Both twins retarded
Twin pairs		
Monozygotic	13	13
Dizygotic	37	9
	$\chi^2 = 5.89$, $P < 0.02$	
White	11	4
Negro	39	18
Total	50	22

exceeded the arbitrary IQ level of 70 by only a few points, while in discordant dizygotic twins the IQ of the non-retarded twin is much more variable. This is clearly evident in Table 11 which shows the means and standard deviations of concordant, and retarded and non-retarded among discordant twins. The mean IQ difference between retarded and non-retarded in the discordant monozygotic pairs is 18.0 points while that in the discordant dizygotic pairs is much larger, 24.4 points; and the difference between standard deviations of the non-retarded members of monozygotic and dizygotic pairs of 5.5 points is highly significant. These differences clearly show that a considerably larger number of monozygotic than dizygotic co-twins have IQs in the 70–80 IQ range. The difference in standard deviations between retarded members of discordant monozygotic and dizygotic pairs is also highly significant and is largely due to some quite low scores in the latter group.

TABLE 11 *Means and standard deviations of IQs of concordant, and retarded and non-retarded among discordant twins*

	Both twins retarded		One twin retarded			
			Retarded twin		Non-retarded twin	
	Mean	SD	Mean	SD	Mean	SD
Twins						
Monozygotic	60.0	6.9	64.5	3.2	81.9	5.4
Dizygotic	59.6	8.2	58.8	11.3	83.2	10.9

Our findings, therefore, do not appear to be too different from those of Allen and Kallmann, if allowance is made for the ascertainment bias of that study. Nor can our results be explained by differences in birth weight. The mean birth weight of retarded twins generally, and when examined by zygosity, race, sex and birth order, is not different from that of non-retarded twins. In fact, the mean birth weight of retarded twins among discordant pairs (2404.1 ± 437.7 g) is slightly though not significantly higher than the mean birth weight of their non-retarded co-twins (2375.7 ± 350.8 g). While, therefore, it is generally accepted that low birth weight is associated with small decrements in IQ, our findings indicate that it is not a deciding factor in mental retardation. The tyranny of small numbers which perennially afflicts twin research frustrates any attempts at a more extensive or sophisticated analysis; but from our small sample it appears that the contribution of heredity in mental retardation must be considerable.

EVENTS OF POSSIBLE SIGNIFICANCE IN THE HISTORY OF RETARDED TWINS

In another, retrospective approach to the problem, we reviewed the records of twins who were concordant and discordant for mental retardation in an effort to uncover any significant socioeconomic, genetic and medical events which might explain, or give any clues about, the occurrence of retardation in these twins. Special attention was paid to the socioeconomic background of the family, parental consanguinity, presence of mental retardation and other gross physical defects in other members of the family; toxemia in the mother during pregnancy, infection, severe anemia and trauma during pregnancy, and abnormal labor; trauma in the child during and after delivery, primary apnea (apnea of more than two minutes' duration), severe infection, severe neurogical or physical defect, cardiovascular disease and genetic defect. A summary distribution of these events in various members of the families of concordant and discordant twins, and in the twins themselves, is given in Table 12; a more

detailed account, showing what these events are qualitatively and in whom and in which families they occurred, is given in the Appendix.

It is immediately apparent that the retarded twins come from families of lower socioeconomic background than that of twins in general. The drop in socioeconomic index is mainly due to the preponderance and contribution of Negro twin pairs. When examined by race, the mean socioeconomic index of concordant Negro twins (28.0) is over 10 points lower than that of all Negro twins, while the mean socioeconomic indices of concordant white twins (52.3), and discordant white (56.6) and Negro twins (34.2) are quite similar to those of all white and and Negro twins. The relatively low socioeconomic background of concordant Negro twins would appear to temper the genetic interpretation of these data, but one should be wary of interpreting the effects of socioeconomic background as purely environmental. These findings, however, underscore the elusive character of this parameter and its influence on intellectual performance.

TABLE 12 *Events of possible socioeconomic, genetic and medical significance in the history of twins concordant and discordant for mental retardation*

	Concordant pairs	Discordant pairs
Socioeconomic index	32.0	39.2
Events of possible significance in:		
both twins	0	6
one twin – retarded	6	6
non-retarded	–	3
mother	4	8
father	0	2
mother and father	1	3
siblings	0	3
other close relatives	0	4
Families with events	10	29
Families without events	12	21

Events that affected both twins are confined to discordant pairs and consist of latent syphilis, malnutrition, erythroblastosis and severe anemia. Twins concordant for mental retardation were discordant for primary apnea, pneumococcal meningitis, congenital heart disease and sicklemia. Such a distribution is certainly not what would be expected if these events were responsible for the mental retardation. It is interesting that in the three discordant cases of primary apnea, it was the second twin who was apneic and that in two of these the apneic has a higher IQ than the non-apneic twin. Twins discordant for mental retardation were also discordant for primary apnea, spinal meningitis, malnutrition, amphetamine poisoning, seizures, cerebral palsy, lipochondrodystrophy and hyperthyroidism. Typical events in the mother were severe anemia during pregnancy, seizures and syphilis; in the mother and father, mental illness; and in siblings and other close relatives, CNS defects, seizures, mental retardation and mental illness. It is evident that these events are relatively more frequent in families of discordant than concordant pairs. These considerations seem to indicate that in concordant twins there is no apparent relation between any specific events in the parents, the family or the environment and the occurrence of mental retardation, and that the retardation is in large measure due to unknown prenatal influences, genetic or environmental, which are apt to affect both twins; while in discordant twins retardation tends to be associated with specific events, genetic and environmental, which could presumably affect the biologically more susceptible twin.

It may be of interest to point out that of the three twins from the concordant group who had an IQ below 50, one had pneumococcal meningitis at age 23 months, the mother of the second had a history of convulsive disorder and mental illness, and there was nothing significant in the history of the third. Of the six twins from the discordant group who had an IQ below 50, one with an IQ of 38 had grand mal seizures from age 4 months to 5 years and a pneumoencephalogram showed mild dilation of the lateral ventricles; a second with an IQ of 47 had grand mal seizures and muscular wasting which after biopsy was diagnosed as muscular dystrophy; a third with an IQ of 37 had severe erythroblastosis fetalis which required three exchange transfusions; his co-twin also had three transfusions but his IQ is 94. There was nothing significant in the history of the other three.

The results of all these investigations indicate that while the role of genetic factors in the etiology of undifferentiated mental retardation is significant, environmental influences are also important. Most of the environmental factors are subtle and unidentified, but some of them, including twinning, are more obvious.

SUMMARY AND CONCLUSIONS

In this study we have compared the intelligence test performance and the frequency and characteristics of mental retardation of twins in the Collaborative Study with those of singletons from the same population.

Twins perform more poorly than singletons, on tests of mental and motor performance, and the detrimental effects of being a twin are greater in Negroes than in whites. The performance of twins, relative to that of singletons, tends to improve as they get older at least from age 4 to 7 years. Whether or not this 'catch up' phenomenon continues into adolescence and adulthood remains to be seen, although Vandenberg's (1968) studies of older twins suggest that it does. Plans are now being made to follow the Collaborative Study twins to age 15 years and to study their mental, physical and behavioral development into adolescence.

Twins are physically and mentally retarded 6–7 times as frequently as singletons at 8 months, and mentally retarded 3 times as frequently as singletons at 4 and 7 years. The poor performance of twins relative to singletons and the increased frequency of retardation in twins are likely due to a poor prenatal environment. This conclusion is supported by the fact that twins brought up as singletons perform at the intelligence level of twins and not of singletons.

Concordance for undifferentiated mental retardation is significantly higher in monozygotic than in dizygotic twins. While mental retardation of this kind in both twins of a pair does not seem to be related to any antecedent events in the family, that in only one twin is often associated with any of a large number of specific events of possible significance. Low socioeconomic status, which is often associated with the so-called 'cultural-familial' mental retardation, is an impressive feature only in the families of the concordant Negro twins.

The distribution of twins concordant for mental retardation and the frequency of mental retardation in twins relative to singletons indicate that both genetic and environmental factors are involved in the etiology of mental retardation for which no specific cause can be found; while changes in performance over time, such as that seen in twins relative to singletons from 4 to 7 years, can be caused by postnatal environmental factors.

ACKNOWLEDGEMENTS

We wish to thank Dr. Alfred Naylor for his help in programming and performing the analysis of variance; and Dr. Gordon Allen for his critical reading of the manuscript.

APPENDIX

Events of possible socioeconomic, genetic and medical significance in the history of twins concordant and discordant for mental retardation

Concordant twins	Family number
One twin	
Primary apnea	8, 10, 20
Pneumococcal meningitis at age 2 months	12
Congenital heart disease	13
Sickle cell trait	22
Mother	
Severe anemia during pregnancy	1
Seizures and mental illness	2
Syphilis	13
Severe viral infection during first trimester	16
Mother and father	
Mental illness	15
Discordant twins	
Both twins	
Seizures	4
Early latent syphilis	19
Severe anemia	46
Severe malnutrition	24
Erythroblastosis fetalis with exchange transfusions	17, 40
Retarded twin	
Seizures and muscular dystrophy	14
Seizures and congenital heart disease	36
Cerebral palsy	41
Amphetamine poisoning at 2 years	43
Lipochondrodystrophy	44
Hyperthyroidism	48
Non-retarded twin	
Primary apnea	15
Spinal meningitis at age 2 months	29
Severe malnutrition	35
Mother	
Severe anemia during pregnancy	11, 13
Toxemia of pregnancy	12
Vaginal bleeding during first trimester	40
Seizures	45
Sickle cell trait	18
Syphilis	18, 22
Mental illness	17
Father	
Anhidrotic ectodermal dysplasia	1
Mental retardation	36
Mother and father	
Mental illness	34, 39, 42
Siblings	
Hydrocephalus	5
Seizures	28, 34

Discordant twins	Family number
Other close relatives	
Hydrocephalus	7
Mental retardation	2
Mental illness	12, 17

REFERENCES

ALLEN, G. and KALLMANN, F. J. (1962): Etiology of mental subnormality in twins. In: *Expanding Goals of Genetics in Psychiatry*, Chapter 21, pp. 174–191. Editor: F. J. Kallmann. Grune and Stratton, New York, N. Y.

BAYLEY, N. (1965): Comparisons of mental and motor test scores for ages 1–15 months by sex, birth order, race, geographical location, and education of parents. *Child Developm., 36*, 379.

BAYLEY, N. (1969): *Bayley Scales of Infant Development (Manual)*. Psychological Corporation, New York, N. Y.

DRILLIEN, C. M. (1961): A longitudinal study of the growth and development of prematurely and maturely born children. Part VII: Mental development 2-5 years. *Arch. Dis. Childh., 36*, 233.

JENSEN, A. R. (1969): How much can we boost IQ and scholastic achievement? *Harvard educ. Rev., 39*, 1.

KOCH, H. L. (1966): *Twins and Twin Relations*. The University of Chicago Press, Chicago, Ill.

MYRIANTHOPOULOS, N. C. (1970): An epidemiologic survey of twins in a large, prospectively studied population. *Amer. J. hum. Genet., 22*, 611.

MYRIANTHOPOULOS, N. C. and FRENCH, K. S. (1968): An application of the U. S. Bureau of the Census socioeconomic index to a large, diversified patient population. *Soc. Sci. Med., 2*, 283.

RECORD, R. G., MCKEOWN, T. and EDWARDS, J. H. (1970): An investigation of the difference in measured intelligence between twins and single births. *Ann. hum. Genet., 34*, 11.

SANDON, F. (1957): The relative numbers and abilities of some ten-year-old twins. *J. roy. statist. Soc., 120*, 440.

SCARR, S. (1969): Effects of birth weight on later intelligence. *Soc. Biol., 16*, 249.

TERMAN, L. M. and MERRILL, M. A. (1960): *Stanford-Binet Intelligence Scale: Manual for the Third Revision, Form L-M*. Houghton-Mifflin Co., Boston, Mass.

VANDENBERG, S. G. (1968): The nature and nurture of intelligence. In: *Genetics*, Chapter 1, pp. 3–58. Editor: D. G. Glass. Rockefeller University Press and Russell Sage Foundation, New York, N. Y.

WECHSLER, D. (1952): *Wechsler Intelligence Scale for Children: Manual*. Psychological Corporation, New York, N. Y.

WILLERMAN, L. and CHURCHILL, J. A. (1967): Intelligence and birth weight in identical twins. *Child Developm., 38*, 623.

B. G. Scally

Muckamore Abbey Hospital, Muckamore, Co. Antrim, Northern Ireland

A survey of mentally defective parents and their offspring

In the field of mental health there are certain topics which have the habit of cropping up now and again, arousing great interest and controversy, and then subsiding. One of these concerns the offspring of the mentally defective. You will all have read about the early alarmist theories of the 1900's when all sorts of fulminating things were said about the mentally defective: that they were the canker of society, that they were responsible for much crime, that female defectives turned almost naturally to prostitution, and so on. Of more profound concern was the fear that, ultimately, society would be overrun by defectives. The argument which underlay this fear was simple and was in two parts, each being treated almost as axioms. The first was that defectives beget defectives. The second was that defectives have large numbers of children. It clearly follows, the argument ran, that it will only be a matter of time before society is swamped by the subnormal. Family histories – the Jukes, the Kallikaks, the Hill Folk – were cited as evidence of what might happen to the community on a large scale. Experts and lay people, worried about the consequences, anxiously advocated measures (some of them extreme and repressive) which would help to stem, if not completely stop, this menace.

In later years there were soothing noises whenever this subject came up. Society is a self-regulating, self-balancing type of machine went one argument. Tall parents have children who are taller than average but shorter than the parents themselves. The children of very bright parents are brighter than average but not as intelligent as the parents. The phenomenon of biological regression-to-the-mean was adduced to counter the older arguments. By this time (the 1940's and 1950's), of course, investigations of this kind were much more sophisticated. Nevertheless, there were nagging doubts. Thomson (1950), reporting on his survey of the Isle of Wight populations, found that the average I.Q. of children decreased as family size increased. He concluded that the I.Q. of the community was declining little by little. In his very readable account of this problem Eysenck (1953) suggests that the question is by no means settled and that, in view of its importance, a large and comprehensive survey should be undertaken.

Very recently another issue has come up which has aroused much heat and controversy. This is the problem of race, intelligence and education (in fact the title of a work by Eysenck, 1971), although only a small segment of the issue is directly relevant here. In a well reasoned argument Eysenck suggests that observed differences in abilities between groups (*e.g.* races) of people may be influenced by many factors which tend to be overlooked. He tentatively suggests, for example, that the people who have emigrated to the United States of America over the years may have been biassed samples of the populations they left. I quote .... 'many other groups came to the U.S.A. due to pressures which make them very poor samples of the original populations; Italians, Spaniards and Portuguese, as well as Greeks, are examples

where the less able, less intelligent were forced through circumstances to emigrate, and where their American progeny showed significantly lower I.Q.'s than would have been shown by a random sample of the original population. Other groups, like the Irish, probably showed the opposite tendency; it was the more intelligent members of these groups who emigrated to the U.S.A. leaving their less intelligent brethren behind' (p.47).

The implication of the latter part of his argument – the selective emigration of the more able – is obvious: the general ability level of those who remain declines as the brighter elements leave. Now, I personally have an interest in this argument since I come from Northern Ireland and it was in Northern Ireland that the survey I propose to describe was carried out. If Eysenck is right, then it might be supposed that the average I.Q. of the Province is lower than in other parts of the United Kingdom. But I have no evidence about this. It could also be suggested that there might be a greater rate of mental subnormality in this area than elsewhere. On this count we do have evidence which, in short, is that the rate of *severe* subnormality is higher in Northern Ireland than elsewhere in the United Kingdom, that it is greatest in areas where there has been mass depletion of the population as a result of emigration and that the prevalence of mongolism is nearly the highest ever quoted in the literature.

Set against this background, it is clear that the problem of the offspring of the mentally subnormal is an important one. Even if the regression-to-the-mean phenomenon operates in their case, it is surely possible that at least some of the children will themselves be defective to the extent that they need special care, training and supervision. Other simple and obvious points arise. We know, for example, that mental deficiency is not a disease entity. It is a feature common to many people of all ages who differ, often very noticeably, in other respects. One very obvious way in which they differ is, of course, aetiology and diagnosis. Is there a relationship, then, between the ability levels of the offspring and the diagnostic category of the parents? (We can make one reasonable guess here: a subnormal woman whose mental handicap is attributable solely to an injury – say, a head injury in childhood – may have children who are normal.)

Investigations into this problem are relatively few. Brandon (1957) in her study of 108 children born to 73 mentally defective mothers found that 13% were themselves defective and 37% 'retarded' or 'backward'. Shaw and Wright (1960) came to a similar conclusion: 12% of the 377 children of defective parents were unsuitable for ordinary education. Girault-L'Herbault and Lafon (1964) classified their sample of 125 children as follows:

I.Q. below 50	30%
I.Q. 51–80	45%
I.Q. normal	25%

Obviously, there are discrepancies here. They might be due to biased samples, *e.g.* accidental or incidental. One of the aims of the present survey was to look at the entire population of subnormals under both community and residential care, identify those who had had children and make every possible effort to trace and examine each and every child.

SUBJECTS AND FINDINGS

I. PARENTS

In Northern Ireland the responsibility for the mentally subnormal of all ages lies with one administrative body, the Special Care Service. The nature of this organization made it easier to trace all relevant cases. At the time of the survey there were 4,631 special care cases in a total general population of 1,435,000. Of the mentally subnormal group, 353 met one of the following criteria:

1. Married, with or without offspring.
2. Single, with offspring alive or deceased.
3. Single, with miscarriages or still births.

These subjects comprised 8% of the subnormal population and 0.025% of the general population. Only 11 cases were not available for the survey. This is a very small number, approximately 3%, of the group of subnormal parents. In nearly all instances they had left the country to live elsewhere. In the actual sample investigated there were 32 males and 310 females. All 32 men were married; for obvious reasons it was impossible to include single males. One hundred and ninety-seven of the women were married and 113 were single. The chronological ages of the males ranged from 21–73 years with a mean of 37.6. The ages of the females ranged from 17–84 years with a mean of 36.6. The difference between the proportion of married cases in the subnormal sample and the proportion of married cases in the general population was highly significant.

Diagnostic classification is notoriously difficult in subnormality, apart from a relatively few well defined syndromes. In the present survey it was made all the more difficult because of two factors. Firstly, many of the well defined clinical groups are to be found amongst the severely or profoundly retarded. The higher the mental grade, the smaller the incidence of such well defined syndromes. The severe and profound mental defectives are almost completely infertile, not in the biological sense but rather in the psychological sense. Most of the cases in the present survey were in the upper reaches of subnormal ability levels and classification was, therefore, problematic. Secondly, no mongols were involved and this meant the omission of yet another relatively well defined subgroup. In the event, the following very simple system was used:

1. *Familial* – where there is at least one other mentally deficient person in the immediate family and the condition of neither has a clearly defined cause.
2. *Undifferentiated* – no other classification of the mentally deficient is appropriate.
3. *All other diagnoses* – this includes mental deficiency due to developmental cranial anomalies, infection, trauma, epilepsy, other diseases of the nervous system and a few patients with undetermined subdiagnoses.

Half the parents were classified as familial, 41% as undifferentiated and 9% as 'others'. Clinical examination revealed no chromosomal abnormalities; only one family was suspected of having a metabolic abnormality. Approximately two-thirds of the subjects lived in the community, the remainder being under hospital or other residential care. The mean I.Q. of the male parents was 66, with a range of 42–84; the mean I.Q. of the subnormal mothers was 57 with a range of 21–90. Both distributions were skewed. Interestingly enough, there was only one family in which both husband and wife were registered as being special care cases. The mean I.Q. of the *spouses* of the married defective subjects was 88, range 66–112.

There seems to be a tendency for defectives to marry people who are brighter than themselves. It would also appear that the opportunity of marriage and/or parenthood is greater for the female defective than for the male. Many male subnormals show their lack of sexual control in perverted forms such as indecent exposure, homosexuality or assaults on young children, whereas the female subnormals show less perverted traits.

II. OFFSPRING

We now turn to the children. There was a total of 887 pregnancies resulting in live births, still births and miscarriages. There were 791 live births. Of these, 71 (9%) had died, most during infancy. The mean family size (extant) was 2.3. In the case of the subnormal mothers, this included 383 illegitimate children. In Northern Ireland the mean family size in the general population was 2.9. Here, then, is the first important finding: the old and vociferous claim that defectives breed a lot of children is not substantiated.

Nearly 95% of all the living offspring in the survey were individually examined. A good

deal of information was collected on each. At this point I want to concentrate on the ability levels of the children. To make it as simple as possible, we shall consider only three broad levels: the mentally subnormal (*i.e.* special care cases), the educationally subnormal (E.S.N.) and the normal. Because the compulsory school leaving age in Northern Ireland is 15, the children were divided into those who were over 15 and under 15. A word about the educationally subnormal. They are the responsibility of the local Education Committee which provides special schooling facilities. A large number of these, however, are eventually notified to the Special Care Service. This often happens after they have left school.

Even with these simple levels there are problems. The main one is that classification of mental handicap in very young children is difficult. Predictors of mental age and I.Q. in test batteries for the young are unreliable. We shall, therefore, look only at offspring of six years of age and over. The principal finding was that 137 of the total of 454 children of six and over were either E.S.N. or special care (defective) cases. That is, about one-third of the offspring were, themselves, subnormal to some extent. I should add that this estimate, if generalized or extrapolated to the entire group of children, would be conservative. This is because some of the younger children could well be defective and die before the age of five or so. The regression-to-the-mean phenomenon was observed, even in the group of children who were subnormal.

We now turn to the question of the relationship, if any, between the diagnostic classification of the parents and the ability levels of the children. Again, we are concerned only with those children over the age of six.

TABLE 1 *Comparison of defect of offspring aged 6 years and over with the diagnostic classification of defective parents*

Defect of offspring	Offspring of familial parents (N = 255)		Offspring of undifferentiated parents (N = 177)		Offspring of all other parents (N = 22)	
	Number	Percentage	Number	Percentage	Number	Percentage
Mentally defective	48	18.8	11	6.2	2	9.1
Educationally subnormal	60	23.5	14	7.9	2	9.1
Both	108	42.4	25	14.1	4	18.2

Table 1 summarizes the findings. The main point to note is that there is a difference, a highly significant difference, between the percentage of children found to be defective or educationally subnormal in the familial group and the percentage in the undifferentiated group. It is interesting that the 14 subnormal parents who had definite organic brain damage had children who were all of normal or above normal intelligence.

We have said that the regression-to-the-mean phenomenon occurred. It is likely, therefore, that there would be a greater proportion of subnormal children born to the non-organic severely handicapped parents than to the non-organic higher grade cases. This finding was confirmed. The percentage of both mentally defective and E.S.N. children born to what we called medium grade parents (that is, those with I.Q.'s roughly between 20 and 49) was 48 compared with 23% in the high grade (*i.e.* I.Q. 50+) sample of parents. This difference was statistically significant. There is one further point. You may be interested to know that the mean I.Q. of all offspring was 90, with a range of 11–127.

CONCLUSION

1. In this investigation it was possible to reduce to some extent the dangers of biased sampling by looking at *all* patients known to have had children. Furthermore, it was possible to examine nearly all of the samples of parents and children. But one very large gap remained. Because of obvious difficulties, the number of male parents was small. Inevitably, bias remains in the groups and generalizations have to be treated cautiously.

2. The notion that defectives propagate with alarming rapidity is not borne out by the results of the investigation. The average family size (*i.e.* average number of living children) was, in fact, slightly smaller than that in the general population.

3. The offspring of the mentally defective tend to be brighter than their parents, but approximately 30% are, themselves, defective or in the educationally subnormal range.

4. There are relationships between the diagnostic category and degree of mental handicap in the parents and the ability levels of the children. A high proportion of the *defective* children had parents of medium grade mental subnormality who were classed as familial. A much smaller percentage had parents of high grade subnormal intelligence in the 'undifferentiated' group. The children of brain damaged parents were normal, although this group was perhaps too small to warrant firm conclusions.

You may feel that more than this was promised in the introduction to the survey. But at least we found out a few things. The implications of the findings are interesting. What will happen in the third generation when it comes along? We might suppose that, if the process of regression-to-the-mean persisted, so the proportion of defective children would decline further. There is evidence to support the view that nature is a self-balancing system. Yet doubts remain. If the percentage of familial subnormality increases (and this is the group where the children were most affected) very slowly as a result of selective emigration, then there may be some cause for alarm about the prevalence of subnormality in years to come. At this stage we can only say that, if asked for advice about marriage by the mentally subnormal, we have at least a little empirical information on which to base our predictions.

REFERENCES

BRANDON, M. W. G. (1957): The intellectual and social status of children of mental defectives. *J. ment. Sci.*, *103*, 710.

EYSENCK, H. J. (1953): *Uses and Abuses of Psychology*. Penguin Books Ltd., Harmondsworth, Middx.

EYSENCK, H. J. (1971): *Race, Intelligence and Education*. Temple Smith, London.

GIRAULT-L'HERBAULT, G. and LAFON, R. (1964): Le problème du mariage et des conséquences chez l'infirme mental. *Evolut. psychiat.*, *29*, 131.

SHAW, C..H. and WRIGHT, C. H. (1960): The married mental defective. *Lancet*, *1*, 273.

THOMPSON, G. H. (1950): The relations between intelligence and fertility. *A Memorandum in Papers of the Royal Commission on Population, Vol. V*. Her Majesty's Stationery Office, London.

Arthur R. Jensen

University of California, Berkeley, Calif., U.S.A.

A two-factor theory of familial mental retardation

VARIETIES OF MENTAL RETARDATION

Persons who are classified as mentally retarded are a highly diverse group. Not only is there great variety in the behavioral and emotional characteristics of the retarded, or in their social adaptability, but careful examination reveals marked difference among them even in their mental abilities, both quantitatively and qualitatively. The causes of mental retardation, as well as their behavioral manifestations, are also diverse. It is our task scientifically to understand this diversity.

Two broad categories of mental deficiency are now generally recognized. The first is comprised of those conditions resulting from (*a*) chromosomal anomalies (*e.g.*, Down's syndrome or 'mongolism'); (*b*) major gene defects whereby a single mutant gene, usually recessive, completely overrides the normal determinants of mental development (*e.g.*, phenylketonuria and microcephaly); (*c*) brain damage due to infectious disease or trauma (*e.g.*, maternal rubella, encephalitis, eclampsia). The vast majority of the most severely retarded, with IQs below 50, belong in this category.

The second category consists of what is now called familial mental retardation. The vast majority of these individuals are mildly retarded, with IQs between 50 and 70. (The upper limit seems quite arbitrary and has been placed anywhere from 70 to 85.) At least 80 to 90% of persons in this IQ range appear clinically normal and show no history or signs of neurological damage.

The first category of retardation, although it is continuous with the normal distribution of intelligence in the population, is in a sense separate from it. It is superimposed upon the normal distribution and creates the 'bulge' at the lower tail of the distribution, that is, the excess over the frequency of low IQs that would be expected from a polygenic and microenvironmental model of the distribution of intelligence in the population.

The second category of retardation, that is, the so-called familial variety, on the other hand, can be viewed as just the lower tail (about 3%) of the normal distribution. Although such retardation constitutes normal variation rather than a pathological condition, for the individual it is usually a severe handicap educationally, occupationally, and socially. Such persons as adults in a modern industrial society can seldom manage on their own and they usually require various social services for their welfare.

The relative frequency of this 'normal', aclinical kind of retardation increases drastically as we move from higher to lower socioeconomic segments of the population. But it is worth noting that when the *mean* IQ of *all* individuals within any given stratum of the population (or a random sample thereof) is determined, this information alone permits a considerably accurate estimate of the frequency of familial retardation within that segment of the population. (The

same thing holds true in predicting the frequency of intellectually gifted persons in a given segment of the population.) It is clear that there is a highly regular relationship between the overall mean IQ of a population (or subgroup thereof) and the frequency of familial retardation (and of giftedness) in that population. This is even more true when we consider only the children in a population group rather than the parental generation, which, with the high degree of social mobility found in modern Western societies, has already become quite sorted out along occupational and socioeconomic lines, creating markedly skewed IQ distributions of the adult populations of the upper and lower socioeconomic status groups. These quite regular relationships to which I have just referred are best described in terms of the properties of the normal curve, and they are highly consistent with a polygenic theory of the distribution of intelligence. Mental retardation of the second kind, therefore, cannot properly be regarded in isolation from parameters of the intelligence distribution in the whole population. Some theories of the etiology of mental retardation and the programs proposed for its amelioration all too often overlook this central fact.

HETEROGENEITY OF ABILITIES IN FAMILIAL RETARDATION

From here on I shall be concerned only with the aclinical variety of mental retardation, which accounts for at least 80% of all mental deficiency. Although individuals in this category span a range of 20 or 30 IQ points and differ in predictable ways as a result, there are actually greater ability differences within this group than one might expect on the basis of IQ differences alone. Indeed, retarded persons having the very same IQ are often seen to differ quite markedly in their abilities, and to differ in ways that do not seem entirely accountable in terms of differences in their experience and training. Children and adults in the IQ range from 50 to 80 are known to differ greatly in vocational aptitudes, in social adaptability, and in various non-scholastic aptitudes, and these differences are only slightly related to their IQs obtained on the best standard tests. What these persons share most in common is an inordinate difficulty in regular school work. Under the usual conditions of class instruction, they lag far behind the average child of the same age, and the gap increases from earlier to later years. In non-academic pursuits, on the other hand, these children show great diversity of ability. I have been concerned with understanding the basis for this diversity. Although my present conclusions have taken shape gradually throughout a series of empirical studies, it will be most efficient to begin by presenting the main points of my formulation as it now stands.

THE BASIC OBSERVATIONS

There are several interrelated empirical observations which my theoretical formulation attempts to explain.

First, there is the fact that retarded children, in the IQ range between 50 and 80, are a relatively homogeneous group in performance on practically all standard intelligence tests. Most individual tests, such as the Stanford-Binet and the Wechsler scales have their highest reliability and concurrent validity in this range of the IQ distribution.

Secondly, there is the fact that within this rather homogeneous group with respect to IQ, there is apparently a very much greater range of other abilities, including cognitive abilities, provided they are non-academic in the traditional sense of the word. These abilities have been noted in the casual observations of parents, teachers, school psychologists, and the like, as great differences in the acquisition of skills in the playground, in social skills, and in practical knowledge and shrewdness in coping with the environment.

Thirdly, there is the fact that children of the lowest socioeconomic status (SES), who comprise by far the largest proportion of the aclinical mentally retarded, show the greatest discrepancy, on the average, between their low IQs and these other kinds of abilities I have

referred to. This seems especially true of Negro children of low SES. Middle-class white children with low IQs, on the other hand, generally show a more all-round retardation. Their poor performance on IQ tests is more consistent with their general behavior, in and out of school, than seems to be the case with low SES retarded children, whose mental handicap often seems confined almost entirely to the more academic aspects of schooling.

These casual observations by teachers and school psychologists have contributed largely to the popular belief that the standard IQ tests are somehow culturally biased against children of low SES and in favor of middle-class white children. The tests are seen as seriously underestimating the intelligence of low SES children. The fact that the IQ predicts scholastic performance equally well for low SES as for middle SES children is usually explained away by saying that schooling itself, both the academic curricula and the methods of instruction, is culturally biased in favor of the middle class. Until a few years ago I had subscribed completely to this commonly held viewpoint, and my research in this area actually began with an attempt to formalize these observations in the psychological laboratory and thereby to demonstrate, by more precise and rigorous scientific methods than had yet been applied, that the much higher incidence of retardation among children of low SES, particularly among minority children, was the fault of the IQ tests and also, possibly, of the schools. My own research in this vein has since led me to reject this view. But the theory I have gradually arrived at to replace it is quite different from the simple alternative that existed before I began my research.

In order to analyse the basic observations which I have just described, a series of laboratory studies were conducted in which we compared retarded and average children of lower and middle SES (including Negro, Mexican and white children) on a number of standard IQ tests and also on a considerable variety of other cognitive tasks. (We were not interested in sensory and motor skills or other abilities outside the cognitive domain.) These studies have been summarized elsewhere in more detail than is possible here (Jensen, 1968*a*, *b*, *c*; 1969*a*, *b*, *c*; 1970*a*, *b*, *c*; 1971*a*; Jensen and Rohwer, 1968, 1970). What these studies show, aside from any theoretical interpretation, are essentially the following points:

1. On a variety of tests of rote learning and short-term memory, retarded children score much less far below children of average IQ than on tests involving abstraction, reasoning, problem solving, and conceptual learning. Consequently, some considerable proportion of children who are retarded in terms of IQ are able to perform at an average level or above on a certain class of tasks that clearly involve mental ability. These are represented in our laboratory studies by (*a*) trial-and-error selective learning with visual and auditory reinforcements for correct responses (these problems have involved the trial-and-error acquisition of anywhere from 2 to 12 S-R associations); (*b*) serial rote learning, using lists of familiar objects, (*e.g.*, cup, comb, pencil, etc.), pictures of familiar objects, colored geometric forms, nonsense syllables, and common nouns; (*c*) paired-associates learning, using the same or similar materials as in the serial learning; (*d*) free recall learning (*e.g.*, presenting 20 familiar objects and asking the subject to recall, in any order they come to mind, the names of as many of the items as possible when they are put out of sight), using the same materials as above; (*e*) digit span memory under different conditions of presentation and recall (*e.g.*, recall immediately after presentation of the string of digits; recall 10 seconds after presentation; and recall after three successive presentations of the same string of digits.

What all these tasks have in common, as contrasted with tasks on which all retardates perform much more poorly, is that they call for little or no transformation of the stimulus input in order for the subject to arrive at the response output. Stimulus and response are highly similar. What the tasks call for essentially is accurate registration of sensory experiences, immediately giving already well learned names or labels to these, and at some later point in time repeating these labels in response to partial stimulus cues. It is a kind of recording and playback on cue, as contrasted with the other class of cognitive tasks, those on which retardates perform most poorly, involving transformation and mental manipulation of the input

in order to produce the answer – the relating and comparing of present stimuli with past learning, generalization and transfer of old learning to the new problem, the abstraction of conceptual and semantic similarities and differences, etc. All of these latter processes especially characterize those kinds of intelligence test items which are most highly loaded with *g*, the general factor common to all intelligence tests, which Spearman characterized as an ability for the 'education of relatives and correlates'. For convenience I have labelled these two broad types of mental ability Level I (for non-transformational learning and retention) and Level II (for intelligence as characterized by *g*).

2. Level I and Level II abilities show an interaction with SES such that retarded low SES children are on the average superior in Level I ability to middle SES children of the same IQ. Those retardates who appear most adequate in non-academic activities are generally average or above average in Level I. It is not uncommon, for example, to find low SES Negro children with IQs below 60 who perform in the average range or above on Level I tests. Yet their counterparts in this respect are exceedingly rare among low IQ middle and upper-middle class white children, who almost always perform well below the average on Level I tests.

Institutionalized retardates (and usually those in 'sheltered workshops'), as contrasted with a representative sample of all retardates in the population, are usually low both in Level I and Level II abilities. It is therefore doubtful if my findings would ever have been made had I tested only institutionalized individuals. There are marked differences between retardates who become more or less self-sufficient out in the world and those who must be cared for. Psychometrically this difference is not much related to IQ but is more markedly related to Level I ability.

In attempting to understand these findings, our first thought was that the Level II tests were more culturally biased against low SES individuals and that therefore, for any given IQ, the low SES person was really more intelligent than the high SES person, and this difference would show up in the presumably less culture-biased Level I tests. In short, I at first thought I had found in my Level I tests a culture-free or a culture-fair means of measuring intelligence. But this idea has proved to be wrong. A variety of Level II tests differing in degree of culture-loading all show highly consistent results: We have found no tests, verbal or non-verbal, with any appreciable complexity or substantial *g* loading on which properly diagnosed retarded children score in the average range. And surprisingly enough, low SES children, especially if they are Negro, actually score slightly higher on the verbal and the more obviously culture-loaded tests than on non-verbal tests of the type that attempt to minimize middle-class cultural content. Also, the experimental manipulation of task variables in laboratory learning experiments so as to either minimize or maximize the role of Level II processes leads me to the conclusion that the Level I-Level II distinction is not a matter of the culture-loading of the tests that measure each type of ability, but of the different kinds of mental processes required in the two classes of tests. Nor is the difficulty of the task the essential basis of distinction. Level I and Level II test items can be made equally difficult in terms of their *p* values (*i.e.*, the percentage of the population that can perform successfully). The essential distinction between Level I and Level II is in the complexity of the mental transformations or operations required for successful performance of the task. Moreover, twin and sibling correlations and estimates of the heritability (*i.e.*, the proportion of the total variance in test scores attributable to genetic factors) of Level I and Level II tests give no indication of significantly lower heritability of Level II than of Level I tests. If Level II tests reflect environmental or cultural influences to a greater extent than Level II tests, one should expect lower heritability values for Level II tests. But this is not the case, and, if anything, slightly the reverse seems to be true.

LEVEL I AND LEVEL II IN THE GENERAL POPULATION

In order to determine just how far below the average of the population retarded children stand on Level I tests, we have given such tests to large, representative samples of the school

age population, now totalling 15,000 children in all. And to study the relationship between Level I and Level II abilities, verbal and non-verbal intelligence tests, representative of Level II, have also been administered to the same large samples. These large-scale data obtained from the general population put our findings with the mentally retarded into a proper perspective and show that they are not isolated phenomena peculiar to retardates, but are a consequence of certain population characteristics.

The regression of Level I test scores on IQ or Level II scores in all samples appears to be linear throughout the IQ range from about 50 to 150. The slope of the regression line and the correlation between Level I and Level II abilities differ from one subpopulation group to another. It is lower in low SES groups and higher in upper SES groups. It is especially lower among Negroes as compared with whites. In various studies the correlations between Levels I and II have ranged from 10 to 40 in low SES groups, comprised largely of Negro children, and from 50 to 70 in middle SES groups comprised largely of white children. (However, a sample of Oriental-American children, although of lower SES than the white sample, showed an even higher correlation between Level I and Level II than was found in the white sample.) Because the regression of Level I on Level II has a steeper slope (higher correlation) in higher than in lower SES groups, the regression lines of lower and upper SES groups must inevitably cross. Consequently, in the region of low IQ that characterizes mental retardation, the lower SES group obtains higher average scores on Level I tests – which is the phenomenon described earlier. These relationships are shown in Figure 1.

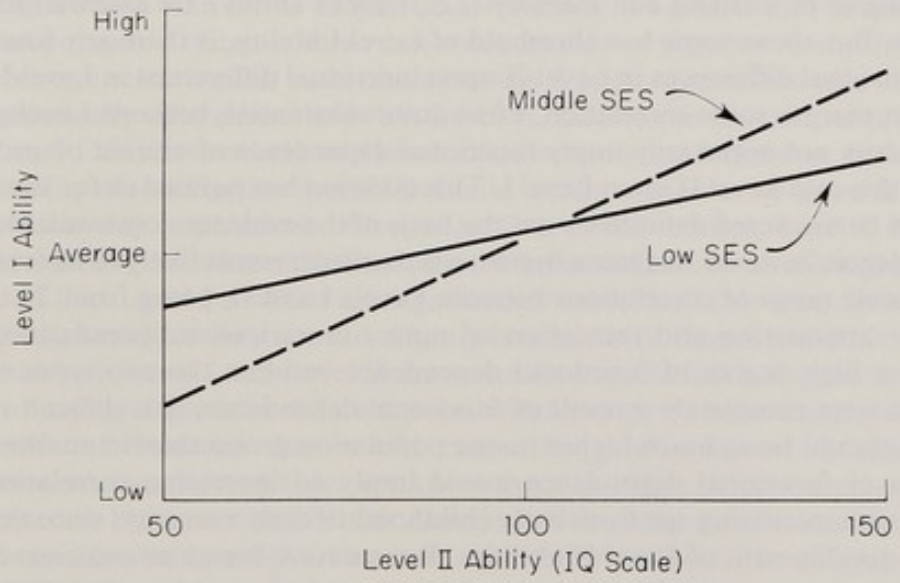


Fig. 1. Typical regression lines of Level I upon Level II ability in middle and low socioeconomic groups.

Thus, the phenomenon of higher Level I ability among lower than among upper SES retardates, on the average, is seen to be a consequence of the lower correlation between Levels I and II in the low SES group as compared with the higher SES group. But what we did not expect to find before we finally tested children in adequately large numbers throughout the entire range of IQ is the *reverse* phenomenon at the upper end of the IQ scale, that is, the finding that low SES children (most of whom are Negro in these studies) with high IQs perform significantly less well than their middle SES counterparts in IQ. This came as something of a surprise, but it is now based on such substantial evidence that its factual status is beyond reasonable doubt. From a scientific, theoretical standpoint it is, of course, a simpler, more

regular picture than we would have if the regression were not linear and the consequent reverse symmetry at low and high ends of the IQ scale did not obtain.

This finding, furthermore, helps to clarify a point about which there was some doubt in the earlier stages of our research. This was the question of whether low SES retardates performed better on Level I tests, relative to those of middle SES, simply because Level I tests were less culturally biased than the IQ tests. This culture-bias hypothesis seems untenable in view of the fact that in the range of IQ above 100, low SES children perform relatively *less* well on Level I tests. Also, when we have given various Level II tests which differ obviously in culture loading, such as the Peabody Picture Vocabulary Test and Raven's Progressive Matrices, and then have examined the regression of the less culture-loaded on the more culture-loaded test, we find no cross-over of the regression lines of the low and middle SES groups; the lines are quite parallel. In short, comparison of lower and upper SES groups on Level I *vs*. Level II tests gives a quite different picture from that of comparing the two groups on culture-loaded *vs*. culture-fair tests.

NATURE OF THE RELATIONSHIP BETWEEN LEVELS I AND II

Does the correlation between Level I and Level II abilities represent a functional dependence of Level II upon Level I? For example, is above-average Level I ability a necessary but not sufficient condition for above-average Level II ability in the sense, say, that knowledge of subtraction is a necessary but not sufficient condition for solving problems in long division? Obviously some degree of learning and memory (*i.e.*, Level I ability) are essential for intellectual development. But above some low threshold of Level I ability, is there any functional dependence of individual differences in Level II upon individual differences in Level I? We know, of course, that there is some correlation, often quite substantial, between Levels I and II. But correlation does not necessarily imply functional dependence of one set of processes upon another, in this case Level II upon Level I. This question has puzzled us for some time. It probably cannot be answered definitively on the basis of the evidence now available. A number of lines of evidence, however, suggest a hypothesis that seems most likely to be true.

In the first place, the wide range of correlations between Levels I and II, going from 20 to 80 (after corrections for attenuation and restriction of range) in various subpopulations, seems inconsistent with a high degree of functional dependence between the two types of ability. If the correlation were completely a result of functional dependence, it is difficult to see why the dependence should be so much higher in one population group than in another. Secondly, a high degree of functional dependence would imply an increasing correlation between Levels I and II with increasing age from early childhood to early maturity, since this is the period of marked development of Level II abilities. But we have found no evidence of greater correlation between Levels I and II with increasing age, and, if anything, slightly the opposite is the case. Subjects with high IQs but low Level I ability are somewhat less common among younger children between the ages 4 and 7 than among children beyond 10 years of age. It is as if Level I ability acts as scaffolding for the development of Level II abilities and then falls away in importance as the Level II abilities are consolidated. The child who is below average in Level I and above average in Level II will appear to be a slow developer in Level II in early childhood; he is in a sense a slow learner who, because of good Level II ability, is able thoroughly to understand and consolidate everything he learns and incorporate it into the cognitive structures we call intelligence. Later in development these Level II cognitive structures become relatively more important in educational attainments, and the child who is relatively low in Level I but high in Level II becomes much less handicapped in school than the child who shows the opposite pattern of abilities. The low I-high II child is one who learns with difficulty in school when the learning is more or less rote and affords little opportunity to grasp concepts and relationships; he is slow in acquiring skills that require sheer repetition;

but once they have been acquired, he can fully bring them to bear on logical reasoning and problem solving. He *understands* what he learns, though he may have learned it slowly. Such children, who often seem to get off to a slow start in the early grades of school, appear to become brighter and intellectually more capable as they progress in school and as the academic subject matter makes increasing demands on conceptual and abstract thinking and involves relatively less sheer acquisition of simple skills and factual information. The high I–low II child, on the other hand, presents a very different picture. In early childhood he may appear quite bright and quick in picking up all kinds of simple skills and verbal knowledge; he may appear linguistically precocious; he may do quite well in scholastic subjects and skills that depend upon learning by repetition such as penmanship, spelling, mechanical arithmetic, memorizing the words of songs, etc., but he experiences increasing difficulty and frustration – sometimes to the point of hating school – as the conceptual and abstract demands of the subject matter increase from earlier to later grades. It becomes increasingly difficult to understand what is learned, and, when ultimately in some academic subjects learning and understanding become one and the same, the pupil with a marked deficiency in Level II is almost totally handicapped. While one can find some small percentage of pupils of below-average Level I ability who are doing very well, say, in algebra or science, there are virtually no below-average Level II pupils who are succeeding in these subjects.

If there is at most only a slight degree of functional dependence of Level II upon Level I, as suggested by the fact that some few older children with very high Level II ability are found to be well below average in Level I, what is the basis for the correlation between Levels I and II and for the fact that it differs so markedly in different populations? The most plausible explanation is in terms of genetic assortment. If Levels I and II are controlled by two different polygenic systems, these can become assorted together to any degree in a given populaton through selective and assortative mating. I have rejected the idea that only Level I ability is genetically determined and that Level II abilities are learned, acquired, or developed out of Level I abilities entirely as a result of environmental influences. If this were the case, the heritability of intelligence (Level II) should not be as high as we know it to be – about 70 to 80 in present-day populations. Also, according to this notion, Level I should have much higher heritability than Level II. But the correlations obtained on siblings and twins give no indication that Level I abilities are significantly more heritable than Level II abilities, and if anything, Level I ability appears slightly less heritable than Level II. It seems much more likely that both Level I and Level II are controlled by distinct polygenic systems and are correlated to varying degrees in different population groups because these groups have differed in the kinds of demands that would cause the genetic factor underlying Levels I and II to become assorted together. We know there is a high degree of assortative mating for intelligence in European and North American Caucasian populations. In fact, in Western society there is probably a higher degree of assortative mating for intelligence than for any other trait.

This should not be too surprising since educational attainments, occupational level, and socioeconomic status, which are the basis for assortative mating, are highly correlated with intelligence. If Level I ability also has some correlation with occupational and socioeconomic status independent of intelligence (Level II), we should expect the genetic factors involved in Levels I and II to become associated through assortative mating. This is consistent with the observation that omnibus-type intelligence tests which involve an admixture of both Level I and Level II (*e.g.*, the Stanford-Binet and Wechsler tests) show a higher correlation with practical criteria such as educational achievement and occupational status than do factorially more pure tests of Level II, such as the Raven Matrices. Populations that have not long been stratified educationally and occupationally would have had less assortative mating for these abilities, and consequently would show a lower correlation between them, as we find, for example, in the American Negro population as contrasted with the white. Also, Level II ability, being more highly related to the academic and intellectual demands of schooling and higher occupational status, is more subject to assortative mating and consequently to genetic

stratification in terms of socioeconomic status. Good Level I ability, on the other hand, is more or less equally advantageous in all cultures and walks of life and would therefore become less differentiated than Level II among various population groups.

PHYSIOLOGICAL BASIS OF LEVEL I AND II ABILITIES

This is quite speculative, but from what we know about the organization of the nervous system it is an interesting hypothesis that the basic locus of Level I abilities is in the electrochemical processes involved in short-term memory and the neural consolidation of memory traces. The biochemical basis of these processes is evinced, for example, in the fact that learning and memory, which involve neural consolidation, can be altered by pharmacological means. Level II abilities, on the other hand, are hypothesized to depend upon the structural aspects of the brain – the number of neural elements and the complexity and organization of their potential interconnections.

The evolution of the nervous system, represented in the hierarchy of phyla, is most evident in the development of Level II processes. The growth of mental ability in the individual similarly reflects largely the gradual emergence of Level II processes from infancy to maturity (Jensen, 1971*b*). G. Stanley Hall's famous dictum that 'ontogeny recapitulates phylogeny' appears to hold true for mental as well as physical development. The growth curves of Levels I and II are quite different, with Level I approaching its developmental asymptote at an earlier age than Level II.

THEORETICAL OVERVIEW

The picture is that of a very fundamental division of mental abilities into Level I (learning and memory) and Level II (intelligence, *i.e.*, analytic understanding, reasoning, abstraction, conceptual thinking). Individual differences in both Levels I and II are viewed as due mainly to independent polygenic factors. The distributions of Level I and II abilities in the population are approximately normal. The correlation between Levels I and II is due mainly to the common assortment of the genes involved in the two types of ability. (But there is also probably some moderate degree of functional dependence of Level II upon Level I.) The genetic correlation differs in various subpopulations, being lower in the low SES segment of the population and higher in the middle and upper-middle class segment. The correlation is lower in the American Negro than in the white population. Because education makes greater demands on Level II than on Level I and the occupational hierarchy and socioeconomic status are highly related to educational attainments in Western societies, there is a much greater mean difference between social classes in Level II than Level I. While Level I is distributed about very similar means in lower and upper SES groups, the means of the Level II distributions may differ by one standard deviation or more. (One standard deviation is equivalent to about 15 IQ points.)

Mental retardation of the type which is a part of the normal distribution of abilities in the population can be described as *primary* retardation if it involves marked deficiency in both Levels I and II and as *secondary* retardation if there is a deficiency only in Level II ability. Secondary retardates often appear normally bright and capable of learning and achievement in many situations, although they invariably experience great difficulties in school work under the traditional curricula and methods of instruction. Many secondary retardates who are regarded as backward children while in school later become socially and economically adequate persons once they are out of the academic situation. Primary retardates, on the other hand, appear to be much more handicapped in the world of work. A serious shortcoming of ordinary IQ tests is that they measure predominantly Level II and fail to distinguish between

primary and secondary retardation. Tests that reliably measure both Levels I and II should be developed for use in schools, in personnel selection, and in the armed forces. This formulation also has important implications for the education of children now popularly called culturally disadvantaged, most of whom have normal Level I ability but are often quite far below average in Level II. Such children might benefit educationally from instructional methods which make the acquisition of scholastic skills less dependent upon Level II abilities and more fully engage Level I abilities as a means of raising their educational attainments.

REFERENCES

JENSEN, A. R. (1968*a*): Social class, race, and genetics: implications for education. *Amer. educ. Res. J., 5/1*, 1.

JENSEN, A. R. (1968*b*): The culturally disadvantaged and the heredity-environment uncertainty. In: *The Culturally Disadvantaged Child, Vol. 2*, pp. 29–76. Editor: J. Hellmuth. Special Child Publications, Seattle, Wash.

JENSEN, A. R. (1968*c*): Patterns of mental ability and socioeconomic status. *Proc. nat. Acad. Sci. USA, 60/4*, 1330.

JENSEN, A. R. (1969*a*): How much can we boost I.Q. and scholastic achievement? *Harvard educ. Rev., 39/1, 1.*

JENSEN, A. R. (1969*b*): Intelligence, learning ability, and socioeconomic status. *J. spec. Educ., 3/1*, 23.

JENSEN, A. R. (1969*c*): Jensen's theory of intelligence: a reply. *J. educ. Psychol., 60/6*, 427.

JENSEN, A. R. (1970*a*): Learning ability, intelligence, and educability. In: *Psychological Factors in Poverty*, pp. 106–132. Editor: V. Allen. Markham, Chicago, Ill.

JENSEN, A. R. (1970*b*): A theory of primary and secondary familial mental retardation. In: *International Review of Research in Mental Retardation, Vol. 4*, pp. 33-105. Editor: N. R. Ellis. Academic Press, New York, N. Y.

JENSEN, A. R. (1970*c*): Hierarchical theories of mental ability. In: *On Intelligence*, pp. 119–190. Editor: B. Dockrell. Ontario Institute for Studies in Education, Toronto, Ont.

JENSEN, A. R. (1971*a*): Do schools cheat minority children? *Educ. Res., 14*, 3.

JENSEN, A. R. (1971*b*): The phylogeny and ontogeny of intelligence. *Perspect. Biol. Med., 15*, 37.

JENSEN, A. R. and ROHWER, W. D., Jr (1968): Mental retardation, mental age, and learning rate. *J. educ. Psychol., 59/6*, 402.

JENSEN, A. R. and ROHWER, W. D., Jr (1970): *An Experimental Analysis of Learning Abilities in Culturally Disadvantaged Children*, pp. 1–181. Office of Economic Opportunity, Contract No. OEO 2404.

Chapter VI Genetics of human enzymes and proteins

Harry Harris

Galton Laboratory, University College, London, United Kingdom

Enzyme and protein variants–some general comments

Inherited variants of many different enzymes and proteins have been discovered in recent years, and in introducing this symposium I thought it might be useful to discuss briefly some of the background to these discoveries, and also the general picture which is beginning to emerge.

Theoretical considerations lead us to believe that a very large number of different mutant alleles may in principle be generated from a single gene by separate mutational events. For example, from a gene containing a sequence of DNA say 900 bases long and coding for a polypeptide of 300 aminoacids, 2,700 different alleles each differing from the original by only a single base change may be formed by separate mutations, since each of the 900 bases may be altered to one of three others in different mutational events.

Furthermore, the functional effects of these different alleles can be expected to vary very widely.

From what is known about the genetic code and about the aminoacid composition of proteins we can estimate that some 20–25% of all mutations of this type will be synonymous. That is to say, they will result in no alteration in the aminoacid structure of the protein because they will simply involve the change of one particular codon to another specifying the same aminoacid.

In about 3–6% of cases, because the mutation involves the alteration of a base triplet coding for an aminoacid to a nonsense triplet which results in chain termination, the mutant allele will cause the synthesis of a shortened polypeptide chain lacking a greater or lesser portion of its carboxyl terminal aminoacid sequence. In most instances this is likely to result in considerable disruption of protein structure with loss of functional activity.

However, in some 70–75% of cases a single base change will result in the synthesis of a protein differing from the original by the substitution of one aminoacid for another at the corresponding point in its aminoacid sequence. Since each of the several hundred aminoacids in a protein may be changed to one of several others by such mutations, a considerable variety of structurally different variants may be produced in this way. Furthermore, they may be expected to differ in their properties one from another, according to the particular aminoacid which has been substituted and the particular site in the protein where the substitution has occurred. In the case of enzyme proteins, for example, such mutations may in some cases lead to a gross reduction or even a complete loss of activity, while in other cases only minor changes in function or perhaps none at all will be produced.

Finally, besides the gene mutations which involve only a change in a single base, an indeterminate number of other kinds of mutational event can also occur. They may result in deletions, duplications, frameshifts or other alterations of the base sequence, and they can be expected to produce corresponding alterations in polypeptide structure. In most cases such mutants probably result in a marked loss of functional activity.

Thus, in theory a great variety of different alleles can be generated by separate mutations within the confines of a single gene. Many of these may be expected to result in the occurrence of structurally variant forms of the corresponding enzyme or protein, and some in complete deficiency. What we would like to know is how many actually occur among the individual members of human populations, what is their incidence and distribution, and what are their functional effects.

In fact a very large number of such enzyme and protein variants and deficiencies have now been identified. Their discovery has stemmed from two quite different, though as it turns out complementary, approaches.

One approach has its origins in the classical work of Garrod on the 'inborn errors of metabolism'. It takes as a starting point particular diseases which appear to be attributable to single gene mutations and aims to identify in each case the specific enzyme or protein defect which gives rise to the metabolic and clinical abnormalities characteristic of the particular condition. The critical enzyme or protein defect is now known in more than one hundred different clinical syndromes, and judging from the rate at which new examples are currently being reported, it is clear that we are only at the beginning.

From the point of view of the present discussion perhaps the most important thing which has emerged in recent years from this line of investigation is the remarkable degree of genetical heterogeneity which may evidently occur even in very rare abnormalities. It is being increasingly found that when sensitive analytical procedures are applied to material from different patients with a specific deficiency of a particular enzyme, but who come from different families or different populations, quite significant differences in the degree or character of the enzyme defect may be demonstrable. In general such differences appear to be due to the occurrence of different mutant alleles of the gene which determines the enzyme in question.

For example, even in a condition as rare as methaemoglobinaemia due to methaemoglobin reductase (NADH diaphorase) deficiency at least five clearly distinct types of abnormality have now been identified and a similar degree of heterogeneity has been observed in studies on patients with haemolytic diseases due to pyruvate kinase deficiency, in hyperuricaemia due to HGPRT deficiency, and in a variety of other cases. Indeed it now seems likely that such heterogeneity is the rule rather than the exception. In other words it appears that for any given gene locus coding for the structure of a particular enzyme or protein a series of different mutant alleles each resulting in marked functional deficiency and hence in a characteristic clinical abnormality may occur. This conclusion is of course completely consistent with what we might have expected on theoretical grounds. However, it does imply a much greater degree of genetical heterogeneity in inherited clinical disorders than would have been thought likely only a few years ago.

The second main approach to the search for inherited variants of enzymes and proteins involves a quite different strategy. It takes as its starting point randomly selected normal healthy individuals in different populations, and aims to see how far they can be differentiated one from another in terms of the characteristics of some particular enzyme or protein.

There are now many techniques which in principle might be used to detect enzyme or protein variants in population surveys, but in practice the technique of electrophoresis is the one which has been most widely used. It happens to be a convenient and sensitive tool for detecting subtle differences in molecular structure, and it usually requires only small amounts of material so it can be readily adapted to screening large numbers of individuals. But it is important to remember that it is, in general, only capable of detecting conditions which involve a change in molecular charge, and it has other limitations as well. So at best one can expect to detect by this procedure only a proportion of all the structural variants of a protein which may be produced by different mutations. Nevertheless, a considerable number of variants attributable to allelic differences have in fact been discovered in the course of such electrophoretic surveys, and a great deal of information about these, the characteristics and incidence, is rapidly accumulating. And from it some important generalisations are beginning to emerge.

It now appears likely that if virtually any enzyme or protein is examined in a sufficiently large number of individuals (say 5,000 to 10,000) at least one structural variant will be detected. And family studies indicate that these are not usually the products of fresh mutations in the immediately preceding generation. Often several different allelic variants are found and in general it seems that as more and more individuals are examined for a particular enzyme or protein an increasing number of different variants are picked up. So probably at most loci a considerable number of different alleles occur in human populations.

The majority of those alleles of course are rare, and many appear to have frequencies of the order of 1 in 10,000 or less. But some are relatively common and give rise to the well known polymorphisms. Our own surveys of arbitrarily chosen enzymes in different populations suggest that two or more relatively common alleles occur at at least 25% of loci coding for enzyme structure.

These findings imply that any single individual is likely to be heterozygous at many different gene loci, and it becomes of some interest to enquire what the average degree of such heterozygosity actually is.

A rough estimate of this parameter can be derived from the data summarised in Table 1.

TABLE 1 *Estimates of average heterozygosity per locus from surveys of 20 arbitrarily chosen enzymes in Europeans and Negroes (only loci where incidence of heterozygotes was >0.02 are included in the calculation)*

	Incidence of heterozygotes	
	Europeans	Negroes
Red cell acid phosphatase	0.51	0.28
Phosphoglucomutase		
Locus PGM_1	0.35	0.33
Locus PGM_3	0.38	0.47
Adenylate kinase	0.09	–
Peptidase A	–	0.16
Peptidase D (prolidase)	0.02	0.10
Adenosine deaminase	0.11	0.06
Average heterozygosity (detected electrophoretically) per locus, assuming 26 loci were screened	0.056	0.054
Average heterozygosity per locus for alleles determining all structural enzyme and protein variants (*i.e.* electrophoretic variants × 3)	0.168	0.162

Data of the MRC Human Biochemical Genetics Unit, Galton Laboratory, University College London.

The data are based on electrophoretic surveys carried out on some 20 arbitrarily chosen enzymes in representative European and Negro populations. For each gene locus at which polymorphism was detected the proportion of the population who are heterozygous has been determined. Some of the enzymes like phosphoglucomutase involve more than one structural locus, and in the enzymes examined in these surveys it appeared probable that the polypeptide products of some 26 different loci were being scrutinised for electrophoretic differences. If so, one can obtain an estimate of average heterozygosity for alleles producing electrophoretic differences by summing the values given in the table and dividing by 26. For the Europeans the average value per locus obtained is 0.056, and for the Negroes it is 0.054.

Now from what is known about the nature of the genetic code and about the aminoacid composition of proteins, it seems unlikely that more than one third of all the possible structural variants that may actually occur can be detected electrophoretically. So it is probable that the average heterozygosity per locus for alleles resulting in structural differences of enzymes is about three times that calculated on the basis of purely electrophoretic differences. This particular set of data therefore suggests that the average heterozygosity per locus in Europeans is about 0.168, and in Negroes about 0.162. The findings thus imply that any one individual may be heterozygous at about 16% of all gene loci coding for the structures of enzymes.

Such calculations are of course very rough, but they do suggest that a very remarkable degree of individual differentiation in the enzymic make-up of different people in a given population must exist. And indeed taking all the data now available in man it seems extremely probable that each individual has his own unique combination of enzymes and proteins.

Now the variants which have been discovered through the study of rare inherited diseases are in certain important respects very different from the majority of those discovered in the course of random population surveys. This is not surprising because if we search for variants in disease states we must expect simply to find those which are characterised by a marked loss of functional activity. Whereas when we screen populations randomly we may expect to find variants covering the whole range of possible functional alteration, from those which show similar activity to the usual form of the enzyme or only differ in minor degree, to those which show markedly defective activity and which, at least in the homozygous state, may give rise to clinical abnormality. In fact, because variants which have a severe degree of activity loss are for various technical reasons difficult to detect by electrophoretic screening procedures, they are probably underrepresented among those so far discovered by population surveys.

So the two approaches may be regarded as giving information about different aspects of the overall problem. Taken together they may be expected to provide a much clearer picture of the whole spectrum of allelic variation which may evidently occur at any gene locus.

The point is perhaps best illustrated by glucose-6-phosphate dehydrogenase. This enzyme has probably been studied more extensively than any other enzyme in man, both by random population surveys and by the deliberate investigation of patients with obscure forms of haemolytic disease. More than 60 different variants have now been identified and they can be attributed to different mutant alleles at a single locus on the X chromosome. They have been shown to differ one from another in their physical and biochemical characteristics as well as in their functional activities and their clinical consequences. Some give rise to persistent haemolytic disease, and are clearly analogous to the enzyme variants which have been found in other types of inherited disorder. Others produce no clinical abnormality under ordinary conditions but render the individual particularly susceptible to certain drugs like primaquine or to dietary ingredients like the Fava bean. Still others appear to have no adverse effects at all.

Now there is no reason to think that the multiplicity of alleles which are found at this particular locus represents a unique or special phenomenon. Almost certainly a similar situation occurs at most other loci coding for enzyme and protein structure. And this perhaps gives us some insight into the immensity of the task ahead if we wish to understand in biochemical terms the nature of inherited variation in human populations.

Magne K. Fagerhol

Blood Bank and Department of Immunohaematology, Ullevaal Hospital, Oslo, Norway

The serum alpha-1-antitrypsin polymorphism

Since alpha-1-antitrypsin (a-1-at) is one of the major protease inhibitors of human blood, the inherited variants of a-1-at have been called the Pi system (Fagerhol and Laurell, 1967). The first variants were detected by paper and agarose gel electrophoresis (Laurell and Eriksson, 1963; Axelsson and Laurell, 1965).

A-1-at is one of the more homogeneous proteins in human serum as judged from the pattern on agarose gels. However, when tested by antigen-antibody crossed electrophoresis, with agarose gel in the first dimension, two minor fractions can be detected in addition to the major peak. One of them results in a small shoulder on the cathodal side of the main peak and contains about 10% of the total a-1-at (Laurell, 1965). The second small fraction (about 3% of total a-1-at) is often not detected unless serum is concentrated or fractionated, *e.g.* by gel filtration on Sephadex G-200 (Ganrot, 1967). This fraction is eluted between the 19S and 7S peaks and migrates with the beta globulins on agarose gel electrophoresis.

Most healthy individuals are homozygous for a gene called Pi^M determining the a-1-at and their sera will give a single distinct alpha-1-globulin zone. A few per cent, however, are heterozygous for faster or slower a-1-ats and their sera may give two distinct alpha-1-globulin zones. Patterns of this kind can only be obtained if the electric charge of the variant protein differs significantly from the normal and the variant protein is present in a sufficiently high concentration. Some variants have a normal mobility on agarose gels, but have clearly increased or decreased mobility on acid starch gel electrophoresis. The presence of variants with an abnormally low concentration of a-1-at may be suspected when the alpha-1-globulin zone is missing or weak.

A much more sensitive method for detection of a-1-at variants is the acid starch gel electrophoresis (Fagerhol, 1968). This method gives also a much more complex pattern: two major and six minor zones. That all these zones are in fact a-1-at can be demonstrated by use of antigen-antibody crossed electrophoresis (Fagerhol and Laurell, 1967; Fagerhol, 1969).

Up to now evidence has been presented that the Pi system consists of at least 11 codominant alleles which have been detected in at least 20 different combinations. Figure 1 is a schematic drawing of most of the Pi phenotypes known at the present. In this figure only the two major zones of each allele product are indicated. The a-1-at patterns on agarose as well as on acid starch gels suggest that each allele product is synthesized and transferred to the plasma as a monomer independently of the other.

The acid starch gel electrophoresis is most easily performed with the gel in the horizontal position. The following buffer is used in the gels: 0.0012 M citric acid and 0.0023 M Tris, which will give a pH of about 4.95. The starch concentration must be adjusted according to the quality of the starch and the separation obtained, but will in most cases be about 12–16%. In the cathodic vessel a buffer with 0.6 M H_3BO_3 and 0.125 M NaOH is used, and in the anodic vessel: 0.05 M citric acid and 0.09 M Tris.

TABLE 1 *Methods for detection of various Pi phenotypes*

Method	EM	FF	FM	FS	FZ	GM	IM	IS	IZ	MP	MS	MV	MW	MX	MZ	SS	SZ	XZ	ZZ
Antigen-antibody crossed electrophoresis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid starch gel electrophoresis	+	+	+	+	(+)	+	+	+	(+)	+	+	+	+	+	(+)	+	(+)	(+)	+
Agarose gel electrophoresis		+	+	+								+	+	+	(+)	+	(+)	(+)	+
Total antitryptic activity or immunochemical estimation of α_1-antitrypsin, paper or cellulose acetate electrophoresis															(+)				+

Three factors seem to be very important for the separation: (1) the quality of the starch, (2) the cathodic vessel should contain borate, and (3) a correct pH in the gel.

The separation of various proteins by starch gel electrophoresis is based upon differences in the net charge of the molecules, their molecular size and probably other factors. In contrast to what might be expected, the starch gel cannot be substituted by polyacrylamide gel for Pi typing. This has been tried without success in several experiments including variation of the pH, ionic strength and pore size in the gel. The reason for this is unknown, but it may suggest that the high resolving power of the starch gel depends in part upon some unknown factor. This theory is supported by the observation that the best results are obtained with relatively freshly prepared starch. After storage for 6–18 months some batches of starch give a poor separation.

Clear-cut Pi patterns can only be obtained if the cathodic vessel contains borate or EDTA. Since the zones are much more distinct with borate, this is preferred for most purposes.

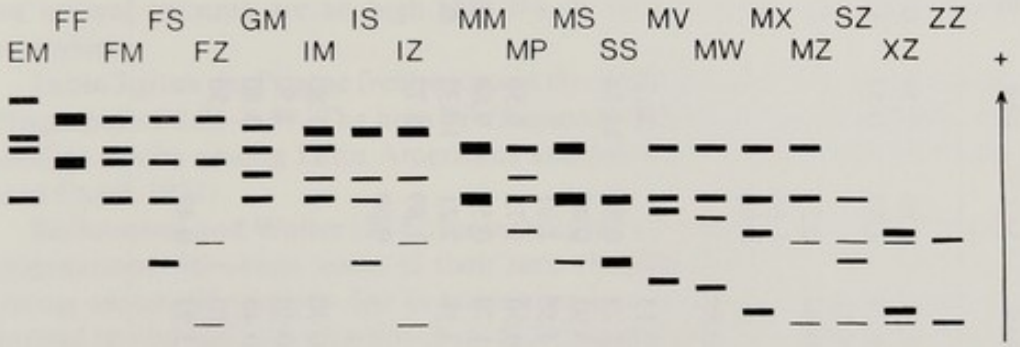


Fig. 1. A schematic drawing of the different Pi phenotypes described up to now.

If all variants are to be detected, both acid starch gel electrophoresis and antigen-antibody crossed electrophoresis must be used. The latter method is often necessary to demonstrate the presence of the Pi^Z allele product which may be covered by other proteins on the starch gels. Since no clinical condition is known which might lower the concentration of a-1-at to less than 30% of the normal, the absence of a distinct alpha-1-globulin zone on paper, cellulose acetate or agarose gel electrophoresis, or a very low total antitryptic activity or alpha-1-antitrypsin concentration (by immunochemical estimation) suggests, with a very high probability, that the subject has the Pi type ZZ. These methods may therefore be useful for studies on the association between a-1-at deficiency and disease. A large number of clinical and non-clinical conditions, including use of oral contraceptives and pregnancy, are associated with markedly increased concentration of a-1-at in serum. Under such conditions subjects with Pi type MZ may erroneously be classified as MM unless the most sensitive methods are used so that the Z protein can be positively identified.

If sera are heavily contaminated or exposed to room temperature or higher for more than 2–3 days, the MM pattern may be altered to a false 'FM' or 'EM' -like pattern on acid starch gel electrophoresis. This pattern can be distinguished from a true FM type since (1) the rapid zones in the false 'FM' are clearly faster than the normal F zones, (2) at a slightly lower pH (about 4.90) the slower major F zone migrates between the two major M zones, while the false 'F' zones both migrate in front of the M zones, and (3) the sera giving a false 'FM' pattern give only one alpha-1-globulin zone on agarose gel electrophoresis while FM sera give two zones.

Table 1 shows which Pi phenotypes can be detected by various methods. The phenotypes EM, GM, IM, MP and MS can only be detected by the most sensitive methods and not on agarose gel electrophoresis. The EM and GM types which were discovered in Norway recently (Fagerhol, 1971) have also been found in several relatives of the probands. Both phenotypes have been detected in several individuals at various times.

TABLE 2 *Pi phenotype frequencies (in per cent) in various populations*

Population	Authors	No. tested	MM	MS	MZ	FM	SS	IM	SZ	FS	ZZ
Norwegians	Fagerhol, 1967	2830	89.75	4.10	2.86	2.54	0.14	0.21	0.14	0.04	0.07
Spaniards	Fagerhol and Tenfjord, 1968	378	75.40	18.78	1.85	0.53	1.59	0.26	0.53		
Portuguese	Fagerhol and Tenfjord, 1968	39	71.79	28.21							
Finnish Lapps	Fagerhol *et al.*, 1969	468	99.15	0.64	0.21						
Norwegian Lapps	Fagerhol *et al.*, 1969	302	98.34		1.66						
Finns	Fagerhol *et al.*, 1969	223	99.10		0.90						
Åland Islanders	Fagerhol *et al.*, 1969	450	99.11	0.89							
Mongolians	Fagerhol and Tenfjord, 1968	68	100,00								
Icelanders	Kellermann and Walter, 1970	94	75.53	2.13		20.21					
Germans	Kellermann and Walter, 1970	516	78.68	3.29	0.58	14.34		0.19		0.97	
Germans	Kueppers, 1971*a*	200	96.05	3.92	0.49						
Germans	Goedde *et al.*, 1970	262	93.51	4.58	1.91						
Greeks	Kellermann and Walter, 1970	400	92.50	0.50	1.75	2.50		1.25			0.75
Hungarians	Kellermann and Walter, 1970	172	82.55	1.74	0.58	9.88	0.58	0.58		0.58	1.16
Iranians	Kellermann and Walter, 1970	271	80.44	2.58	2.21	10.33	0.36	0.73		1.11	
Pakistanis	Kellermann and Walter, 1970	53	92.45		1.89	3.77					
Indians	Kellermann and Walter, 1970	430	99.06		0.69						0.23
Koreans	Kellermann and Walter, 1970	90	98.89								1.10
Negroes	Kellermann and Walter, 1970	274	97.08	0.30		1.80					
Japanese	Harada and Omoto, 1970	965	96.80	0.50		2.50					
Ainus	Harada and Omoto, 1970	238	95.80	0.40		3.80					
USA, white	Harada and Omoto, 1970	261	91.20	3.10		4.50				0.40	
USA, white	Kueppers, 1971*a*	188	96.65	3.93	0.49						

The EM phenotype is remarkable since it cannot be detected by agarose gel electrophoresis, although the E protein is very fast on acid starch gel electrophoresis. Most of the a-1-at variants have probably arisen from single amino acid substitutions due to point mutations, but the E protein may have a different origin.

Even the Pi^{I} allele product is peculiar as the distribution of a-1-at among the 8 Pi zones differs markedly from that of other allele products.

All published observations as well as unpublished studies in our laboratory suggest that the a-1-at is determined by a series of at least 11 codominant alleles at a single locus. This theory is supported by the distribution of phenotypes in various populations and in selected families, and the Pi zone patterns on starch gels. No evidence has been presented in favour of linkage to other genetic markers or that more than one locus might be involved. Since variants have been transmitted from father to son and since the frequency of variants is the same in both sexes, the Pi locus is not sex-linked.

Table 2 shows the Pi phenotype distribution in various populations. The frequencies of several variants are so high that the Pi system fulfils the requirements of a true polymorphism.

Table 3 gives the Pi gene frequencies in the same populations. In most populations Pi^{M} has a frequency of 0.85–0.99. The high Pi^{S} frequency in Iberians is remarkable, and is supported by similar results among Latin Americans and people living in southern France (Robinet-Levy and Cazal, 1971).

Kellermann and Walter (1970) have reported surprisingly high frequencies of Pi^{F} in various populations. However, some of their sera classified as FF or FM may in fact be MM sera giving abnormal patterns due to contamination or storage as mentioned above. This is supported by the fact that Goedde *et al.* (1970) and Kueppers (1971*a*) found no FM type among 462 Germans living in the Hamburg area, while Kellermann and Walter found 74 FM subjects among 516 Germans supposed to be representative for Western Germany.

A large number of publications have confirmed the original observation by Laurell and Eriksson (1963) that the Pi type ZZ is associated with chronic obstructive pulmonary disease (COPD). During the subsequent years the ZZ type has been found in patients with various diseases, but only the association with infantile cirrhosis of the liver seems to be well established (Sharp *et al.*, 1969; Gans *et al.*, 1969).

The frequency of Pi type ZZ among patients with COPD varies from 1 to 2% in Scandinavia (Eriksson, 1965; Fagerhol and Hauge, 1969; Varpela and Saris, 1971) and France (Robinet-Levy and Cazal, 1970; Vidal *et al.*, 1970) to 25% in California, USA (Lieberman, 1969). The frequencies found will to a large extent depend upon the criteria of diagnosis and the age groups of the patients. In a series of patients studied by Hepper *et al.* (1969) a-1-at deficiency was found in about 30% of those in whom a diagnosis of COPD was made at an age of 43 years or earlier. Furthermore, the frequency of a-1-at deficiency will be higher in a group of patients who have developed early idiopathic emphysema than in patients with other types of COPD. The morbidity (for COPD) among ZZ subjects may be 50–80%, but detailed pulmonary physiology studies will probably disclose some pulmonary affection in all adults. Since some ZZ subjects may reach an age of 70 years or more without clinical symptoms, it is reasonable to assume that other factors, genetic or environmental, in addition to a low level of a-1-at, may be necessary to produce clinical emphysema. The pathogenesis of this disease is thought to be a progressive degradation of pulmonary elastic tissue due to attack by proteolytic enzymes. This may be due to a disturbance of the normal metabolism of pulmonary elastic tissue or to attack by proteases originating from leucocytes, macrophages or microorganisms. It has been shown by Lieberman and Gawad (1971) that a-1-at can inhibit the major leucocytic proteases which can be extracted from purulent sputum. These enzymes were also able to digest human lung tissue.

Children with Pi type ZZ have a considerable risk of cirrhosis of the liver shortly after birth or in early childhood. The morbidity in ZZ subjects for this disease is probably as high as for

TABLE 3 *Pi gene frequencies in various populations*

Population	Authors	No. tested	Pi^M	Pi^S	Pi^Z	Pi^F	Pi^I
Norwegians	Fagerhol, 1967	2830	0.9463	0.0230	0.0157	0.0133	0.0012
Spaniards	Fagerhol and Tenfjord, 1968	378	0.8664	0.1124	0.0119	0.0026	0.0013
Portuguese	Fagerhol and Tenfjord, 1968	39	0.8590	0.1410			
Finnish Lapps	Fagerhol *et al.*, 1969	468	0.9957	0.0032	0.0010		
Norwegian Lapps	Fagerhol *et al.*, 1969	302	0.9917		0.0083		
Finns	Fagerhol *et al.*, 1969	223	0.9955		0.0045		
Åland Islanders	Fagerhol *et al.*, 1969	450	0.9958	0.0042			
Mongolians	Fagerhol and Tenfjord, 1968	68	1.0000				
Icelanders	Kellermann and Walter, 1970	94	0.8723	0.0106		0.1170	
Germans	Kellermann and Walter, 1970	516	0.8789	0.0213	0.0087	0.0901	0.0010
Germans	Kueppers, 1971*a*	200	0.9800	0.020	0.0025		
Germans	Goedde *et al.*, 1970	262	0.9670	0.0230	0.0095		
Greeks	Kellermann and Walter, 1970	400	0.9587	0.0025	0.0162	0.0125	0.0062
Hungarians	Kellermann and Walter, 1970	172	0.8924	0.0174	0.0145	0.0698	0.0029
Iranians	Kellermann and Walter, 1970	271	0.8838	0.0221	0.0221	0.0682	0.0037
Pakistanis	Kellermann and Walter, 1970	53	0.9528		0.0094	0.0377	
Indians	Kellermann and Walter, 1970	430	0.9942		0.0058		
Koreans	Kellermann and Walter, 1970	90	0.9889		0.0111		
Negroes	Kellermann and Walter, 1970	274	0.9818	0.0018		0.0164	
Japanese	Harada and Omoto, 1970	965	0.9830	0.0030		0.0140	
Ainus	Harada and Omoto, 1970	238	0.9790	0.0020		0.0190	
USA, white	Harada and Omoto, 1970	261	0.9520	0.0170		0.0290	
USA, white	Kueppers, 1971*a*	188	0.9380	0.0430	0.0160		

emphysema, and the prognosis is very bad. Most cirrhotic patients die either within a few months or before the age of 16.

The pathogenesis of the liver disease is also obscure. Immunofluorescence studies of the liver biopsies in such cases have revealed a peculiar accumulation of a-1-at in the liver cells (Sharp, 1971; Aagenaes and Munthe, 1970). Sera from such children have given ordinary ZZ patterns on acid starch gel electrophoresis and antigen-antibody crossed electrophoresis. On the other hand, sera from patients with other types of cirrhosis (adults), even from those with very severe impairment of liver functions, give a normal MM pattern in the majority of cases. Due to the accumulation of a-1-at in the liver cells, it has been suggested that the low concentration of this protein in serum is due to a block in the secretion, or transportation, from the liver cells to the plasma. We have, however, found no abnormal accumulation of a-1-at in the liver of a child, Pi type MZ, with cirrhosis. This argues against the theory that the structure of the Z protein in itself is responsible for the defective secretion or transportation.

Since not every ZZ individual develops cirrhosis other factors must be of importance.

Although the number of patients studied was relatively small, I would like to mention the possible relationship between the Pi system and sex chromosome aberrations. Aarskog and Fagerhol (1970) found Pi types other than MM in the patient and/or the parents in 5 out of 7 families where sex chromosome mosaics occurred. In these families the Pi types MS, FM and SS were found and in 4 out of 5 cases the unusual type was found in the mother.

Moghissi *et al.* (1964) have suggested that proteases play a role in the process of sperm penetration. Lundblad (1954) has shown that this is the case for sea urchins. Kueppers (1971*b*) has shown that rabbit sperm contains a protease which reacts with the protein corresponding to a-1-at in rabbit serum. It has been shown by Hagström (1961) and Lönning (1967) that treatment of sea urchin eggs with relatively high concentrations of trypsin inhibits the formation of the fertilization membrane and increases the tendency towards polyspermy. Moreover, it has been demonstrated that the cortical reaction following insemination is both delayed and inhibited in the presence of trypsin inhibitors, resulting in the formation of an incomplete fertilization membrane and increased susceptibility to polyspermy (Hagström, 1957; Lönning, 1967). Proteolytic enzymes may also be involved in the phenomenon of anaphase lag since it has been shown that the first cell divisions after fertilization are considerably accelerated by trypsin (Hagström and Lönning, 1963). Schumacher and Pearl (1968) have demonstrated that the concentration of a-1-at in cervical mucus from women decreases considerably (in some cases to only 10% of the original level) shortly before ovulation. These observations may suggest that: (1) abnormal protease activity (for instance due to low level of inhibitors or presence of abnormal inhibitors) may contribute to the development of sex chromosome aberrations; (2) abnormal protease activity, due to the Pi phenotype of the individual, may be responsible for a slightly increased fertility and thus contribute to the selection in favour of Pi variants.

Some support for the latter hypothesis may be found in the increased frequency of Pi variants among mothers with many children (Fagerhol and Gedde-Dahl, 1969).

Recently Omoto *et al.* (1970) have shown that 6 species of the Macaques (*Macaca irus*, *mulatta*, *cyclopis*, *nemestrina*, *speciosa* and *fuscata*) exhibit a Pi polymorphism closely resembling that of man. This has opened new possibilities for experimental studies on several aspects of the Pi system. The finding of a phenotype corresponding to ZZ, *i.e.* a-1-at deficiency, in 2 samples of *Macaca irus* from Malaysia may be of special interest to those working on the pathogenesis of COPD and cirrhosis of the liver in ZZ subjects.

REFERENCES

AAGENAES, Ö. and MUNTHE, E. (1970): Personal communication.

AARSKOG, D. and FAGERHOL, M. K. (1970): Protease inhibitor (Pi) phenotypes in chromosome aberrations. *J. med. Genet.*, 7/4, 367.

AXELSSON, U. and LAURELL, C.-B. (1965): Hereditary variants of serum α_1-antitrypsin. *Amer. J. hum. Genet.*, *17/6*, 466.

ERIKSSON, S. (1965): Studies in α_1-antitrypsin deficiency. *Acta med. scand.*, *177*, *Suppl. 175*.

ERIKSSON, S. and LAURELL, C.-B. (1963): A new abnormal serum globulin α_1-antitrypsin. *Acta chem. scand.*, *17*, 150.

FAGERHOL, M. K. (1967): Serum Pi types in Norwegians. *Acta path. microbiol. scand.*, *70*, 421.

FAGERHOL, M. K. (1968): The Pi-system. *Series Haematologica, 1/1*, 153.

FAGERHOL, M. K. (1969): Quantitative studies on the inherited variants of serum α_1-antitrypsin. *Scand. J. clin. Lab. Invest.*, *23*, 97.

FAGERHOL, M. K. (1971): Genetics of the Pi system. *Proceedings, International Symposium on Proteolysis and Pulmonary Emphysema, Duarte, California, 1971.* Academic Press, In press.

FAGERHOL, M. K., ERIKSSON, A. W. and MONN, E. (1969): Serum Pi types in some Lappish and Finnish populations. *Hum. Hered.*, *91/4*, 360.

FAGERHOL, M. K. and GEDDE-DAHL, T., Jr (1969): Genetics of the Pi serum types. *Hum. Hered.*, *19/4*, 354.

FAGERHOL, M. K. and HAUGE, H. E. (1969): Serum Pi types in patients with pulmonary diseases. *Acta allerg. (Kbh.)*, *24*, 107.

FAGERHOL, M. K. and LAURELL, C.-B. (1967): The polymorphism of 'prealbumins' and α_1-antitrypsin in human sera. *Clin. chim. Acta*, *16*, 199.

FAGERHOL, M. K. and TENFJORD, O. W. (1968): Serum Pi types in some European, American, Asian and African populations. *Acta path. microbiol. scand.*, *72*, 601.

GANROT, P. O. (1967): Demonstration of an α_1-antitrypsin complex of high molecular weight in human serum. *Clin. chim. Acta*, *15*, 373.

GANS, H., SHARP, H. L. and TAN, B. H. (1969): Antiprotease deficiency and familial infantile liver cirrhosis. *Surg. Gynec. Obstet.*, *129*, 289.

GOEDDE, H. W., BENKMANN, H.-G., CHRIST, I., SINGH, S. and HIRTH, L. (1970): Gene frequencies of red cell adenosine deaminase, adenylate kinase, phosphoglucomutase, acid phosphatase and serum α_1-antitrypsin (Pi) in a German population. *Humangenetik*, *10*, 235.

HAGSTRÖM, B. E. (1957): Studies on polyspermy in sea urchins. *Ark. Zool.*, *10*, 307.

HAGSTRÖM, B. E. (1961): Experiments on hybridization of sea urchins. *Ark. Zool.*, *12*, 127.

HAGSTRÖM, B. E. and LÖNNING, S. (1963): The effect of trypsin on the early development of the sea urchin egg. *Ark. Zool.*, *15*, 377.

HARADA, S. and OMOTO, K. (1970): Electrophoretic variants of human serum α_1-antitrypsin in Japan. *J. anthrop. Soc. Nippon*, *78/1*, 22.

HEPPER, N. G., BLACK, L. F., GLEICH, G. J. and KUEPPERS, F. (1969): The prevalence of alpha$_1$-antitrypsin deficiency in selected groups of patients with chronic obstructive lung disease. *Mayo Clin. Proc.*, *44*, 697.

KELLERMANN, G. and WALTER, H. (1970): Investigations on the population genetics of the α_1-antitrypsin polymorphism. *Humangenetik*, *10*, 145.

KUEPPERS, F. (1971*a*): Alpha$_1$-antitrypsin: physiology, genetics and pathology. *Humangenetik*, *11*, 177.

KUEPPERS, F. (1971*b*): Isolation and characterization of rabbit antitrypsin. *Proceedings, International Symposium on Proteolysis and Pulmonary Emphysema, Duarte, California, 1971.* Academic Press, In press.

LAURELL, C.-B. (1965): Electrophoretic microheterogeneity of serum α_1-antitrypsin. *Scand. J. clin. Lab. Invest.*, *17/3*, 271.

LAURELL, C.-B. and ERIKSSON, S. (1963): The electrophoretic α_1-globulin pattern of serum in α_1-antitrypsin deficiency. *Scand. J. clin. Lab. Invest.*, *15/2*, 132.

LIEBERMAN, J. (1969): Heterozygous and homozygous alpha$_1$-antitrypsin deficiency in patients with pulmonary emphysema. *New Engl. J. Med.*, *281*, 279.

LIEBERMAN, J. and GAWAD, M. A. (1971): Inhibitors and activators of leukocytic proteases in purulent sputum. Digestion of human lung and inhibition by alpha$_1$-antitrypsin. *J. Lab. clin. Med.*, *77/5*, 713.

LÖNNING, S. (1967): Electron microscopic studies of the block to polyspermy: the influence of trypsin, soy bean trypsin inhibitor and chloral hydrate. *Sarsia*, *30*, 107.

LUNDBLAD, G. (1954): *Proteolytic Activity in Sea Urchin Gametes.* Almqvist and Wiksell, Uppsala.

MOGHISSI, K. S., DABICH, D., LEVINE, J. and NEUHAUS, O. W. (1964): Mechanism of sperm migration. *Fertil. Steril.*, *15*, 15.

OMOTO, K., HARADA, S., TANAKA, T., NIGI, H. and PRYCHODKO, W. (1970): Distribution of the electrophoretic variants of serum alpha$_1$-antitrypsin in six species of the Macaques. *Primates, 11*, 215.

ROBINET-LEVY, M. and CAZAL, P. (1970): Les groupes sériques du système Pi (alpha-1-antitrypsine). *Rev. franç. Transfus., 13/4*, 389.

ROBINET-LEVY, M. and CAZAL, P. (1971): Personal communication.

SCHUMACHER, G. F. B. and PEARL, M. J. (1968): Alpha-antitrypsin in cervical mucus. *Fertil. Steril., 19/1*, 91.

SHARP, H. L. (1971): Familial cirrhosis. *Proceedings, International Symposium on Proteolysis and Pulmonary Emphysema, Duarte, California, 1971.* Academic Press, In press.

SHARP, H. L., BRIDGES, R. A., KRIVIT, W. and FREIER, E. F. (1969): Cirrhosis associated with alpha-1-antitrypsin deficiency: a previously unrecognized inherited disorder. *J. Lab. clin. Med., 73/6*, 934.

VARPELA, E. and SARIS, N.-E. (1971): Hereditary alpha$_1$-antitrypsin deficiency. *Ann. clin. Res., 3*, 46.

VIDAL, J., CAZAL, P., ROBINET-LEVY, M. and MICHEL, F. B. (1970): Déficits en alpha$_1$-antitrypsine, groupes Pi et bronchopneumopathies chroniques. *Presse méd., 78/17*, 783.

John S. O'Brien

Department of Neurosciences, University of California at San Diego, La Jolla, Calif., U.S.A.

Genetic defects of lysosomal enzymes

Presently, there are 35 syndromes which can be classified as lysosomal storage diseases or leucodystrophies (Table 1). Twenty of these are associated with a marked deficiency of a specific lysosomal hydrolase enzyme. The diagnosis of many of the lysosomal storage diseases listed is possible by assaying the appropriate lysosomal enzyme in serum, leucocytes, or in cultured fibroblasts from the affected patient. Heterozygotes can often be detected by enzyme assays. Prenatal diagnosis of homozygotes for many of the lysosomal storage diseases listed is possible by amniocentesis and assays of the appropriate enzyme.

In this single presentation time does not permit a thorough discussion of more than one lysosomal hydrolase deficiency disease. I have decided to discuss Tay-Sachs disease as an example of progress in this field.

For almost 100 years Tay-Sachs disease was the only ganglioside storage disease known. The disease involves:

1. Progressive mental and motor deterioration with onset in infancy and fatal outcome in early childhood.
2. Autosomal recessive inheritance.
3. Neuronal lipidosis secondary to storage of ganglioside GM_2 and the asialo derivative of GM_2, asialo-GM_2.
4. Absence or severe deficiency of a specific lysosomal glycohydrolase, hexosaminidase A.

CLINICAL PICTURE

Tay-Sachs disease was described in 1881 by a British ophthalmologist, Waren Tay, and was later expanded upon by an American neurologist, Bernard Sachs (1896). The infant presents with weakness beginning within the first six months of life, progressive mental and motor deterioration, blindness, paralysis, dementia and death by three years of age.

Pathological changes include ballooning of neurons throughout the nervous system. By electron microscopy, lipid bodies were seen to accumulate within neurons (Terry and Weiss, 1963). These are spirally wound structures which stain heavily with osmium.

GENETICS

Tay-Sachs disease appears to be transmitted as an autosomal recessive trait for the following reasons: parents of probands have been clinically normal; sex ratios are nearly equal; analysis of sibships, correcting for incomplete ascertainment, gives ratios of affected to non-

TABLE 1

Disease	Compound stored	Enzyme defect	Best way to diagnose	Carrier detection	Prenatal diagnosis
1. Tay-Sachs (GM_2 Type 1)	Ganglioside GM_2	Hexosaminidase A	Serum enzyme assay	Serum enzyme assay	Established
2. Sandhoff's (GM_2 Type 2)	Ganglioside GM_2	Hexosaminidase A and B	Serum enzyme assay	Skin fibroblast enzyme assay	Possible
3. Juvenile GM_2 (GM_3 Type 3)	Ganglioside GM_2	Partial deficiency of hexosaminidase A	Serum enzyme assay	Serum enzyme assay	Possible
4. Generalized gangliosidosis (GM_1 Type 1)	Ganglioside GM_1	GM_1, β-galactosidase	WBC enzyme assay	WBC enzyme assay	Possible
5. Juvenile GM_1 (GM_1 Type 2)	Ganglioside GM_1	GM_1, β-galactosidase	WBC enzyme assay	WBC enzyme assay	Possible
6. Infantile Gaucher's	Glucocerebroside	Glucocerebrosidase	WBC enzyme assay	WBC or skin fibroblast enzyme assay	Possible
7. Adult Gaucher's	Glucocerebroside	Glucocerebrosidase	WBC enzyme assay	WBC or skin fibroblast enzyme assay	Possible
8. Mannosidosis	Mannolipids and mannoproteins	α-mannosidase	WBC enzyme assay	?	?
9. Fucosidosis	Fucolipids and fucoproteins	α-fucosidase	Serum or WBC enzyme assay	?	?
10. I-cell disease	Various lipids in fibroblasts	Multilysosomal enzyme deficiency	Skin fibroblast enzyme assays	?	?
11. Metachromatic leucodystrophy late infantile type	Cerebroside sulfate	Arylsulfatase A	WBC enzyme assay, skin fibroblast enzyme assay	Skin fibroblast or WBC enzyme assay	Possible
12. Juvenile type	Cerebroside sulfate	Arylsulfatase A	WBC enzyme assay, skin fibroblast enzyme assay	Skin fibroblast or WBC enzyme assay	Possible
13. Adult type	Cerebroside sulfate	Arylsulfatase A	WBC enzyme assay, skin fibroblast enzyme assay	Skin fibroblast or WBC enzyme assay	Possible
14. Hurler's (MPS I)	Heparatin sulfate Chondroitin sulfate B	α-iduronidase	Urinary AMPS SO_4 turnover in fibroblasts à la Neufeld	?	Possible

TABLE 1 *(continued)*

Disease	Compound stored	Enzyme defect	Best way to diagnose	Carrier detection	Prenatal diagnosis
15. Hunter's (MPS II) (X-linked)	Heparatin sulfate Chondroitin sulfate B	?	Urinary AMPS SO_4 turnover in fibroblasts à la Neufeld	?	Possible
16. Sanfillippo (MPS III)	Heparatin sulfate Chondroitin sulfate B	Type A – heparatin sulfate sulfatase; Type B – α-acetylglucosaminidase	Urinary AMPS SO_4 turnover in fibroblasts à la Neufeld	in type A; fibroblast enzyme assay in type B	in type A; possible in type B
17. Morquio	Heparatin and keratan sulfate	?	Urinary AMPS SO_4 turnover in fibroblasts à la Neufeld	?	?
18. Scheie	Heparatin sulfate CSB	α-iduronidase	Urinary AMPS SO_4 turnover in fibroblasts à la Neufeld	?	?
19. Niemann-Pick infantile cerebral type (Crocker Type A)	Sphingomyelin	Sphingomyelinase	WBC enzyme assay, skin fibroblast enzyme assay	WBC or fibroblast enzyme assay (still not established)	Possible
20. Niemann-Pick chronic non-cerebral type (Crocker Type B)	Sphingomyelin	Sphingomyelinase	WBC enzyme assay, skin fibroblast enzyme assay	WBC or fibroblast enzyme assay (still not established)	Possible
21. Acid phosphatase deficiency (Nadler's disease)	?	Acid phosphatase	Skin fibroblast enzyme assay	Skin fibroblast enzyme assay	Possible
22. Wolman's disease	Triglycerides and cholesterol ester	Acid lipase	Skin fibroblast or WBC enzyme assay	?	?
23. Fabry's disease (X-linked)	Ceramide trihexoside	α-galactosidase	Skin fibroblast or WBC enzyme assay	? (not always reliable due to varying mosaicism)	Possible
24. Pompe's disease (glycogenosis Type II)	Glycogen	α-1-4 glucosidase	Skin fibroblast or WBC enzyme assay	Skin fibroblast or WBC enzyme assay	Established
25. Ceramide lactosidosis (Dawson's disease)	Ceramide lactoside	Ceramide lactoside-β-galactosidase	Skin fibroblast or WBC enzyme assay	?	?

TABLE 1 *(continued)*

Disease	Compound stored	Enzyme defect	Best way to diagnose	Carrier detection	Prenatal diagnosis
26. Krabbe's disease (globoid cell leucodystrophy)	Galactocerebroside	Galactocerebroside-β-galactosidase	Skin fibroblast enzyme assay	Skin fibroblast enzyme assay	Possible
27. Jansky-Bielschowsky late infantile lipidosis	Lipofuscin	?	Brain or rectal biopsy, light and EM	No	No
28. Spielmeyer-Vogt juvenile lipidosis	?	?	Brain or rectal biopsy, light and EM, WBC morphology	Possibly by examination for hypergranulation of polymorphs	No
29. Lafora's disease (infantile hereditary myoclonus)	Polysaccharide in neurons	?	Brain biopsy, rectal biopsy (?)	No	No
30. Spongy sclerosis (Van Bogaert-Bertrand-Canavan)	Cerebral edema	?	Brain biopsy, light and EM	No	No
31. Alexander's disease (megalen-barencephaly)	Eosinophilic stuff in astrocytes	?	Brain biopsy, light and EM	No	No
32. Shilder's sudanophilic leucodystrophy	Cholesterol esters, demyelination	?	Brain biopsy, pathology, lipid analyses	No	No
33. Sudanophilic leucodystrophy (X-linked)	None known, demyelination	?	Brain biopsy, clinical picture of Addison's disease plus demyelination in childhood	No	No
34. Pelizaeus-Merzbacher's disease (X-linked)	Hypomyelination	?	Brain biopsy	No	No
35. Menkes syndrome (kinky hair disease) (X-linked)	Autofluorescent lipid in neurons	?	Brain biopsy and demonstrated deficiency of 22:6 fatty acid	No	No

affected children which are consistent with those expected for autosomal recessive inheritance; and finally, both parents have activities of the pivotal enzyme which are intermediate between those of patients and normal controls (*vide infra*).

In the United States the frequency of Tay-Sachs disease has been estimated by case ascertainment from mortality records (Aronson, 1964; Myrianthopoulos, 1962; Kozinn *et al.*, 1957) (Table 2). These studies give a heterozygote frequency of 0.026 for Ashkenazi Jewish individuals and 0.0029 for non-Jewish individuals. Using these frequencies, the 1970 census figures for North America, a birth rate of 17.4 per thousand, and assuming that intermarriage between Jews and non-Jews is an uncommon event, one can calculate that 30 children with Tay-Sachs disease are born annually in North America, 22 of whom are Jewish. The world figure is probably five to seven times this number.

CHEMISTRY OF STORAGE SUBSTANCE

In 1942, Ernst Klenk reported a remarkable increase in the cerebral ganglioside content in Tay-Sachs disease. The specific ganglioside stored was identified as ganglioside GM_2 in 1962 by Lars Svennerholm, a structure that was confirmed in more detail by Ledeen and Salzman (1965). Ganglioside GM_2 has a ceramide backbone to which are linked glucose, galactose, N-acetylgalactosamine, and N-acetylneuraminic acid in the sequence shown below:

ceramide-glucose-galactose-N-acetylgalactosamine
|
N-acetylneuraminic acid

Cerebral levels of ganglioside GM_2 are increased 100–300 times normal in Tay-Sachs disease (O'Brien *et al.*, 1971*b*). The asialo derivative of ganglioside GM_2, asialo-GM_2, is also increased to levels 20 times normal. Ganglioside GM_2 also accumulates in visceral organs in Tay-Sachs disease, but the amount of storage is too small to cause visible cytoplasmic lipidosis or organomegaly.

ENZYMIC DEFECT

The accumulation of ganglioside GM_2 in Tay-Sachs disease can be explained by increased synthesis, decreased degradation, or a combination of both. Deficiency of a degradative enzyme, a lysosomal hydrolase, is a satisfactory explanation for the primary enzymic defect since a pathway for ganglioside GM_2 breakdown involving sequential cleavage of sugars by specific lysosomal hydrolases is known to be present in the normal brain (Gatt, 1967) and the stored ganglioside accumulates within structures which have the ultrastructural and histochemical characteristics of altered lysosomes (Wallace *et al.*, 1964). Furthermore, storage of ganglioside GM_1, in generalized gangliosidosis, another inborn error of ganglioside metabolism involving the storage of ganglioside GM_1, was shown by Shintaro Okada and myself (1968) to be due to a profound deficiency of a β-galactosidase which cleaves the terminal galactose from the stored ganglioside (GM_1-β-galactosidase).

In 1969, Dr. Okada and I reported a striking deficiency of a β-N-acetylhexosaminidase in Tay-Sachs disease. This appears to be the primary enzyme defect. Two hexosaminidase components, both of which cleave β-galactosaminides and β-glucosaminides, were first demonstrated by Robinson and Stirling (1968) in human spleen. These hexosaminidases possess similar Michaelis-Menten constants, have acid pH optima, are both present in the lysosomal fraction, and are readily separated from one another by ion exchange column chromatography and starch gel electrophoresis. One of these, called hexosaminidase A, is more negatively charged and more heat labile than the other, hexosaminidase B.

Dr. Okada and I (1969) demonstrated that both hexosaminidase A and hexosaminidase B are normally present in human liver, brain, skin, kidney, leucocytes, serum, and cultured skin fibroblasts. In nine patients with Tay-Sachs disease we found that hexosaminidase A was absent in all these tissues (Fig. 1). The deficiency was demonstrated using para-nitrophenyl, 4-methylumbelliferyl, and naphthol derivatives of both β-D-N-acetylglucosamine, and β-D-N-acetylgalactosamine as substrates. The activity of hexosaminidase B was found to be 10 times higher than normal in the brain of patients with Tay-Sachs disease, explaining the elevated total cerebral hexosaminidase activity; but in the same tissue hexosaminidase A was absent.

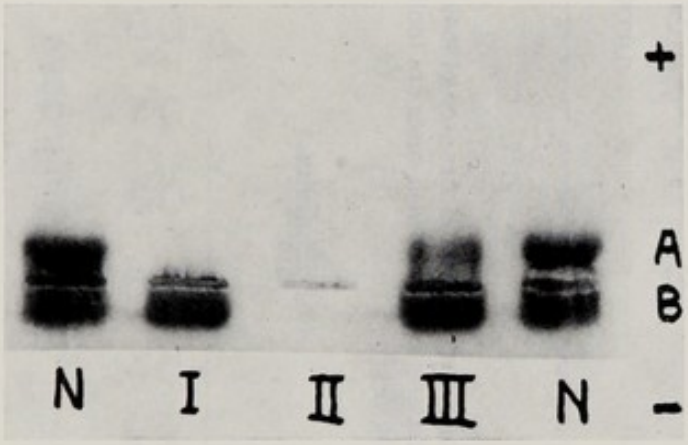


Fig. 1. Starch gel electrophoresis of liver hexosaminidases, stained with naphthol-ASBI-N-acetyl-β-D-glucosaminide (Okada and O'Brien, 1969). Lane I – Tay-Sachs disease; II – Sandhoff's disease; III – juvenile GM_2 gangliosidosis; N – two control subjects.

I now believe that the elevated activity of hexosaminidase B is probably due to stimulation of lysosomes secondary to cerebral ganglioside storage, since the activities of the other lysosomal hydrolases including β-glucosidase, β-galactosidase, and acid phosphatase are also increased. Moreover, hexosaminidase B activity is not increased in tissues such as liver and spleen, where lipid storage is not prominent.

The enzyme defect is specific for Tay-Sachs disease. No deficiency of hexosaminidase A occurs in related neuronal lipidosis. Mixing experiments indicate that soluble inhibitors are not responsible for the absence of the enzyme. The enzymic defect is present in fresh tissues including leucocytes and venous blood, as well as in cultured skin fibroblasts propagated for many cellular generations.

DETECTION OF HOMOZYGOTES AND HETEROZYGOTES BY SERUM ASSAY

Hexosaminidase A and B are present in normal human serum and Tay-Sachs patients have profound deficiencies of the enzyme in serum. Our laboratory then set out to determine whether heterozygotes could be identified by reductions of hexosaminidase A in serum. We devised a fluorometric assay for the estimation of both hexosaminidase A and B in 100 μl of serum (O'Brien *et al.*, 1970). The assay exploits the different thermal stabilities of the two hexosaminidases; the activity of hexosaminidase A is rapidly destroyed by heat at 50° whereas the activity of hexosaminidase B remains nearly unchanged under the same conditions.

This assay has been very useful in the clinical diagnosis of Tay-Sachs disease, especially in atypical cases without cherry red spots, and in the differentiation of Tay-Sachs disease from infantile Gaucher's disease, metachromatic leucodystrophy, Niemann-Pick's disease and Batten-Spielmeyer-Vogt disease. We have demonstrated that the enzyme is absent in serum from

TABLE 2 *Estimation of frequency of Tay-Sachs disease in North America*

	Total population*	Annual no. of births**	Homozygote frequency***	Annual no. of probands	Annual no. of total cases†	Heterozygote frequency***	Total no. of heterozygotes
Jewish	6,182,000	107,567	0.0017	18	22	0.026	160,732
Non-Jewish	215,393,326	3,747,844	0.0000018	7	8	0.0029	624,641

* 1970 census figures for the U.S. and Canada.
** Assumes a birth rate of 17.4 per thousand for each group.
*** Data from case ascertainment of probands only by Kozinn *et al.* (1957), Myrianthopoulos (1962), and Aronson (1964).
† Probands represent 82% of the cases, post-probands 18% (Kozinn *et al.*, 1957). Estimates of non-Jewish cases are too high, since they include Sandhoff's disease as well.

a Tay-Sachs fetus as early as 18 weeks' gestation (O'Brien *et al.*, 1971*a*). Thus far, we have demonstrated the deficiency of the enzyme in serum from 24 patients with Tay-Sachs disease (Fig. 2).

Obligate heterozygotes have nearly normal values for serum hexosaminidase B, but reductions in the activity of hexosaminidase A. Thus far, we have carried out serum assays in 56 parents of Tay-Sachs children, comparing their activity with 77 control subjects. The results demonstrate a small degree of overlap between heterozygotes and controls. The overlap amounts to 5% of the controls and 5% of the heterozygotes. When these indeterminate subjects are retested, the overlap diminishes to less than 1%.

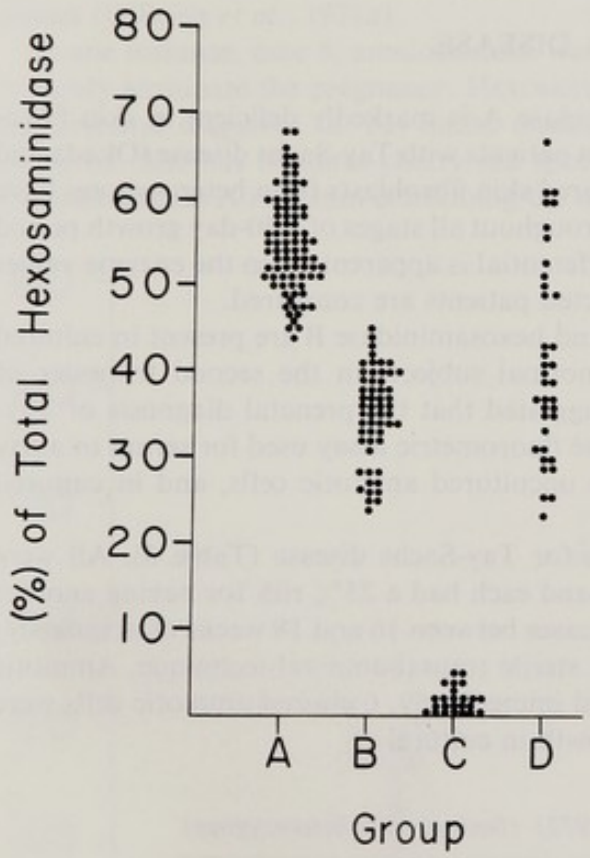


Fig. 2. Activity of hexosaminidase A in serum from control subjects (A), parents of patients with Tay-Sachs disease (B), patients with Tay-Sachs disease (C), and relatives of probands (D).

We have found the assay to be of great value in families in which the disease has previously appeared. In the same study we have determined the activity of the enzyme in 44 relatives of probands, including grandparents, uncles and aunts, first cousins, and siblings. Fourteen of these had activity of the enzyme in the normal range and 30 had activity of the enzyme in the range for heterozygotes. The mean and range of activity of the enzyme in serum from presumptive heterozygotes are nearly identical to the mean and range of the activity of the enzyme in serum from obligate heterozygotes, again emphasizing the usefulness of the assay in heterozygote detection. Thus far, the proportion of heterozygotes among relatives is close to that expected for autosomal recessive inheritance, although the total number tested is still small.

We designed the serum hexosaminidase A assay for use in a routine clinical chemistry laboratory. It is simple and inexpensive to perform and is very sensitive, requiring 100 μl of serum. The enzyme in serum is stable at room temperature for one day, at refrigerated temperature (5°) for ten days, and at freezer temperature (—20°) for many months (O'Brien *et al.*, 1970).

The assay has been applied in a pilot project for population screening by Dr. Michael Kaback at Johns Hopkins Medical School. Dr. Kaback has used the serum hexosaminidase A assay to detect heterozygotes for Tay-Sachs disease in the Jewish population living in the Baltimore-Washington area, a population of 200,000 people, 80,000 of whom are between age 18 and 43. Thus far, he has tested 8,000 of these people. The results he has obtained are consistent with the conclusions we drew from testing a smaller population; that is, the assay is reliable, reproducible, and the overlap between the heterozygotes and controls, after retesting indeterminate individuals, is less than 1%. Thus, it appears that mass population screening for heterozygotes by serum assay is now feasible.

PRENATAL DIAGNOSIS OF TAY-SACHS DISEASE

Previously, we demonstrated that hexosaminidase A is markedly deficient in skin fibroblasts grown for many cellular generations from patients with Tay-Sachs disease (Okada and O'Brien, 1969). We also demonstrated that cultured skin fibroblasts from heterozygotes have intermediate reductions of hexosaminidase A throughout all stages of a 30-day growth period after subculture (Okada *et al.*, 1971). A large differential is apparent when the enzyme values obtained from controls, heterozygotes and affected patients are compared.

We also found that both hexosaminidase A and hexosaminidase B are present in cultured amniotic cells obtained by amniocentesis of normal subjects in the second trimester of pregnancy (Okada and O'Brien, 1969). This suggested that the prenatal diagnosis of Tay-Sachs disease was possible. We then modified the fluorometric assay used for serum to assay hexosaminidase A and B in amniotic fluid, in uncultured amniotic cells, and in cultured amniotic cells (O'Brien *et al.*, 1971*a*).

To date, we have monitored 24 pregnancies for Tay-Sachs disease (Table 3). All were women who had one or more affected children and each had a 25% risk for having another (Fig. 3). Amniocentesis was carried out in most cases between 16 and 18 weeks of pregnancy; 10 to 20 ml of amniotic fluid were obtained by sterile transabdominal technique. Amniotic fluid and uncultured amniotic cells were assayed immediately. Cultured amniotic cells were assayed within ten days to four weeks after growth in culture.

TABLE 3 *Prenatal diagnosis of Tay-Sachs (Jan. 1972) (both parents heterozygous)*

Number monitored	24	
Homozygotes	8	
Aborted	7	(6 analyzed and diagnosis confirmed, 1 unavailable)
1 monitored too late (27 weeks) now 24 months, has TSD		
Not Tay-Sachs	16	
Born	16	(all have adequate hex A)

All diagnoses made by assays of amniotic fluid, uncultured cells and cultured cells.

We have diagnosed Tay-Sachs disease prenatally in eight of the 24 pregnancies. In each, the results on amniotic fluid, uncultured amniotic cells and cultured amniotic cells were consistent; marked deficiencies of the enzyme were found in each sample. However, the results obtained on cultured amniotic cells and on uncultured amniotic cells are more reliable than those on amniotic fluid due to a larger spread of values between homozygotes and controls (Fig. 3). In seven of these eight cases, amniocentesis was carried out early enough to safely terminate the pregnancy, and all seven fetuses were aborted.

The diagnosis of Tay-Sachs disease was confirmed in each fetus by electron microscopy, ganglioside analysis, and enzyme assays (O'Brien *et al.*, 1971*a*). Ultrastructural studies demonstrated the presence of characteristic cytoplasmic bodies in the spinal cord neurons in all aborted fetuses. Ganglioside analysis of the brain revealed striking increases in the concentrations of ganglioside GM_2 in each fetus as early as 100 days' gestation. The cerebral levels of ganglioside GM_2 in the affected fetuses were approximately 30 times higher than controls; levels of the ganglioside at 3 years of age are 300 times normal. The absence of major neurological symptoms in the first six months of life may be explained by the gradual accumulation of cerebral ganglioside GM_2 with age. Enzyme assays of fetal tissues demonstrated the absence of hexosaminidase A and normal activity of hexosaminidase B in fetal tissues (O'Brien *et al.*, 1971*a*).

In one instance, case 5, amniocentesis was carried out at 27 weeks of pregnancy, too late to safely terminate the pregnancy. Hexosaminidase A assay indicated an affected child, and the prenatal diagnosis of Tay-Sachs disease was made. A girl, now 16 months old, was delivered. She has bilateral cherry red spots, mental and motor deterioration, and absent hexosaminidase A in serum confirming the diagnosis of Tay-Sachs disease. In the remaining

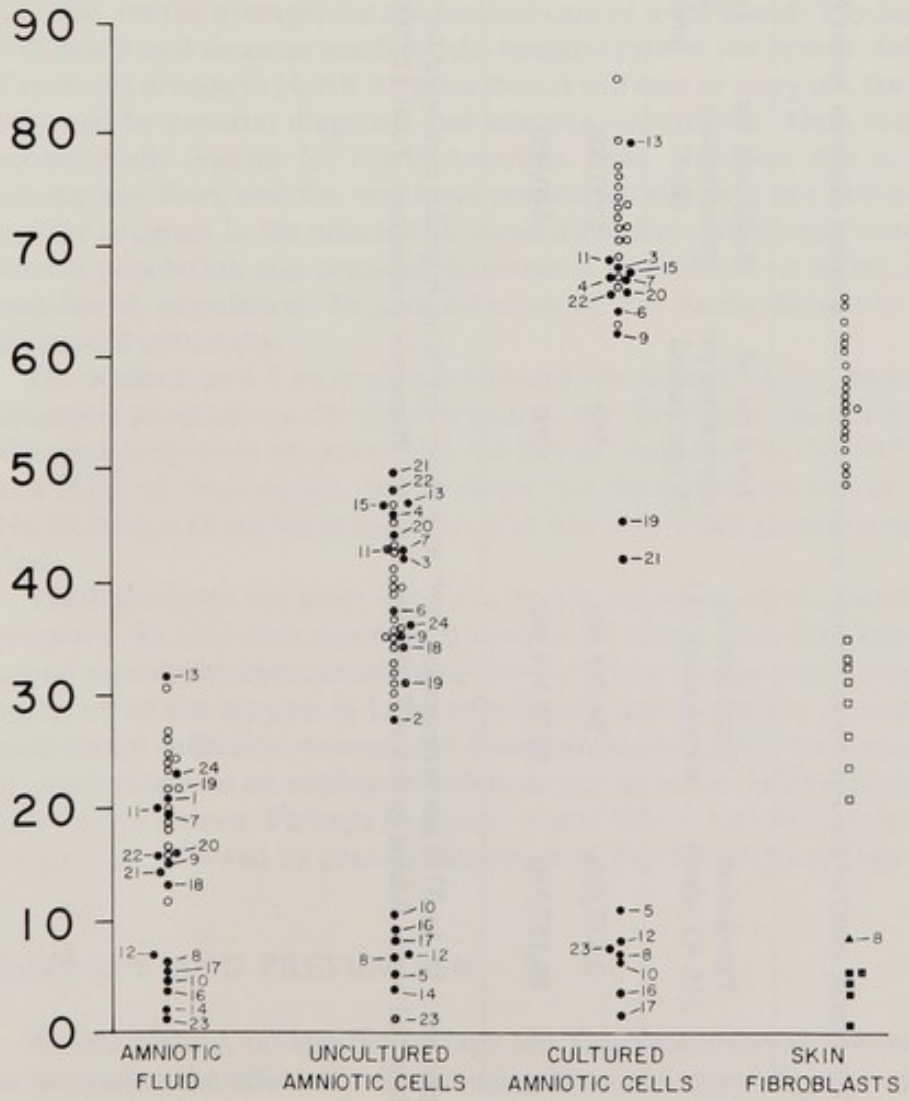


Fig. 3. Hexosaminidase A activity in high-risk pregnancies expressed as per cent of total hexosaminidase. Values from the 24 high risk pregnancies (closed circles) are compared with controls (open circles). Values from cultured skin fibroblasts from patients with Tay-Sachs disease (closed squares), their parents (open squares), controls (open circles), and an affected fetus with Tay-Sachs disease (case 8, closed diamond) are also shown.

TABLE 4 *Cost benefit analysis for Tay-Sachs disease: serum screening and prenatal diagnosis versus hospital costs in Jewish and non-Jewish populations in North America*

	Population (18–43 years)	Total cost for serum assay (A)	Total no. of homozygotes per 25-year period	Total cost for amniocentesis (B)*	Total cost for therapeutic termination (C)**	Total medical cost***	Sum of A, B, C
Jewish	2,472,800	$4,945,600	550	$220,000	$192,500	$13,750,000	$5,358,100
Non-Jewish	86,157,330	$172,314,660	200	$80,000	$70,000	$5,000,000	$172,464,660

* At $100 per test. ** At $350 per termination. *** At $50 per day in an intensive care ward for the period from 14 months to 40 months = $24,000 per child for hospital costs. Added to this is $1,000 per child for diagnostic evaluations. Total $25,000.

16 pregnancies prenatal assessment indicated the absence of Tay-Sachs disease. This was confirmed postnatally in all infants who have been born by clinical examinations and enzyme assays of leucocytes, cultured skin fibroblasts or serum.

In 1933, Slome published data on 88 sibships in which 130 cases of Tay-Sachs disease had occurred. Of these, 82% of the cases were probands and 18% were post-probands, demonstrating that less than one-fifth of the cases of Tay-Sachs disease can be diagnosed prenatally if one waits for the birth of a proband to occur before pregnancies are monitored. In order to diagnose probands prenatally, detection of heterozygotes by population screening is necessary. Such a program is now under way in the Baltimore-Washington area using the serum enzyme assay I have described. Similar programs will soon be implemented in the Jewish population of Toronto, Montreal, and Los Angeles.

Before carrying out mass screening for heterozygotes, it is interesting to determine whether this approach is economically feasible. Let us consider the economic feasibility of carrying out heterozygote detection by serum assay of hexosaminidase A in the North American Jewish population, restricting screening to those individuals between 18 and 43 years of age.

I estimate that it will cost $2 for the serum assay, $100 for amniocentesis and enzyme assay, and $350 for a therapeutic abortion of an affected fetus. In the United States it costs $25,000, on the average, for the medical care of a child with Tay-Sachs disease (Table 4). It will cost 3 to 4 times as much for the hospital care of the Jewish children who are born with Tay-Sachs disease in North America than it will take to carry out the mass screening program followed by prenatal diagnosis and selective termination. Thus, the program appears to be economically feasible for North American Jews. However, due to the low gene frequency among non-Jews, and the very large population size, it is not economically feasible to carry out the program in the non-Jewish population. Even so, if mass screening is restricted to the Jewish population and prenatal diagnosis is carried out to detect all post-probands in the non-Jewish population, 80% of all cases of Tay-Sachs disease in North America can be diagnosed prenatally.

Dr. Kaback and I have also calculated the impact of the mass screening and prenatal diagnosis program on the gene frequency for Tay-Sachs disease in North American Jews. We have calculated an increase in the carrier frequency by 0.0189% per 25-year generation as a result of the program. We conclude that the genetic control of Tay-Sachs disease in the North American Jewish population is of low risk to the genetic composition of this population.

These estimates are given only as a rough indication of the feasibility of carrying out the program. As yet, such practical questions as actual gene frequency of Tay-Sachs disease, actual overlap between carriers and non-carriers in a large population, extent of polymorphic variation of the enzyme in large populations and feasibility of coping with the logistics of mass serum collection, storage and dissemination of results have yet to be answered. Obviously, pilot programs on smaller populations are necessary before the practical feasibility of the approach is known. Perhaps in several years' time it will be known whether mass screening for carriers followed by prenatal diagnosis of Tay-Sachs disease is a worthwhile thing to do.

THERAPY AND PREVENTION

At this writing, no specific therapy for Tay-Sachs disease is known. We have attempted to determine the effect of administering hexosaminidase A in serum of children with Tay-Sachs disease by giving the enzyme in matched plasma from normal donors. No clinical improvement has been noted after dozens of plasma infusions in patients at various stages of their disease. Given in this manner, hexosaminidase A rapidly disappears and is totally gone within 24 hours after administration. It appears unlikely that serum enzyme replacement will benefit the child with Tay-Sachs disease, in view of the rapid disappearance of the enzyme,

the need for it to cross the blood-brain barrier, and the likelihood that it must be administered *in utero* in order to be effective. Obviously, the most straightforward approach to diminish the frequency of Tay-Sachs disease is limitation of reproduction of heterozygotes or ethnic intermarriage. The next most feasible approach is heterozygote detection by serum assay in the Jewish population, followed by prenatal diagnosis of affected fetuses. Obviously, continued attempts to bypass the metabolic block should be sought.

Let me now emphasize the positive aspects of amniocentesis. The first child who was monitored by amniocentesis for Tay-Sachs disease is a little girl. Her mother is a Los Angeles woman who lost a child from Tay-Sachs disease 8 years previously. She called me and told me she was now pregnant. She had read in the Los Angeles Times newspaper that we had discovered the cause of the disease and were able to diagnose the disease *in utero*. She told me that if we could determine that her baby was not going to have Tay-Sachs disease, she would carry it. However, if no answer was forthcoming, she was considering a therapeutic abortion because she did not want to take the 25% risk of having another affected child. We monitored the pregnancy by amniocentesis and determined that there were adequate levels of hexosaminidase A in the amniotic fluid. She carried the baby to term and a little girl was born. By serum assay she is heterozygous. This story illustrates as best I can the great importance of amniocentesis in families who have had an affected child.

NATURE OF THE GENETIC DEFECT

At present, the evidence strongly suggests that hexosaminidase A participates in cleaving the terminal N-acetylgalactosamine from ganglioside GM_2. Major difficulties have arisen, however, in demonstrating this *in vitro*. Thus far, all investigators have found extremely low (or absent) cleavage of the terminal N-acetylgalactosamine from ganglioside GM_2 when homogenates of normal tissue or purified preparations of hexosaminidase A from normal tissue have been tested. These low activities in normal tissues may be due to failure to find the optimal conditions for assay. Until conditions have been found in which respectable rates of hydrolysis of the terminal galactosamine have been achieved in normal tissues, the absence of such activity in tissues from patients with Tay-Sachs disease is not overly impressive. Nonetheless, the overwhelming body of circumstantial evidence suggests that hexosaminidase A is involved in the breakdown of ganglioside GM_2.

It is not known whether the genetic defect involves a structural mutation in hexosaminidase A in which a protein is produced but is catalytically inactive, or whether the enzyme is not synthesized due to a regulator or operator gene mutation, or failure to convert an inactive form of the enzyme to an active one. Robinson and Stirling (1968) have published some evidence to suggest that hexosaminidase A may be derived from hexosaminidase B by addition of sialic acid residues to the B polypeptide. Failure to convert B to A by a sialo transferase might be considered as the defect in Tay-Sachs disease. However, I believe that a 'sialo transferase defect' is very unlikely for the following reasons:

Obligate heterozygotes for the gene for Tay-Sachs disease have reductions of hexosaminidase A in their sera and fibroblasts which are very close to 50% of normal (Okada *et al.*, 1971). If a sialo transferase defect was present, it is unlikely that hexosaminidase A activity would be maintained at 50% in cultured fibroblasts from heterozygotes during the growth period of cultured cells. During this period the specific activity of hexosaminidase A is increasing two- to three-fold (Okada *et al.*, 1971). If the 'sialo transferase defect' was the primary defect, in order to maintain 50% activity of hexosaminidase A over such widely varying conditions of synthesis, the activity of the sialo transferase would have to parallel the concentration of hexosaminidase B and the sialo transferase would have to be rate-limiting at all time points. These conditions seem so unlikely to me that I have dismissed the 'sialo transferase defect' as a likely one. Much more likely is a structural mutation (or an operator or regulator gene

mutation). Further work on the purified enzyme is now under way in our laboratory to decide this point.

In conclusion, I have briefly discussed the clinical picture, the pathology, the genetics, the chemistry, the enzyme defect, and one possible avenue to prevention and genetic control of Tay-Sachs disease.

ACKNOWLEDGEMENTS

I wish to thank those people in my laboratory who have worked diligently on this project: Candy Randolph, Shintaro Okada, Lois Veath, Klaus Zielke, Linda Tennant, Jole Bernett, David Wenger, Mae Wan Ho, Karen Adams and Dorothy Fillerup.

REFERENCES

ARONSON, S. M. (1964): Epidemiology. In: *Tay-Sachs Disease*, p. 118. Editor: B. W. Volk. Grune and Stratton, Inc., New York, N.Y.

GATT, S. (1967): Enzymatic hydrolysis of sphingolipids. V. Hydrolysis of monosialogangliosides and hexosyl ceramides by rat brain α-galactosidase. *Biochim. biophys. Acta, 137*, 192.

KLENK, E. (1942): Über die Ganglioside des Gehirns bei der infantilen amaurotischen Idiotie vom Typ Tay-Sachs. *Ber. dtsch. chem. Ges., 75*, 1632.

KOZINN, P. J., WIENER, N. and COHEN, P. (1957): Infantile familial amaurotic idiocy. *J. Pediat., 51*, 58.

LEDEEN, R. and SALZMAN, K. (1965): Structure of the Tay-Sachs ganglioside. *Biochemistry (Wash.), 4*, 2225.

MYRIANTHOPOULOS, N. C. (1962): Some epidemiologic and genetic aspects of Tay-Sachs disease. In: *Cerebral Sphingolipidoses: A Symposium on Tay-Sachs Disease and Allied Disorders*, p. 375. Editors: S. M. Aronson and B. W. Volk. Academic Press, New York, N.Y.

O'BRIEN, J. S., OKADA, S., CHEN, A. and FILLERUP, D. L. (1970): Tay-Sachs disease: detection of heterozygotes and homozygotes by serum hexosaminidase assay. *New Engl. J. Med., 283*, 15.

O'BRIEN, J. S., OKADA, S., FILLERUP, D. L., VEATH, M. L., ADORNATO, B., BRENNER, P. and LEROY, J. G. (1971*a*): Tay-Sachs disease: prenatal diagnosis. *Science, 172*, 61.

O'BRIEN, J. S., OKADA, S., HO, M. W., FILLERUP, D. L., VEATH, M. L. and ADAMS, K. (1971*b*): Ganglioside storage diseases. *Fed. Proc., 30/3*, 956.

OKADA, S. and O'BRIEN, J. S. (1968): Generalized gangliosidosis: beta-galactosidase deficiency. *Science, 160*, 1002.

OKADA, S. and O'BRIEN, J. S. (1969): Tay-Sachs disease: generalized absence of a beta-D-N-acetyl-hexosaminidase component. *Science, 165*, 698.

OKADA, S., VEATH, M. L., LEROY, J. G. and O'BRIEN, J. S. (1971): Ganglioside GM_2 storage diseases: hexosaminidase deficiencies in cultured fibroblasts. *Amer. J. hum. Genet., 23*, 55.

ROBINSON, D. and STIRLING, J. L. (1968): N-Acetyl-β-glucosaminidases in human spleen. *Biochem. J., 107*, 321.

SACHS, B. (1896): A family form of idiocy, generally fatal, associated with early blindness. *J. nerv. ment. Dis., 21*, 475.

SLOME, D. (1933): The genetic basis of amaurotic family idiocy. *J. Genet., 27*, 363.

SVENNERHOLM, L. (1962): The chemical structure of normal human brain and Tay-Sachs ganglioside. *Biochem. biophys. Res. Commun., 9*, 436.

TAY, W. (1881): Symmetrical changes in the region of the yellow spot in each eye of an infant. *Trans. ophthal. Soc. U.K., 1*, 155.

TERRY, R. D. and WEISS, M. (1963): Studies on Tay-Sachs disease. II. Ultrastructure of the cerebrum. *J. Neuropath. exp. Neurol., 22*, 18.

WALLACE, B. J., VOLK, B. W., and LAZARUS, S. S. (1964): Fine structural localization of acid phosphatase activity in neurons of Tay-Sachs disease. *J. Neuropath. exp. Neurol., 23*, 676.

D. A. Hopkinson

M.R.C. Human Biochemical Genetics Unit, The Galton Laboratory,
University College, London, United Kingdom

Genetic aspects of isozymes

The phenomenon of isozyme formation is undoubtedly common and widespread throughout a large number of species, including man. A great many different human enzymes have been studied by zone electrophoresis and multiple molecular forms of most of these enzymes have been found to occur; thus isozymes appear to be the rule rather than the exception.

During the investigation of these various enzymes in man and also from the results of studies carried out on other organisms it has become apparent that isozymes may be generated in a variety of different ways and also that in the case of any one particular enzyme, more than one kind of cause may be operating in the production of the isozymes.

One approach, originally described by Harris (1969), which has been found to be useful in analysing complex isozyme systems and one which is particularly convenient from the genetical point of view is to divide isozymes into three main groups. And this grouping is based on the three main ways in which isozymes may arise.

1. Multiple gene loci Isozymes may result due to the occurrence of two or more separate gene loci, each of which codes for a different polypeptide chain. Perhaps the best known example is lactate dehydrogenase where three distinct loci are concerned in the determination of the multiple isozymes (Markert, 1968). Other similar examples are aldolase (Penhoet *et al.*, 1966, 1967), phosphoglucomutase (Hopkinson and Harris, 1969) and alcohol dehydrogenase (Smith *et al.*, 1971), where in each case at least three distinct loci determine the structures of the various isozymes which may be detected in human tissues. Similarly, two loci are concerned, in each case, in the determination of the isozymes of human amylase (Kamaryt and Laxova, 1965, 1966; Vacikova and Blochova, 1969; De la Lande and Boettcher, 1969), pyruvate kinase (Koler *et al.*, 1968), malate dehydrogenase (Davidson and Cortner, 1967*a*, *b*) and glutamate-oxaloacetate transaminase (Davidson *et al.*, 1970) to name just a few examples.

2. Multiple allelism The occurrence of more than one allele at a particular gene locus is another cause of isozymes. Variant alleles may be relatively rare in the general population or they may be quite common and give rise to genetic polymorphism. Each allele codes for a structurally distinct polypeptide chain and in heterozygotes with two such alleles at the same gene locus the two polypeptides may separately form two isozymes, or they may combine together with each other in different proportions to give a more complex series of isozymes, or they may combine with other polypeptides from other gene loci to form an even more complex series of isozymes.

An important difference between multiple loci and multiple allelism as a cause of isozymes

is that the *former* determines the basic pattern of isozymes common to all members of the species, whereas the *latter* leads to individual differences in isozymes between members of the species.

3. Other causes The third category of causes of isozyme formation is heterogeneous. There appear to be several different processes operating within the cells which can result in two or more isozymes being generated from an enzyme protein. These processes may be divided into three broad subgroups: secondary chemical modification of the enzyme protein occurring within the cell after primary synthesis, the occurrence of enzymatically active conformational isomers, as was first postulated in the case of chicken mitochondrial malate dehydrogenase (Kitto *et al.*, 1966; Kaplan, 1968), and the possibility that isozymes may occur due to aggregation of the enzyme protein molecules to give different multimeric states, e.g. dimers, trimers, tetramers, etc. of different electrophoretic mobilities.

The first subgroup is the most interesting category since isozymes of this kind appear to be quite common and it is possible to envisage many different ways in which secondary chemical modification of an enzyme protein might occur. For example, removal of amide groups from asparagine or glutamine residues, oxidation of reactive sulphydryl groups on cysteine residues, addition of phosphate groups or carbohydrate side chains, partial degradation due to cleavage by proteolytic enzymes and consequent loss of small fragments from the chain could all lead in principle to the generation of multiple 'secondary' isozymes. Such secondary changes may occur gradually, affecting some but not all of the enzyme protein molecules, or they may occur in a stepwise manner thus generating a characteristic set of isozymes, and it seems likely that many examples of isozymes which have been observed in studies of different enzymes simply represent successive early stages in the normal processes by which the enzyme protein is gradually broken down *in vivo*.

Secondary isozymes of this kind can usually be distinguished from their primary isozymes quite easily, but on occasion difficulties occur. In red cell lysates, for example, secondary isozymes are often prominent, presumably a reflexion of the anucleate nature of these cells and the absence of fresh protein synthesis within them. In such circumstances examination of the isozyme patterns in relatively 'young' red cells obtained from patients with reticulocytosis or obtained by density gradient centrifugation may assist in deciding which are primary and which are secondary forms. Also examination of the isozyme patterns in different tissues may help since the secondary isozymes may be less prominent in cells which are turning over relatively rapidly.

A final general point which should not be overlooked is that this method of classifying isozymes is, like any other biological classification, not rigid, and that in the case of any one particular enzyme several different processes may be operating and interacting in the production of the isozymes that are actually observed.

The principal technique which has been used in the study of isozymes is the method of zone electrophoresis, usually in starch or acrylamide gels, together with a specific sensitive stain for the detection of the isozymes after electrophoresis. At first the staining methods available were fairly limited and simple, but gradually during the last ten years or so newer staining methods have been developed and now a very large number of different enzymes can be studied by zone electrophoresis. However, the demonstration of multiple isozymes on an electrophoretic strip is usually only the beginning. The detailed elucidation of the nature of a particular isozyme system often involves the use of several other techniques and a combination of both biochemical and genetical analysis.

The ways in which this may be accomplished vary of course according to the particular isozymes under analysis, but some recent work (Edwards *et al.*, 1971) with the isozymes of human nucleoside phosphorylase provides a useful specific example with which to illustrate a general method for the analysis of complex isozyme systems and also shows the value of the method of classification of isozymes given above.

NUCLEOSIDE PHOSPHORYLASE

When red cell haemolysates are examined electrophoretically for nucleoside phosphorylase (NP) a series of seven or eight NP isozymes are seen (Fig. 1) and it is not immediately obvious from the appearance of this multibanded pattern in different individuals whether the isozymes are determined by several loci or by several alleles or whether the isozymes are 'secondary' forms. Some clues which give information may be obtained, however, by testing other tissues. In extracts of liver and kidney for example (Fig. 1) the isozyme patterns are

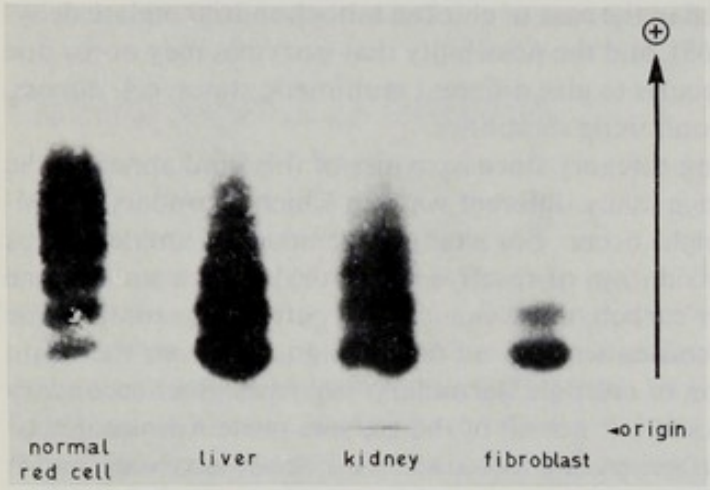


Fig. 1. Photograph of starch gel showing the NP isozyme patterns of adult red cells, liver, kidney and cultured fibroblasts.

somewhat simpler; usually only four or five isozymes are observed, having the same electrophoretic mobilities as the four or five slowest isozymes of the red cell pattern. Also, the distribution of the enzyme activity among the isozymes is different from that exhibited by red cells since the least anodal isozyme is much the most intense in staining activity and the others show progressively decreasing intensity with increasing anodal mobility. Similar isozyme patterns were obtained using extracts of other tissues including extracts of white cells. In extracts of cultured fibroblasts grown in tissue culture an even simpler pattern is observed with a single isozyme corresponding to the least anodal isozyme of the red cell pattern accounting for virtually all of the nucleoside phosphorylase activity, though much weaker isozymes with mobilities corresponding to the next one or two in the series are also sometimes observed.

These findings suggest that the least anodal NP isozyme is the primary form of the enzyme synthesised in all cells and the multiple series of discrete more rapidly moving isozymes, which are observed in varying amounts in different tissues, represent secondary forms of the enzyme protein which are generated in a stepwise manner from the primary form and from one another as the enzyme protein 'ages'. Red cells, in which the more anodal isozymes are the most prominent, have on average a long life span and lose their nuclei and presumably cease enzyme synthesis at an early stage. The cells of other tissues, where the least anodal isozyme is relatively much more active, are nucleated and presumably carry on some enzyme synthesis throughout their life span; thus the primary isozyme is constantly replenished. Cultured fibroblasts are dividing rapidly and very actively synthesising new enzyme protein up until the time they are harvested, and in extracts of these cells the least anodal isozyme (the postulated primary isozyme) is much the most active form present and the more anodal (postulated secondary isozymes) are relatively very weak.

This hypothesis was tested by examining red cell samples from individuals with chronic haemolytic disease or other similar conditions, which have a greater proportion of 'young'

red cells and reticulocytes than normal blood samples. In such cases the slower moving NP isozymes were found to be relatively much more active than in normal individuals and the faster moving isozymes were relatively weaker. Also when 'old' and 'young' red cell samples prepared from normal blood by density gradient centrifugation were compared, the more anodal isozymes were relatively more prominent in the 'old' cell samples and the less anodal isozymes were relatively more prominent in the 'young' cell samples, the least anodal isozyme being the most active.

Thus, there seems to be a direct correlation between the relative intensities of the various nucleoside phosphorylase isozymes and cell 'age'.

Associated with the changing electrophoretic properties of nucleoside phosphorylase there is also evidence that the kinetic properties of the enzyme alter as it 'ages'. This has been deduced from kinetic studies recently carried out (Turner *et al.*, 1971) with nucleoside phosphorylase from red cells and from cultured lymphocytoid cells.

The kinetic profile of nucleoside phosphorylase from red cell haemolysates is shown in Figure 2 in the form of a double reciprocal Lineweaver-Burke plot. The graph is linear at low inosine concentrations, but curves downwards at inosine concentrations greater than about 0.2 mM suggesting that the nucleoside phosphorylase in red cells consists of two or more kinetically different forms: one form with a low Michaelis constant (K_m) mainly active at low substrate concentration and one or more forms with a high Michaelis constant (K_m),

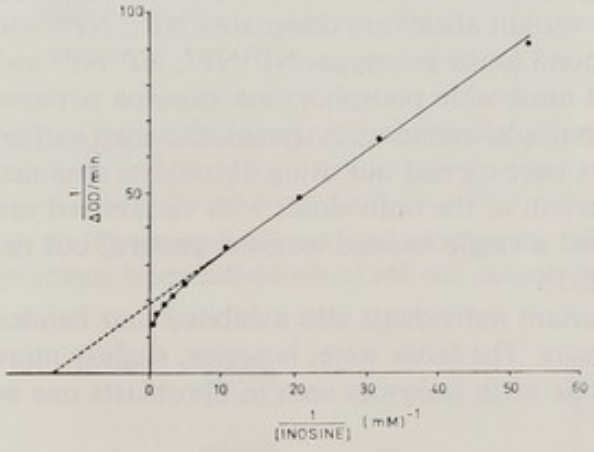


Fig. 2. Plot of reciprocal initial reaction velocity (OD change per minute) against reciprocal inosine concentration (millimolar) for nucleoside phosphorylase in an unfractionated red cell lysate.

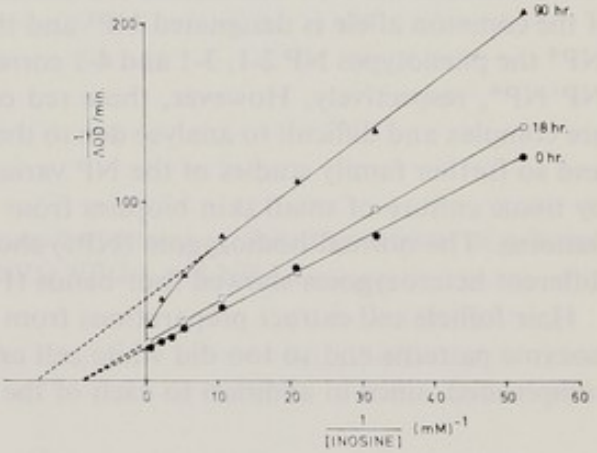


Fig. 3. Plots of reciprocal initial reaction velocity (OD change per minute) against reciprocal inosine concentration (millimolar) for nucleoside phosphorylase in an extract of cultured human lymphocytoid cells stored at 4°C for 0 (●–●), 18 (○–○) and 90 (▲–▲) hours.

active at high substrate concentrations and responsible for the curvature on the kinetic plot shown.

In contrast, the kinetic profile of nucleoside phosphorylase from fresh preparations of cultured lymphocytes is a straight line - essentially similar to the linear portion of the red cell curve (Fig. 3) suggesting the occurrence of the low K_m form only in these cells. This finding correlates with the electrophoretic pattern of NP in fresh cultured lymphocytes which consists of a single main isozyme – a pattern very similar to that seen in fresh extract of cultured fibroblasts. When these lymphocyte cell preparations were aged artificially, however, by allowing them to stand in the refrigerator at 4 °C the kinetic profile changed gradually until it came

to resemble the profile exhibited by red cell haemolysates with the characteristic curve at inosine concentrations of >0.2 mM. Associated with this was a progressive change in the electrophoretic pattern of the NP isozymes from the single banded pattern seen in the fresh cells to a multibanded pattern in the aged cells, very similar in appearance to red cell haemolysate NP.

These results indicate that the least anodal (*i.e.* primary) isozyme of NP has a linear kinetic profile and a relatively low Michaelis constant and that the more anodal (secondary) isozymes are kinetically different, are responsible for the curved portion of the kinetic profile and have a relatively high Michaelis constant. This has been tested directly by investigating the kinetic properties of the separate isozymes obtained by ion exchange chromatography of red cell lysates and the results obtained are in agreement with the findings shown here.

Thus, the evidence derived from the electrophoretic and kinetic studies on NP suggests that the multiple isozymes of nucleoside phosphorylase seen in most blood samples are secondary isozymes and are not due to multiple loci or multiple allelism.

Evidence for the occurrence of multiple allelism in the NP isozyme system was, however, obtained during the electrophoretic study, since in a population survey of red cell haemolysates three different variant NP isozyme patterns were discovered. These variants are shown in Figure 4 together with the usual pattern (NP1) which has been discussed earlier. Family studies carried out using red cell samples showed that these complex variant isozyme patterns were genetically determined and the pedigrees were consistent with the simple hypothesis that each of the variant phenotypes is heterozygous for a different variant allele at an autosomal locus and that the NP1 individuals are homozygous for a common allele at this locus. Thus, if the common allele is designated NP^1 and the variant alleles are designated NP^2, NP^3 and NP^4 the phenotypes NP 2-1, 3-1 and 4-1 correspond to the genotypes NP^1NP^2, NP^1NP^3 and NP^1NP^4, respectively. However, these red cell nucleoside phosphorylase isozyme patterns are complex and difficult to analyse due to the multiple secondary isozymes discussed earlier, and so further family studies of the NP variants were carried out using fibroblasts obtained by tissue culture of small skin biopsies from certain of the individuals with variant red cell patterns. The normal homozygote (NP1) showed a single banded isozyme pattern, but the different heterozygotes showed four bands (Fig. 5).

Hair follicle cell extract preparations from variant individuals also exhibited four banded isozyme patterns and so too did white cell extracts. The latter were, however, slightly more complicated, since in addition to each of the four main isozymes seen in fibroblasts one or

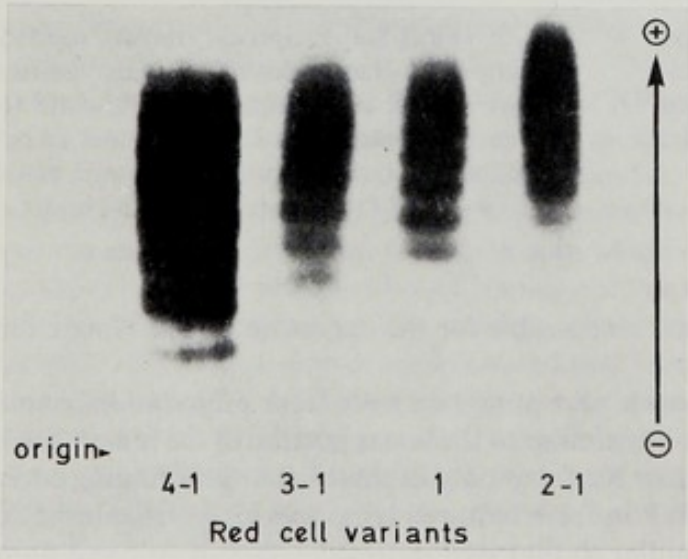


Fig. 4. Photograph of starch gel showing the red cell NP isozyme patterns in the common phenotype NP 1 and the rare variant phenotypes NP 2-1, NP 3-1 and NP 4-1.

two weak minor, presumably secondary, isozymes could also be seen just anodal to each of the four main zones.

The occurrence of these four banded isozyme patterns in the fibroblasts, white cells and hair follicle cell extracts of these individuals suggests that nucleoside phosphorylase is a trimeric molecule and that in normal homozygotes the primary form synthesised contains three identical polypeptide subunits. If this is so the two outer isozymes of the heterozygous patterns would represent the homogeneous products of two separate alleles, the variant allele and the common allele; the two inner isozymes would represent hybrid forms containing both types of subunit but in different combinations.

This interpretation, which is illustrated in Figure 6, could also explain the observed differences in the relative staining intensities of the four isozymes in the heterozygous pattern. If equal amounts of the two subunits are present in such cases and if the subunits have the same specific activity then one would expect that the hybrid isozymes would occur three times more often than the isozymes consisting of like subunits, so that the relative activities of the four isozymes would be in the ratio 1:3:3:1 and this is roughly what is observed.

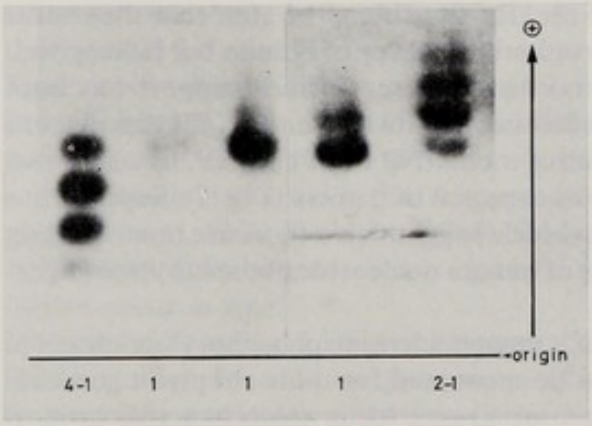


Fig. 5. Photograph of starch gel showing the NP isozyme patterns exhibited by extracts of cultured fibroblasts from individuals of red cell phenotype NP1, NP 2-1 and NP 4-1.

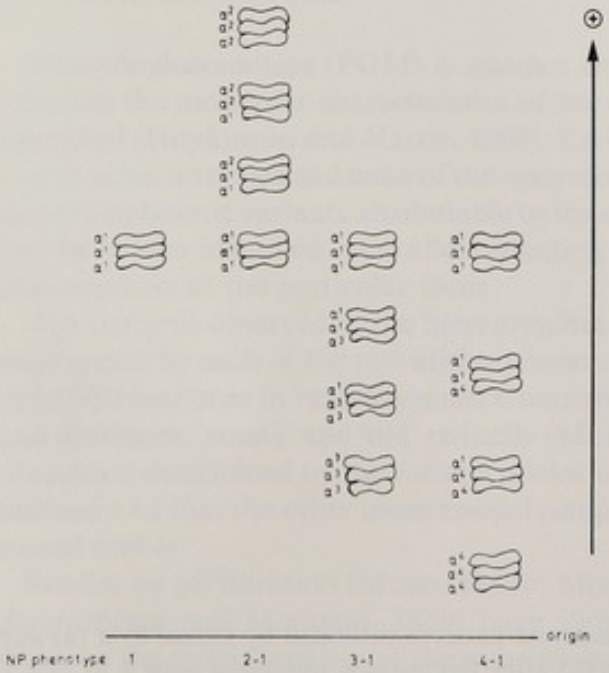


Fig. 6. Diagram illustrating the postulated subunit composition of the principal isozymes of the NP 1, NP 2-1, NP 3-1 and NP 4-1 phenotypes.

The four banded isozyme patterns of the fibroblasts also provide a retrospective explanation for the complex red cell isozyme patterns seen in the variants, since the red cell patterns appear to be made up of the four 'primary' isozymes seen in the fibroblasts and also four series of 'secondary' isozymes derived from each of them.

In the homozygous type NP1 red cell lysates at least six secondary isozymes can be recognised in addition to the primary isozyme, thus one might expect to find as many as 28 isozymes in red cell lysates from individuals with the variant heterozygous patterns. In practice about 15 bands were identified in the red cell NP 4-1 pattern and about 11 in the NP 2-1 and 3-1 patterns. However, one would not expect to observe the full complement of isozymes because some, although being different in structure, may have similar mobilities and also the weaker isozymes of each set would be difficult to detect as separate bands.

The possibility that human NP is a trimer was first suggested by electrophoretic studies carried out by Dr. P. S. Gerald and his colleagues (1971) on human-mouse somatic cell hybrids grown in tissue culture in which four banded isozyme patterns were obtained. The two outer isozymes corresponded to the single isozymes seen separately in the cell lines from which the hybrids were derived, while the two inner isozymes were peculiar to the hybrid cells. Also the relative activities of the isozymes were roughly 1:3:3:1; thus it can be seen that the results of these experiments and the results of the electrophoretic survey of human NP fit very well together and provide convincing evidence that NP is a trimer. Further support has been obtained subsequently by *in vitro* hybridisation experiments (Edwards *et al.*, 1971) carried out using preparations of human NP and preparations of mouse NP or of calf NP, in which two new isozymic forms were generated with properties expected of trimers (Fig. 7). Support has also come from independent biochemical studies which suggests that there are three binding sites for the substrate, hypoxanthine, per molecule of human nucleoside phosphorylase (Agarwal and Parks, 1969).

Thus, the complexities of the isozyme patterns of human nucleoside phosphorylase observed in various tissues and in different individuals can be accounted for quite simply in genetical terms. This enzyme is determined by a single autosomal gene locus which is active in most tissues and two distinct types of isozyme can be recognised: isozymes due to allelic variation and isozymes due to secondary modification of the primary isozymic form(s) of NP.

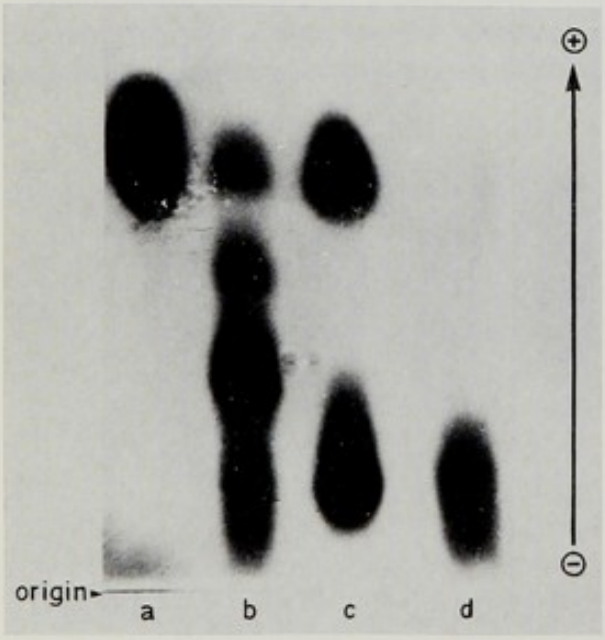


Fig. 7. Photograph of starch gel showing the NP isozyme patterns exhibited by mouse liver (a) and human liver (d) and a mixture of these two tissues (b) after freeze-thaw treatment with 2 M NaCl. Sample (c) is the control mixture which was frozen and thawed in the absence of NaCl. Electrophoresis carried out with phosphate buffer system, pH 6.5.

MULTIPLE GENE LOCI

Where more than one gene locus is involved in the determination of a particular enzyme the isozyme patterns are usually correspondingly more complex than if just one locus is concerned. The polypeptide products of the different loci may separately form the various members of a set of isozymes *or* the different polypeptides may combine together perhaps in different proportions to form a rather more complex series of isozymes. Also there may be marked differences between the cells of different tissues in the quantities of the polypeptide products of the different loci.

1. LACTATE DEHYDROGENASE

In the case of lactate dehydrogenase (LDH) there appear to be three loci (A, B and C) which code for structurally distinct polypeptide subunits of the isozymes, which are themselves tetrameric (Markert, 1968). The products of the A and the B loci are formed in most tissues and give rise by their different combinations to a set of five isozymes with the subunit structures A_4, A_3B, A_2B_2, AB_3 and B_4.

However, the relative amounts of the five isozymes vary considerably from tissue to tissue. This may be due to differences in the relative rates of synthesis of the A and B subunits from one cell type to another and also differences in the rate of degradation of the isozymic products may be important (Fritz *et al.*, 1969).

The C locus is unusual in that it appears to be active only in primary spermatocytes (Bianco and Zinkham, 1963; Zinkham, 1968). Isozymic tetramers containing C subunits as well as A or B subunits can be generated *in vivo* by dissociation and recombination techniques but they do not occur *in vivo*.

Several rare variant LDH isozyme patterns attributed to rare alleles at the A or B loci have been identified. In each case quite complex isozyme patterns were observed, but it was possible to assign the particular mutant to either the A or the B locus with confidence (Boyer *et al.*, 1963; Nance *et al.*, 1963; Kraus and Neely, 1964; Davidson *et al.*, 1965; Vesell, 1965; Mourant *et al.*, 1968; Blake *et al.*, 1969; Das *et al.*, 1970).

2. PHOSPHOGLUCOMUTASE

Phosphoglucomutase (PGM) is another enzyme where three distinct structural gene loci affecting the molecular characteristics of the different PGM isozyme components have been identified (Hopkinson and Harris, 1969). Each PGM locus determines a characteristic set of two or more isozymes and none of the isozymes observed appear to represent hybrids. A series of electrophoretic variants attributable to the occurrence of multiple alleles of each of the three loci have been identified, each allele resulting in the appearance of an altered set of isozymes characteristic of the particular locus.

The patterns observed in the heterozygotes correspond to mixtures of the sets of isozymes determined by each of the two alleles present. There is no evidence to suggest the occurrence of hybrid isozymes in heterozygotes. Comparisons of the isozyme patterns in different tissues and studies on 'young' and 'old' red cells (Monn, 1969*a*) suggest that the least anodal isozyme of each set determined by the various alleles at each of the three loci is the primary form synthesised and that the other more anodal components are 'secondary' isozymes of the kind discussed earlier.

Studies by gel filtration (Monn, 1969*b*; McAlpine *et al.*, 1970*a*) and by ultracentrifugation (Santachiara and Modiano, 1969) have shown that the multiple isozymes determined by alleles at the same locus are of the same molecular size. However, there are significant differences in molecular size between the isozymes of the different loci. The PGM_2 isozymes are 15–20% larger than the PGM_1 and PGM_3 isozymes.

The PGM_2 isozymes are also different in their thermostability characteristics from the PGM_1 and PGM_3 isozymes (McAlpine *et al.*, 1970*b*). The isozymes determined by each of the two common alleles at the PGM_1 locus are more thermolabile than those determined by the common allele of PGM_2. Also the isozymes determined by each of the common alleles at PGM_3 are more labile than those determined by the common alleles at PGM_1.

A further point of some interest is the apparent variation in the relative activities of the isozymes from the three loci in different tissues (McAlpine *et al.*, 1970*c*).

In most tissues the PGM_1 isozymes predominate and account for 90–95% of the total activity; the PGM_2 isozymes account for about 5–10% and usually less than 2% of the total activity is contributed by PGM_3. The much greater contribution to the total activity of the PGM_1 isozymes than the PGM_2 and PGM_3 isozymes in most tissues is presumably attributable to differences in the relative rates of synthesis, but there is also the possibility that the different sets of isozymes differ markedly in specific activity. In red cells, however, only about 50% of the total activity is due to the PGM_1 locus and the rest is contributed by PGM_2. No PGM_3 isozymes occur. This unusual distribution of PGM isozyme activity is probably related to the stability differences between the three sets of isozymes and the anucleate state of the mature red cells. Weak PGM_3 isozyme activity has been found in 'young' red cells and reticulocytes, but presumably because of their apparent instability this set of isozymes disappears as the red cells age. Similarly, relatively large contribution of the PGM_2 isozymes to the total activity in red cells is probably related to their apparently greater stability.

CONCLUSIONS

It is evident from the brief discussion of just one complex isozyme system and the sprinkling of examples which have been merely mentioned that the multiple isozymes of any one enzyme that may occur in any one particular individual can be generated in several different ways. Also in the case of any one enzyme several different processes may be operating to produce isozymes.

First, multiple loci leading to the occurrence of multiple isozymes appear to be quite common. It seems from a survey of the recent literature, that out of about 50 complex isozyme systems analysed so far in human tissues, about a quarter are the products of two or more separate loci. The different loci may give rise to separate sets of isozymes or the polypeptide products may combine to give hybrid isozymes, although it should be noted that the failure to detect hybrid isozymes by electrophoresis does not preclude their existence *in vivo*. Quite often the tissue distribution of the isozymes attributable to different loci varies considerably and this presumably reflects the different metabolic roles of these multiple forms of the same enzyme activity, though in certain cases it may reflect differences in the stability characteristics. Clearly it would be of interest to determine exactly how isozymes determined by different loci differ in their functional activities and also what processes are responsible for the particular tissue distribution of isozymes which may be observed.

An important practical point is that the polypeptide products of alleles at the same locus are likely to differ by only single aminoacid substitutions, whereas polypeptides coded at separate loci are likely to differ at quite a number of residues along the chain. Thus, in general, isozymes coded at the same locus tend to resemble one another in their biochemical and physical properties, whereas isozymes coded at separate loci tend to differ considerably in their properties and this was illustrated by the recent work on the PGM isozymes. In practice such differences are useful in unravelling complex isozyme systems particularly where formal genetic analysis using segregation data from pedigrees is not available (*e.g.* human alcohol dehydrogenase – Smith *et al.*, 1971).

The occurrence of multiple isozymes due to allelic variation is well established. A large number of loci have now been screened by electrophoresis of blood, placentae and other tis-

sue samples and in many cases several different alleles resulting in electrophoretically distinct variants of the same enzyme have been found. Some of these alleles are very rare, but at a significant proportion of loci coding for enzyme structure there are at least two alleles each occurring with an appreciable frequency (>0.01) and thus giving rise to genetic polymorphism.

Putting together the data from several different centres (Hopkinson and Harris, 1971) it was found that out of 56 loci studied in detail by electrophoresis, structural enzyme variants were detected in 37 cases (*i.e.* 66%). At 18 of these loci at least two alleles with frequencies >0.01 have been found to occur in one or more major human population. At 19 loci no certain variants have been reported so far, but it should be noted that at many of these loci the numbers of individuals screened were often relatively small (<500–1000) and also in some cases isozyme discrimination by electrophoresis was limited by technical problems.

Finally, isozymes may arise due to secondary modification of the enzyme proteins subsequent to their primary synthesis. Isozymes of this kind are ubiquitous but probably heterogeneous in origin and require special attention in genetic studies. In particular it is clearly essential to distinguish variation in secondary isozyme formation from genetic variation, but it should also be appreciated that secondary isozymes may occasionally prove helpful in detecting and characterising particular genetic variants. For example, unusual secondary isozyme patterns have led to the recognition of at least two variants – a unique peptidase variant Pep A 5–1 (Lewis *et al.*, 1968; Sinha and Hopkinson, 1969) and a unique phosphohexose isomerase variant PHI 5–1 (Hopkinson, 1970) – and have also aided in the characterisation of a G6PD variant, Gd Tel Hashomer (Kirkman *et al.*, 1969).

REFERENCES

Agarwal, R. P. and Parks, R. E. (1969): Purine nucleoside phosphorylase from human erythrocytes. IV. Crystallisation and some properties. *J. biol. Chem., 244*, 644.

Bianco, A. and Zinkham, W. H. (1963): Lactate dehydrogenases in human testes. *Science, 139*, 601.

Blake, N. M., Kirk, R. L., Pryke, E. and Sinnett, P. (1969): Lactate dehydrogenase electrophoretic variant in a New Guinea Highland population. *Science, 163*, 701.

Boyer, S. H., Fainer, D. C. and Watson-Williams, E. J. (1963): Lactate dehydrogenase variant from human blood: evidence for molecular subunits. *Science, 141*, 642.

Das, S. R., Mukherjee, B. N., Das, S. K., Ananthakrishnan, R., Blake, N. M. and Kirk, R. L. (1970): LDH variants in India. *Humangenetik, 9*, 107.

Davidson, R. G. and Cortner, J. A. (1967*a*): Genetic variant of human erythrocyte malate dehydrogenase. *Nature (Lond.), 215*, 762.

Davidson, R. G. and Cortner, J. A. (1967*b*): Mitochondrial malate dehydrogenase: a new genetic polymorphism in man. *Science, 157*, 1569.

Davidson, R. G., Cortner, J. A. and Rattazzi, M. C. (1970): Genetic polymorphisms of human mitochondrial glutamic oxaloacetic transaminase. *Science, 169*, 3943.

Davidson, R. G., Fildes, R. A., Glen-Bott, A. M., Harris, H., Robson, E. B. and Cleghorn, T. E. (1965): Genetical studies on a variant of human lactate dehydrogenase (subunit A). *Ann. hum. Genet., 29*, 5.

De la Lande, F. A. and Boettcher, B. (1969): Electrophoretic examination of human serum amylase isoenzymes. *Enzymologia, 37*, 335.

Edwards, Y. H., Hopkinson, D. A. and Harris, H. (1971): Inherited variants of human nucleoside phosphorylase. *Ann. hum. Genet., 34*, 395.

Fritz, P. J., Besell, E. S., White, E. L. and Pruitt, K. M. (1969): The roles of synthesis and degradation in determining tissue concentrations of lactate dehydrogenase-5. *Proc. nat. Acad. Sci. USA, 62*, 558.

Gerald, P. S. (1971): Cited by Edwards *et al.* (1971).

Harris, H. (1969): Genes and isozymes. (Review lecture). *Proc. roy. Soc. B, 174*, 1.

Hopkinson, D. A. (1970): The investigation of reactive sulphydryls in enzymes and their variants by

starch gel electrophoresis: studies on the human phosphohexose isomerase variant PHI 5–1. *Ann. hum. Genet., 34*, 79.

HOPKINSON, D. A. and HARRIS, H. (1969): Red cell acid phosphatase, phosphoglucomutase and adenylate kinase. In: *Biochemical Methods in Red Cell Genetics*, p. 337. Editor: J. J. Yunis. Academic Press, New York, N.Y.

HOPKINSON, D. A. and HARRIS, H. (1971): Recent work on isozymes in man. *Ann. Rev. Genet., 5*,5.

KAMARYT, J. and LAXOVA, R. (1965): Amylase heterogeneity. Some genetic and clinical aspects. *Humangenetik, 1*, 579.

KAMARYT, J. and LAXOVA, R. (1966): Amylase variants in man. *Humangenetik, 3*, 41.

KAPLAN, N. O. (1968): Nature of multiple molecular forms of enzymes. *Ann. N.Y. Acad. Sci., 151*, 382.

KIRKMAN, H. N., RAMOT, B. and LEE, J. T. (1969): Altered aggregational properties in genetic variant of human glucose 6-phosphate dehydrogenase. *Biochem. Genet., 3*, 137.

KITTO, G. B., WASSARMAN, P. G. and KAPLAN, N. O. (1966): Enzymatically active conformers of mitochondrial malate dehydrogenase. *Proc. nat. Acad. Sci. USA, 56*, 578.

KRAUS, A. P. and NEELY, C. L., JR (1964): Human erythrocyte lactate dehydrogenase: four genetically determined variants. *Science, 145*, 595.

KOLER, R. D., BIGLEY, R. H. and STENZEL, P. (1968): Biochemical properties of human erythrocyte and leucocyte pyruvate kinase. In: *Hereditary Disorders of Erythrocyte Metabolism*, p. 249. Editor: E. Beutler. Grune and Stratton, New York, N.Y.

LEWIS, W. H. P., CORNEY, G. and HARRIS, H. (1968): Pep A 5–1 and Pep A 6–1: two new variants of peptidase A with features of special interest. *Ann. hum. Genet., 32*, 35.

MARKERT, C. L. (1968): The molecular basis for isoenzymes *Ann. N. Y. Acad. Sci., 151*, 14.

MCALPINE, P. J., HOPKINSON, D. A. and HARRIS, H. (1970*a*): Molecular size estimates of the human phosphoglucomutase isozymes by gel filtration chromatography. *Ann. hum. Genet., 34*, 177.

MCALPINE, P. J., HOPKINSON, D. A. and HARRIS, H. (1970*b*): Thermostability studies on the isozymes of human phosphoglucomutase. *Ann. hum. Genet., 34*, 61.

MCALPINE, P. J., HOPKINSON, D. A. and HARRIS, H. (1970*c*): The relative activities attributable to the three phosphoglucomutase loci (PGM_1, PGM_2, PGM_3) in human tissues. *Ann. hum. Genet., 34*, 169.

MONN, E. (1969*a*): Relation between blood cell phosphoglucomutase isoenzymes and age of cell population. *Scand. J. Haemat., 6*, 133.

MONN, E. (1969*b*): Chromatographic studies on human red cell phosphoglucomutase. *Protein Res., 1*, 1.

MOURANT, A. E., BECKMAN, L., BECKMAN, G., NILSSON, L. O. and TILLS, D. (1968): Erythrocyte lactate dehydrogenase variants in the Icelandic and Swedish populations. *Acta genet. (Basel), 18*, 553.

NANCE, W. E., CLAFLIN, A. and SMITHIES, O. (1963): Lactic dehydrogenase: genetic control in man. *Science, 142*, 1075.

PENHOET, E. E., KOCHMAN, M., VALENTINE, R. and RUTTER, W. J. (1967): The subunit structure of mammalian fructose diphosphate aldolase. *Biochemistry (Wash.), 6*, 2940.

PENHOET, E. E., RAJKUMAR, T. V. and RUTTER, W. J. (1966): Multiple forms of fructose diphosphate aldolase in mammalian tissues. *Proc. nat. Acad. Sci. USA, 56*, 1275.

SANTACHIARA, A. S. B. and MODIANO, G. (1969): Ultracentrifuge studies of red cell phosphoglucomutase. *Nature (Lond.), 223*, 625.

SINHA, K. P. and HOPKINSON, D. A. (1969): The investigation of reactive sulphydryls in enzymes and their variants by starch gel electrophoresis. *Ann. hum. Genet., 33*, 139.

SMITH, M., HOPKINSON, D. A. and HARRIS, H. (1971): Developmental changes and polymorphism in human alcohol dehydrogenase. *Ann. hum. Genet., 34*, 251.

TURNER, B. M., FISHER, R. A. and HARRIS, H. (1971): An association between the kinetic and electrophoretic properties of human purine nucleoside phosphorylase isozymes. *Eur. J. Biochem., 24*, 288.

VACIKOVA, A. and BLOCHOVA, L. (1969): Isoamylases in blood donors. *Humangenetik, 8*, 162.

VESELL, E. S. (1965): Genetic control of isozyme patterns in human tissues. In: *Progress in Medical Genetics*. Editors: A. G. Steinberg and A. G. Bearn. Grune and Stratton, New York, N.Y.

ZINKHAM, W. H. (1968): Lactate dehydrogenase isozymes of testis and sperm: biological and biochemical properties and genetic control. *Ann. N. Y. Acad. Sci., 151*, 598.

Chapter VII Immunogenetics

R. R. Race

Medical Research Council Blood Group Unit, The Lister Institute, London, United Kingdom

Is the Xg blood group locus subject to inactivation?

Late in 1961 when the Xg groups disclosed themselves many people hoped they would give clear confirmation of Lyon's theory which was then not so well established as it now is: but Xg did not at first give a clear answer. Then, as the truth of the theory became more and more firmly established, the contribution of Xg became limited to the question whether inactivation involves all the loci on the X. Each successive piece of evidence from Xg had, however, to be qualified because of the theoretical possibility that the antigen Xg^a, unlike nearly all other red cell antigens, might be merely adsorbed on the red cell surface.

If the antigen Xg^a were not made by the ancestors of the red cells but were made elsewhere and secondarily taken up by the red cells, then Xg could give no information about lyonization because random inactivation of Xs in an Xg^a heterozygote would presumably allow sufficient antigen to be made to supply all the red cells. There was never any evidence for such a theoretical Xg^a factory separated from the red cell line, but its non-existence took a long time to prove. The factory idea would be disproved if two populations of red cells, Xg(a+) and Xg(a−), could be found in one circulation – the two kinds of cells launched from one marrow. This we had sought for some years in twin chimeras and cases of dispermy without success, though in 1968 we were encouraged by the results of tests on a case of dispermy being investigated by Professor Ceppellini and Professor Carbonara of Turin.

After separation, one population of cells gave the reaction of Xg(a+) and the other of Xg(a−). We were, however, not absolutely confident about our results since the separations had to be done with the not very avid antibodies of the MNSs system.

However, after my summary was submitted, a paper by Professor Ducos and his colleagues at Toulouse (1970) came out which described a chimera twin pair whose two populations of red cells were easily separable because of differences in their ABO groups. The blood of each twin was a mixture of A_1B, Xg(a−) and O, Xg(a+) cells. This finding of two Xg populations in one circulation seemed so important that we thought it should be confirmed in a second laboratory and, through the kindness of Prof. Ducos and Dr. Kling of Metz, we were given a sample from the male twin and, as expected, confirmed the results of Professor Ducos and his colleagues (1971). So the important point is now established that Xg^a is made in the red cell precursors and therefore, as had been assumed all along by most people, is indeed capable of giving evidence about X-inactivation.

If the locus were involved in inactivation then, on the average, half the cells of heterozygous women should be agglutinated by anti-Xg^a and half not. But the answer was not crisp, for by the normal test there were indeed unagglutinated cells in the heterozygote but they were to be seen also in the Xg(a+) hemizygote. However, Gorman and his colleagues (1963) devised a technique which failed to show a mixture of Xg(a+) and Xg(a−) cells in known heterozygous Xg^aXg females even though the technique easily detected artificial 50:50 mix-

tures of hemizygous Xg(a+) and Xg(a−) cells; this observation, which we were able to confirm in our Unit, was the first indication that the *Xg* locus was not concerned in the inactivation.

Another approach involved the number of cells present in the embryo at the time of inactivation. Gandini and his collaborators (1968), from a study of women known to be heterozygous at the locus for g-6-pd, estimated that at the time of inactivation the primordial cells destined to form the erythroid series number '8 or less'. If the *Xg* locus were subject to inactivation then there should be a slight excess of Xg(a−) females in adequately large random series when analysed by the gene frequencies derived from the males of that series, because some heterozygotes would be grouped as negative. In a sample of over 3000 unrelated females tested in our Unit (Sanger *et al.*, 1971*a*) there was, on the contrary, a slight shortage of Xg(a−). This was somewhat incompatible with inactivation at an 8-cell stage and completely incompatible with it happening at, for example, a 4-cell stage. So there was some further evidence against the inactivation of *Xg*.

In testing over 1300 normal families we found four in which an Xg(a−) mother had an Xg(a+) son, and a fifth was found in Canada. Some writers have hailed such Xg(a−) mothers as $Xg^{a}Xg$ heterozygotes whose Xg^{a} alleles had by chance been almost totally inactivated. However, the latest example (Buckton *et al.*, 1971*a*) to be found had a clear explanation. The mother was a mosaic: two thirds of her lymphocytes were 46,XX and the rest 45,X but when her marrow came to be tested it was over 90% 45,X. The children showed her genotype to be heterozygous $Xg^{a}Xg$, though her red cells were Xg(a−). It remains to be seen whether mosaicism is the explanation also of the other four families in which Xg(a−) mothers have Xg(a+) sons – my guess is that the most likely background to the previous four families is neither 45,X mosaicism nor lopsided inactivation, but some cytological or immunological event yet to be identified.

Further evidence against the inactivation of *Xg* came from the Xg grouping of two series of women with chronic myeloid leukaemia carried out by Fialkow and his colleagues (1970) in Seattle and by Lawler and Sanger (1970) in London. It appears to be accepted that the marrow cells of these patients are clonal, being descended from a single stem cell. Assuming that this is correct then, if *Xg* were subject to inactivation, females with the disease should have the male distribution of the Xg groups because they would each have, in all their red cells, the product of one and the same functional *Xg* locus. But in both series the Xg distribution was female and differed significantly from that of the male. This we consider very powerful evidence against the inactivation of the *Xg* locus.

Another piece of evidence against the inactivation of *Xg* was gathered by Fialkow (1970) from the literature about the Lesch-Nyhan X-linked disease. Heterozygous carriers can be detected by an intermediate amount of the enzyme called HGPRTase in their fibroblasts, but their red cells have the normal enzyme activity. It is thought that there is selective inactivation in the marrow of the X carrying the abnormal gene for the lack of the enzyme. Three *Xg* heterozygotes were found who by pedigree evidence had Xg^{a} only on the inactivated chromosome, nevertheless they had Xg^{a} antigen on their red cells.

Further but more direct evidence is illustrated by a remarkable family investigated by Buckton and her colleagues (1971*b*) which almost certainly gives direct evidence that the allele Xg^{a} when carried on an inactive late-labelling X can nevertheless produce its antigen. A mother and daughter have a balanced translocation t(Xp−;14q+), the short arm of one X being translocated onto the long arm of a No. 14. In both these female members of the family who have the translocation balanced it is the normal X which is late-labelling. The mother is Xg(a−); the father is Xg(a+) and his X is late-labelling in his daughter – yet it has made her Xg(a+). I said this family 'almost certainly' gives direct evidence against inactivation of *Xg*; the 'almost' was put in because, as Buckton and her colleagues explain in their paper, an unknown and purely theoretical phenomenon can be invented that would allow the mother to have an Xg^{a} allele which would negate this, as I see it, very convincing demonstration.

In 1968 Lee and his colleagues at Salt Lake City produced evidence, which received a lot of attention, appearing to show that the *Xg* locus was subject to inactivation. The evidence was based on the Xg groups of the separated parts of two morphologically distinct populations of cells in two sisters heterozygous for X-linked sideroachrestic anaemia. However, Weatherall and his colleagues (1970) testing a similar family could not confirm the Salt Lake City results which they considered in any case to contain internal inconsistency: we think the trouble had been due to technical difficulties of Xg grouping.

So we are convinced that the *Xg* locus when carried on a normal X does escape inactivation.

Now to turn to the question whether the *Xg* locus is subject to inactivation when carried on a structurally abnormal X. We have tested four families of the following kind: father Xg(a+), mother Xg(a+), XXqi daughter Xg(a−); and the simplest interpretation seemed to be that the short arm of the daughter's paternal Xs had gone astray taking with them the locus *Xg* carrying the allele Xg^a. That was to say the *Xg* locus is on the short arm. But then Professor Polani and his colleagues (1970) found a family of this kind: father Xg(a+), mother Xg(a+), XXq− daughter Xg(a−) and, by the same argument, the *Xg* locus is on the long arm. (In none of the five daughters was any sign of 45,X mosaicism detected.)

One way out of this paradox was to suppose that the *Xg* locus when carried on a deleted late-labelling X is subject to inactivation. Inactivation would explain the unquestionably male Xg distribution of people lacking either the short or the long arm of an X who show no sign of 45,X mosaicism. We have tested 44 such propositi lacking a short arm and 10 lacking a long arm and the Xg distribution in both groups is male and differs very significantly from the female (Sanger *et al.*, 1971*b*). It might be wondered whether most propositi with deleted Xs have, or develop, a 45,X line, even though unrecognized, and that 45,X is favoured in the marrow, as it was in the propositus mentioned above. If this were so, deleted Xs would give no information about inactivation or about *Xg* location on the long or the short arm. However, we do not think that 45,X cells are preferentially favoured in the marrow because 46,XX/45,X mosaics, of whom we have tested about 50, have a female distribution of the Xg groups, and not the distribution expected of 45,X marrows (Sanger *et al.*, 1971*b*). So we think Xg is inactivated when carried on a deleted late-labelling X.

It is not very tidy to conclude that *Xg* is not inactivated on a normal X but is inactivated on a deleted X. Perhaps the inactivation process of a normal X differs from that of a deleted X; I do not know.

REFERENCES

Buckton, K. E., Cunningham, C., Newton, M. S., O'Riordan, M. L. and Sanger, R. (1971*a*): Anomalous Xg inheritance with a probable explanation. *Lancet, i/7695*, 371.

Buckton, K. E., Jacobs, P. A., Rae, L. A., Newton, M. S. and Sanger, R. (1971*b*): An inherited X-autosome translocation in man. *Ann. hum. Genet., 35/2*, 171.

Ducos, J., Colombies, P., Marty, Y., Blanc, M., Daver, J. and Emond, J. (1970): Double population cellulaire chez deux jumeaux hétéro-caryotes. *Rev. franç. Transfus., 13/3*, 261.

Ducos, J., Marty, Y., Sanger, R. and Race, R. R. (1971): Xg and X chromosome inactivation. *Lancet, ii/7717*, 219.

Fialkow, P. J. (1970): X-chromosome inactivation and the Xg locus. *Amer. J. hum. Genet., 22/4*, 460.

Fialkow, P. J., Lisker, R., Giblett, E. R. and Zavala, C. (1970): Xg locus: failure to detect inactivation in females with chronic myelocytic leukaemia. *Nature (Lond.), 226*, 367.

Gandini, E., Gartler, S. M., Angioni, G., Argiolas, N. and Dell'Acqua, G. (1968): Developmental implications of multiple tissue studies in glucose-6-phosphate dehydrogenase-deficient heterozygotes. *Proc. nat. Acad. Sci. USA, 61/3*, 945.

Gorman, J. G., Di Re, J., Treacy, A. M. and Cahan, A. (1963): The application of $-Xg^a$ antiserum to the question of red cell mosaicism in female heterozygotes. *J. Lab. clin. Med., 61/4*, 642.

Lawler, S. D. and Sanger, R. (1970): Xg blood groups and clonal-origin theory of chronic myeloid leukaemia. *Lancet, i*, 584.

LEE, G. R., MACDIARMID, W. D., CARTWRIGHT, G. E. and WINTROBE, M. M. (1968): Hereditary, X-linked, sideroachrestic anemia. The isolation of two erythrocyte populations differing in Xg^a blood type and porphyrin content. *Blood, 32/1*, 59.

POLANI, P. E., ANGELL, R., GIANNELLI, F., DE LA CHAPELLE, A., RACE, R. R. and SANGER, R. (1970): Evidence that the *Xg* locus is inactivated in structurally abnormal *X* chromosomes. *Nature (Lond.), 227/5258*, 613.

SANGER, R., TIPPETT, P. and GAVIN, J. (1971*a*): The X-linked blood group system Xg: tests on unrelated people and families of northern European ancestry. *J. med. Genet., 8/4*, 427.

SANGER, R., TIPPETT, P. and GAVIN, J. (1971*b*): Xg groups and sex abnormalities in people of northern European extraction. *J. med. Genet., 8/4*, 417.

WEATHERALL, D. J., PEMBREY, M. E., HALL, E. G., SANGER, R., TIPPETT, P. and GAVIN, J. (1970): Familial sideroblastic anaemia: problem of Xg and X chromosome inactivation. *Lancet, ii*, 744.

Ch. Salmon

Centre Départemental de Transfusion Sanguine de Paris et Groupe de Recherches U 76, Institut National de la Santé et de la Recherche Médicale, 53, boulevard Diderot, Paris, France

Le complexe Cis AB et le locus ABO

Parmi les données récentes concernant les groupes sanguins ABO, la découverte par Seyfried et coll. (1964) dans une famille polonaise d'un complexe génique transmettant à la fois un caractère A et un caractère B est certainement la plus spectaculaire car elle pourrait remettre en question les conceptions classiques concernant la structure et le fonctionnement du locus ABO.

A notre avis cependant, deux autres développements récents doivent être confrontés à cette découverte. Il s'agit des résultats concernant l'analyse de la variation génétique des produits du locus et des effets d'interaction chez l'hétérozygote AB.

L'objet de ce rapport est de rassembler ces trois ordres de faits et d'en discuter la signification mais il faut souligner qu'aucune de ces voies d'approche n'étudie le produit primaire lui-même du locus ABO, à savoir les transférases spécifiques et que de ce fait, l'interprétation ne peut être que constituée d'hypothèses.

En allant du plus simple vers le plus complexe, nous analyserons successivement la variation génétique des produits du locus, puis certains effets d'interaction chez l'hétérozygote AB et enfin, le complexe Cis AB lui-même.

I. ANALYSE DE LA VARIATION GÉNÉTIQUE

Le locus ABO intervient par ses enzymes à la fin d'une séquence d'activités génétiques complexes où sont déjà intervenus d'autres gènes selon les cellules différenciées (Fig. 1). Finalement, c'est sur le substrat H que sont accrochées les structures réactives proprement construites par les enzymes du locus ABO. Pour le moment, ces structures réactives peuvent être définies à trois niveaux de précision, par l'analyse biochimique, par l'analyse immunogénétique, comportant éventuellement des mesures quantitatives, et enfin par l'analyse biophysique, c'est-à-dire la mesure des bilans thermodynamiques.

1. La biochimie définit les sucres immunodominants: pour les structures réactives A, c'est la N-acétylgalactosamine (Fig. 2), pour les structures réactives B, c'est le galactose (Fig. 3). On voit que la seule différence définie par la biochimie est un $NHCOCH_3$ ou un radical OH. Il faut insister sur cette faible différence dont nous aurons à tenir compte dans la discussion concernant le Cis AB.

2. L'immunologie, associée à l'analyse des ségrégations dans les familles, reconnaît déjà des différences relativement plus subtiles. C'est ainsi que l'on a identifié plusieurs variétés d'antigènes A (A_1, A_2, A_3, A_x, A_{end}, A_m, A_{el}) qui se transmettent intégralement avec leurs caractéristiques propres, d'une génération à l'autre. Une appréciation quantitative de ces phénotypes apparaît sur le Tableau 1.

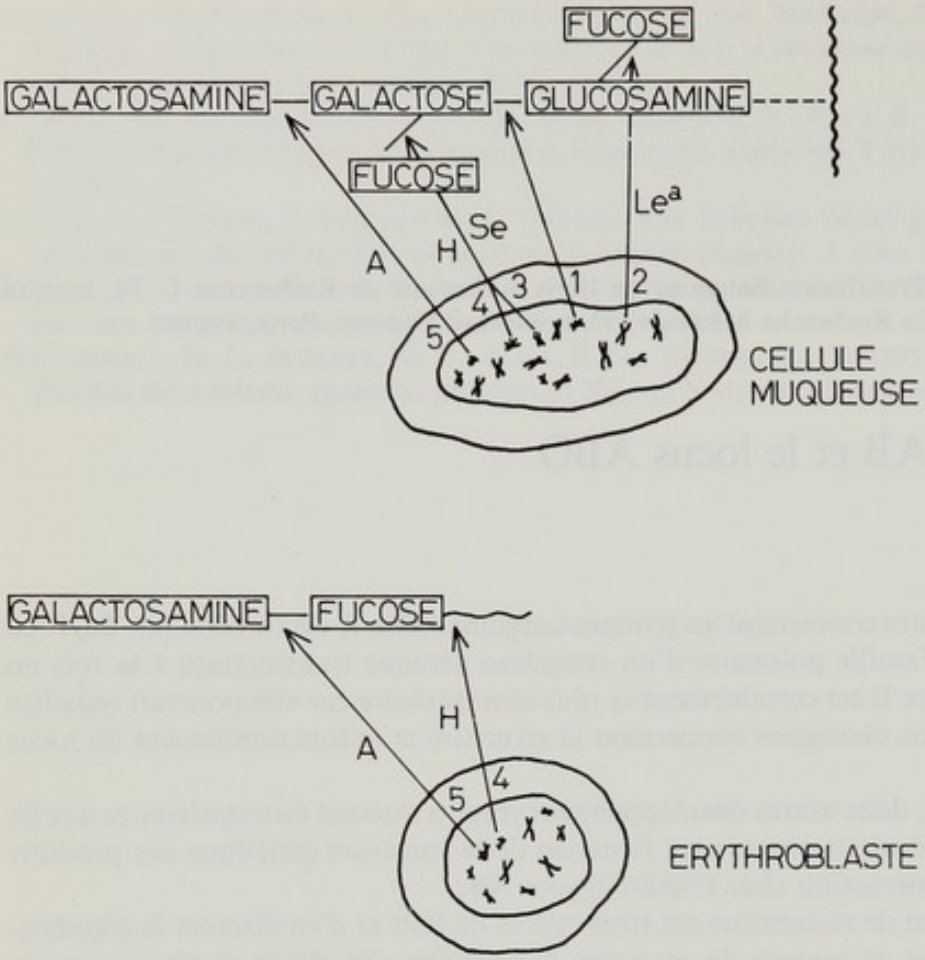


Fig. 1. Activité génétique conduisant à la synthèse des substances de groupes ABO dans la cellule muqueuse et l'érythroblaste.

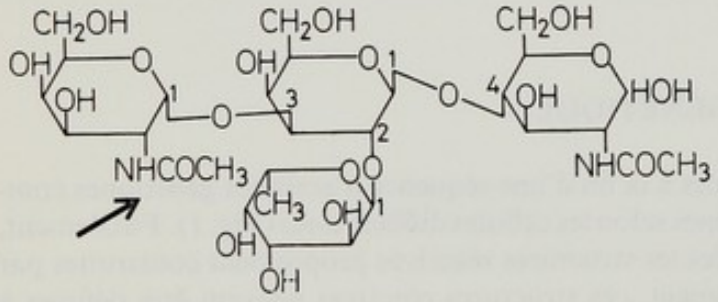


Fig. 2. N-acétylgalactosamine(1–3)αL-fucose (1–2). Galactose(1–4)N-acétylgluc.

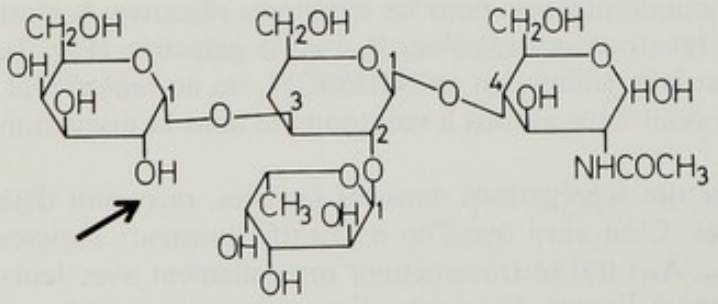


Fig. 3. D-galactose(1–3)αL-fucose(1–2). Galactose(1–4)N-acétylgluc.

TABLEAU 1 *Phénotypes A faibles*

Phénotypes	Détermination classique du groupe sanguin					Mesures quantitatives (%)	Substances sécrétées dans la salive
	Globules rouges			Sérum			
	β	α	$\alpha\beta$	anti-A	anti-B		
A_1 et A_2	−	+++	+++	−	+++	100	A H
A_3	−	±±	±±	+ ou −	+++	63	A H
A_x	−	(+)	+	+	+++	33	[A] H
A_{end}	−	±	±	+ ou −	+++	5 à 15	H
A_m	−	−	−	−	+++	0	A H
A_{el}	−	−	−	+ ou −	+++	0	H

De la même manière, on peut classer les phénotypes B faibles en plusieurs groupes (Tableau 2). Pour le moment, nous les avons appelés B_{60}, B_{20}, B_0, selon le pourcentage d'agglutinabilité mesuré dans des conditions constantes. Voici ces trois catégories de phénotypes: 27 sujets B_{60}, 5 sujets B_{20}, 3 sujets B_0 (Fig. 4). Ces pourcentages sont obtenus en utilisant 2.000.000 d'unités N_4 d'anticorps contre 200.000 hématies par mm^3. En regard figure la distribution des pourcentages pour 107 sujets A faibles (A_3, A_x, A_{end}, A_m ou A_{el}).

TABLEAU 2 *Phénotypes B faibles*

Phénotypes	Détermination classique du groupe sanguin					Mesures quantitatives (%)	Substances sécrétées dans la salive
	Globules rouges			Sérum			
	β	α	$\alpha\beta$	anti-A	anti-B		
B	+++	−	+++	+++	−	100	**B** H
B_{60}	±±	−	±±	+++	−	60	B H
B_{20}	±	−	±	+++	−	15 à 23	[B] H
B_0	−	−	−	+++	−	0	B H ou H

3. La biophysique, en réalité, va plus loin dans la définition de la variation génétique. Lorsqu'on mesure les bilans thermodynamiques, par la méthode de Filitti-Wurmser et coll. (1950), on aperçoit en effet des différences parmi les divers antigènes d'un même groupe. La méthode est la suivante (Fig. 5): lorsqu'en présence d'une quantité constante d'anticorps, N_4, on fait varier la quantité d'antigène, N_t, ici en abscisse, la loi d'action-de masse démontre que la variation du rapport N_4/N'_4 (anticorps total sur anticorps résiduel) en fonction de l'antigène N_t est représentée par une droite dont la pente est proportionnelle à la constante moyenne d'association. Ainsi, en travaillant à deux températures, le rapport des pentes permet de calculer la chaleur de réaction (Fig. 6).

Par ces méthodes de mesure de $-\Delta H$, on voit apparaître des différences dans les structures réactives, à l'intérieur des B_{60}, à l'intérieur des B_{20}, à l'intérieur des B_0.

Voici (Fig. 7) deux types de B_{60} qui ne sont identifiables que par ces méthodes. On voit que la position des pentes et leur variation avec la température sont tout à fait différentes.

Voici de la même manière (Fig. 8), deux types de B_0, le premier avec une chaleur de réaction considérable, de l'ordre de 20.000 cal/mole alors que la chaleur du second n'est pas mesurable par ces méthodes.

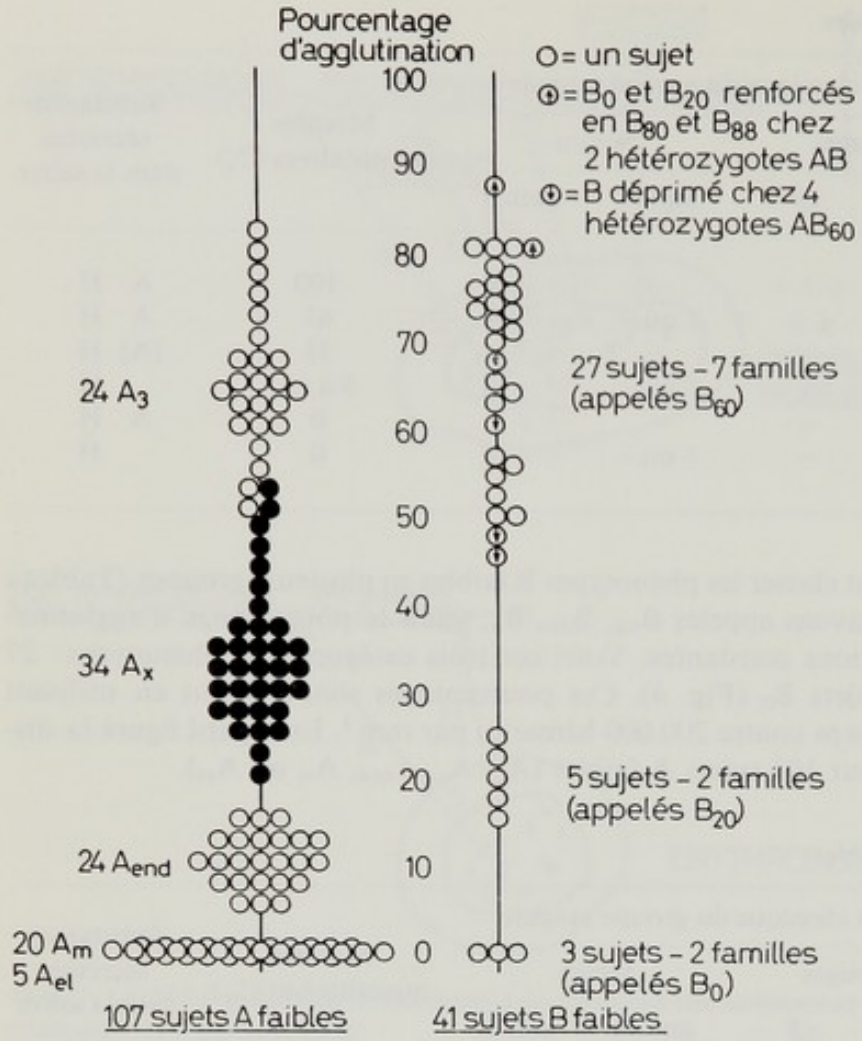


Fig. 4. Résultats des mesures de pourcentage d'agglutination des hématies A faibles et B faibles (2.10^6 unités N_4 d'anti-A de B ou d'anti-B de A_1 contre 2.10^5 hématies par mm^3).

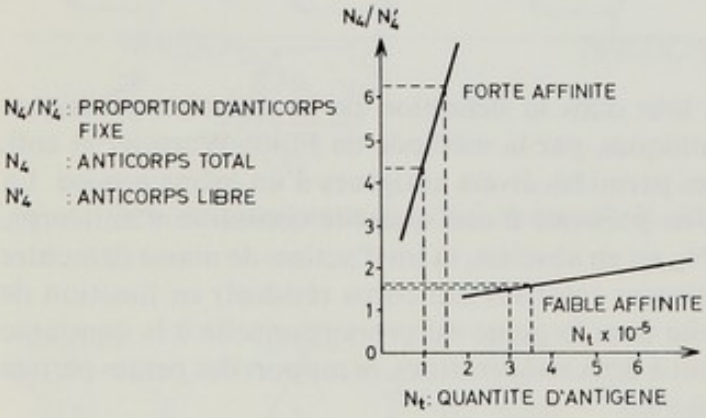


Fig. 5

$$P = \frac{N_4/N'_4 - 1}{N_t \times 10^{-5}}$$

à t_1 °C: P_1, à t_2 °C: P_2

$$\frac{K_1}{K_2} = \frac{P_1}{P_2}$$

$$-\Delta H = 4{,}575 \times \log \frac{K_1}{K_2} \times \frac{T_1 \times T_2}{T_2 - T_1}$$

$$-\Delta H = 4{,}575 \times \log \frac{P_1}{P_2} \times \frac{T_1 \times T_2}{T_2 - T_1}$$

Fig. 6

Fig. 5. Principe de la mesure d'affinité par $N_4/N'_4 \times N_t\ 10^{-5}$.

Fig. 6. Formule de Van 't Hoff. Calcul de $-\Delta H$ à partir des pentes P_1 et P_2 exprimant N_4/N'_4 en fonction de N_t déterminé à deux températures. K_1 et K_2: constantes d'associations à deux températures. T_1 et T_2: températures absolues.

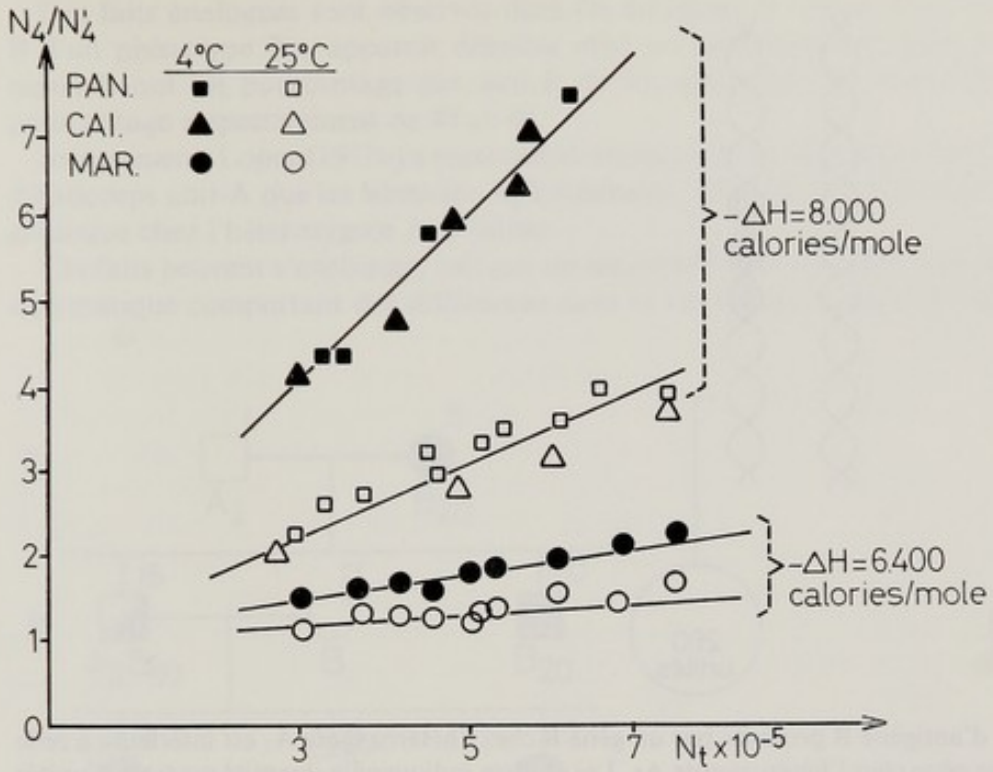


Fig. 7. Droites de régression à deux températures de N_4/N'_4 sur N_t. (Anticorps: sérum anti-B d'A_1O LIB.; antigène: B_{60} PAN. et CAI. et B_{60} MAR.) Ces deux types de B_{60} ne sont identifiables que par les mesures thermodynamiques.

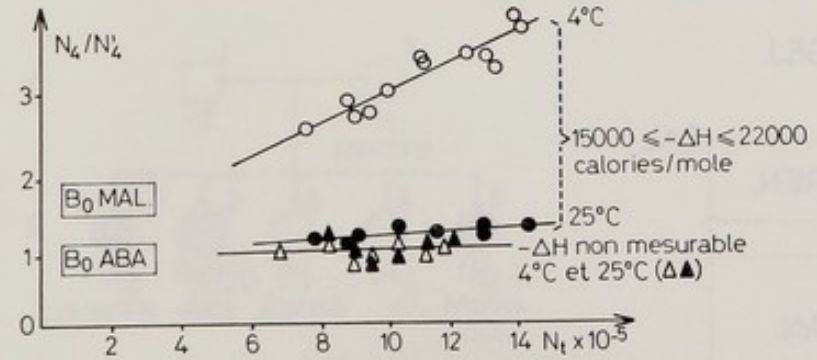


Fig. 8. Droites de régression mesurées à deux températures, de N_4/N'_4 en fonction de N_t pour les deux antigènes B_0 MAL. et B_0 ABA. Dans les deux cas, l'anticorps utilisé est le sérum anti-B d'A_1O LIB. L'affinité vis-à-vis de l'antigène B_0 ABA. (△ et ▲) est si faible que les pentes, aux deux températures, ne s'écartent pas significativement ni de l'abscisse, ni entre elles. $-\Delta H$ n'est donc pas mesurable. Au contraire, la température occasionne une variation considérable des pentes observées pour l'antigène B_0 MAL. (○ et ●). $-\Delta H$ est ici très élevé, compris entre 15.000 et 22.000 calories/mole. Ces deux types de B_0 MAL. et ABA. ne sont donc identifiables que par les mesures thermodynamiques.

II. LES EFFETS D'INTERACTION CHEZ LES HÉTÉROZYGOTES

a. Chez l'hétérozygote AB, il existe un équilibre entre la quantité d'antigène A et la quantité d'antigène B. On sait depuis longtemps que la quantité d'antigène B produite chez un sujet A_2B ou B est plus grande que celle produite par un sujet A_1B, même s'il s'agit de la production d'un même gène B étudié dans une famille. On voit ici (Fig. 9) la quantité maxima d'unités N_4 d'anticorps anti-B que l'on peut fixer sur les hématies A_1B et A_2B.

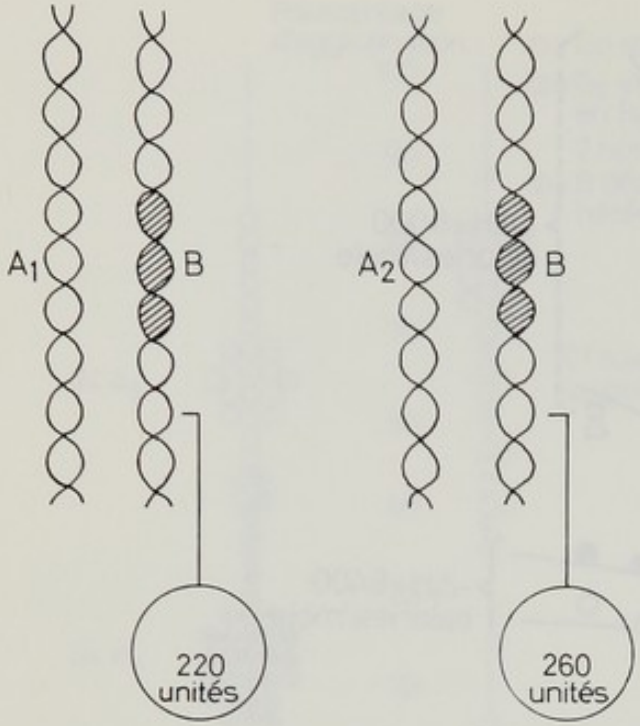


Fig. 9. La quantité d'antigène B produite par un gène B chez l'hétérozygote A_1 est inférieure à celle produite par un même gène chez l'hétérozygote A_2. Les chiffres indiquent la quantité maxima d'unités d'anticorps anti-B que l'on peut fixer sur les hématies A_1B et A_2B.

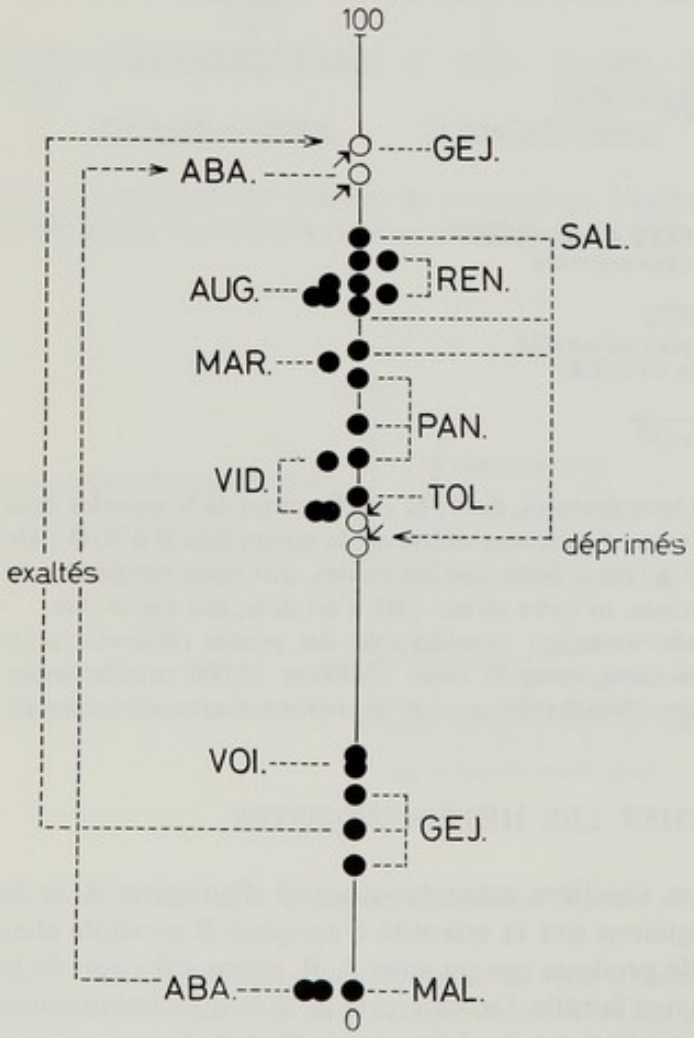


Fig. 10. Pourcentage d'agglutination des hématies avec anti-B (2.10^6 unités N_4 d'anticorps anti-B de A contre 2.10^5 hématies/mm^3).

Des faits analogues sont observés dans les antigènes B faibles (Fig. 10). Ainsi, l'antigène B d'un phénotype B_{60} apparaît déprimé chez un hétérozygote. Dans la famille SAL., les sujets B ont un pourcentage par anti-B de 75, alors que les deux hétérozygotes ont un pourcentage respectivement de 47 et 48.

Inversement, Lopez (1971*a*) a récemment montré que les hématies A_1B faibles fixaient plus d'anticorps anti-A que les hématies A_1B normales, mettant ainsi en évidence une interaction analogue chez l'hétérozygote A_1B faible.

Ces faits peuvent s'expliquer, soit par un encombrement stérique, soit par une compétition enzymatique comportant des différences dans la vitesse de réaction des deux enzymes.

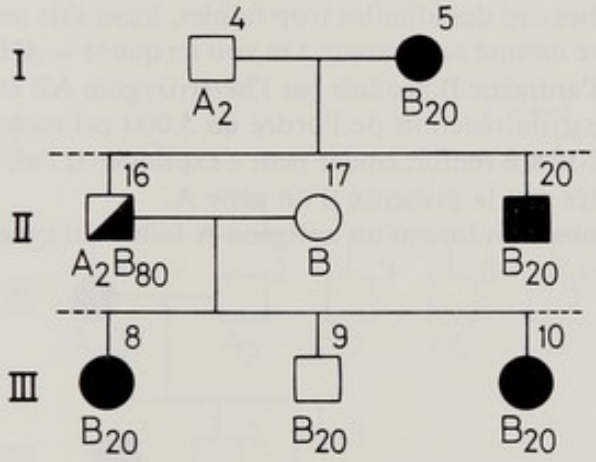


Fig. 11. Famille GEJ. (fragment).

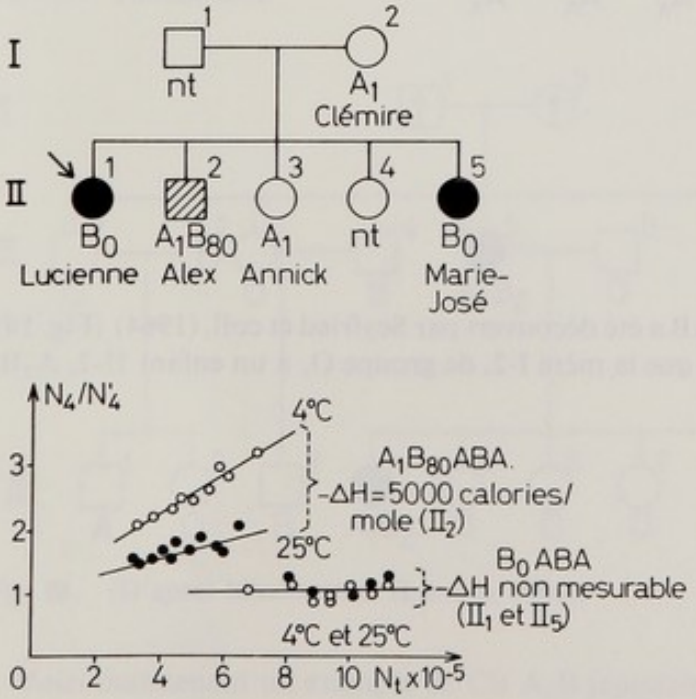


Fig. 12. La famille ABA. où un gène B faible s'exprime par un antigène B_0 ou un antigène B_{80} selon l'appariement allélique.

Les antigènes B_0 et B_{80} ont été étudies par l'anti-B d'A_1O LIB. L'antigène B_{80} du sujet A_1B_{80} donne une chaleur de réaction de 5.000 calories/mole; la chaleur de réaction de l'antigène B_0 n'est pas mesurable (*cf.* Fig. 4). Une telle différence pourrait être due à des différences dans le nombre de sites. Aucune conclusion certaine d'une différence d'ordre qualitatif ne peut donc être tirée de ces mesures.

b. Mais il est beaucoup plus difficile d'expliquer le phénomène inverse qui est le renforcement chez l'hétérozygote AB d'un antigène B faible. Dans la famille GEJ., un B_{20} s'exalte en B_{80} chez l'hétérozygote. Dans la famille ABA., un B_0 s'exalte en B_{80} chez l'hétérozygote.

Voici la famille GEJ. (Fig. 11) où l'on voit dans la première génération un antigène B_{20}, dans la seconde un antigène B_{80} chez l'hétérozygote AB, puis à nouveau un antigène B_{20} dans la troisième, lorsque le gène B est apparié à un gène O.

Voici l'arbre de la famille ABA. (Fig. 12) où un même gène produit soit un antigène B_0 chez le propositus, soit un antigène B_{80} chez l'hétérozygote AB.

L'un des points actuellement à l'étude est de savoir si ces différences sont d'ordre qualitatif. La méthode thermodynamique qui définit des structures quantitatives mole à mole, indépendamment du nombre de sites réactifs, devrait permettre de répondre à cette question. Malheureusement, les antigènes jusqu'à présent étudiés ont des affinités trop faibles, lorsqu'ils ne sont pas renforcés, pour que le $-\Delta H$ puisse être mesuré sans erreur. On voit ici que le $-\Delta H$ du B_0 ABA. n'est pas mesurable. Au contraire, l'antigène B produit par l'hétérozygote AB et qui s'exprime comme un B_{80} dégage une chaleur de réaction de l'ordre de 5.000 cal/mole lorsqu'on le met en présence du même sérum anti-B. Ce renforcement peut s'expliquer si l'enzyme qui a produit l'antigène a été complémentée par la présence d'un gène A.

Des renforcements analogues pouvaient être observés lorsqu'un antigène A faible du type A_x s'associe à un gène B normal (Fig. 13).

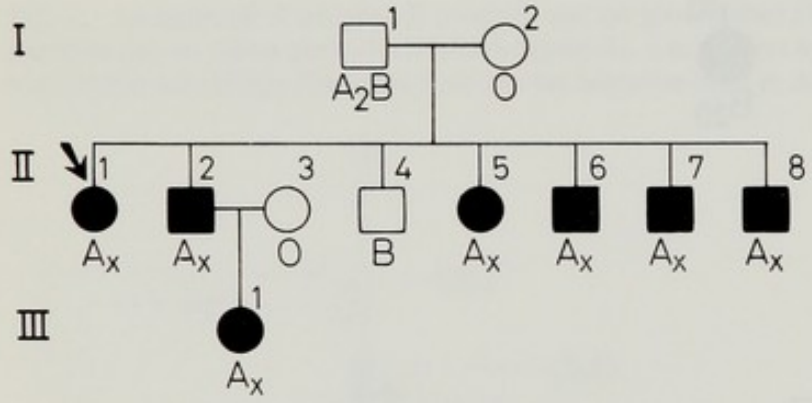


Fig. 13. Famille DUV.

III. LE COMPLEXE Cis AB

Le premier exemple de complexe Cis AB a été découvert par Seyfried et coll. (1964) (Fig. 14) dans cette famille polonaise où l'on voit que la mère I-2, de groupe O, a un enfant II-2, A_2B,

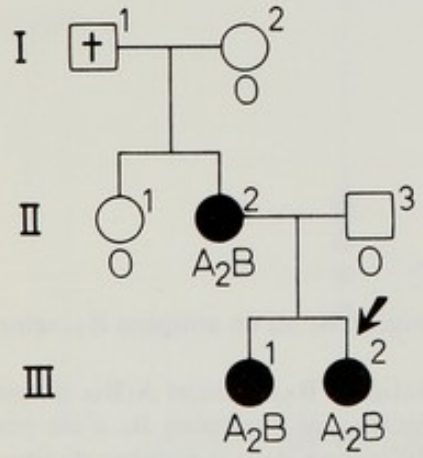


Fig. 14. Famille SL (Seyfried et coll., 1964).

qui lui-même marié à un sujet O a deux enfants A_2B: la transmission du caractère A et celle du caractère B se fait donc à partir d'un seul complexe génique. D'autres familles analogues ont été découvertes au Japon (Yamaguchi et coll., 1965), en Corée (Madsen et Heisto, 1968) et en France (Reviron et coll., 1968).

Voici l'une des familles françaises (Fig. 15), étudiée pour la première fois par Moullec et Le Chevrel (1959) où l'on voit qu'une mère (II-2) de groupe A_2B faible, mariée à un sujet O, a deux enfants A_2B faible (III-1 et III-8) et deux enfants O (III-3 et III-6). Ces deux exemples peuvent être appelés Cis A_2B.

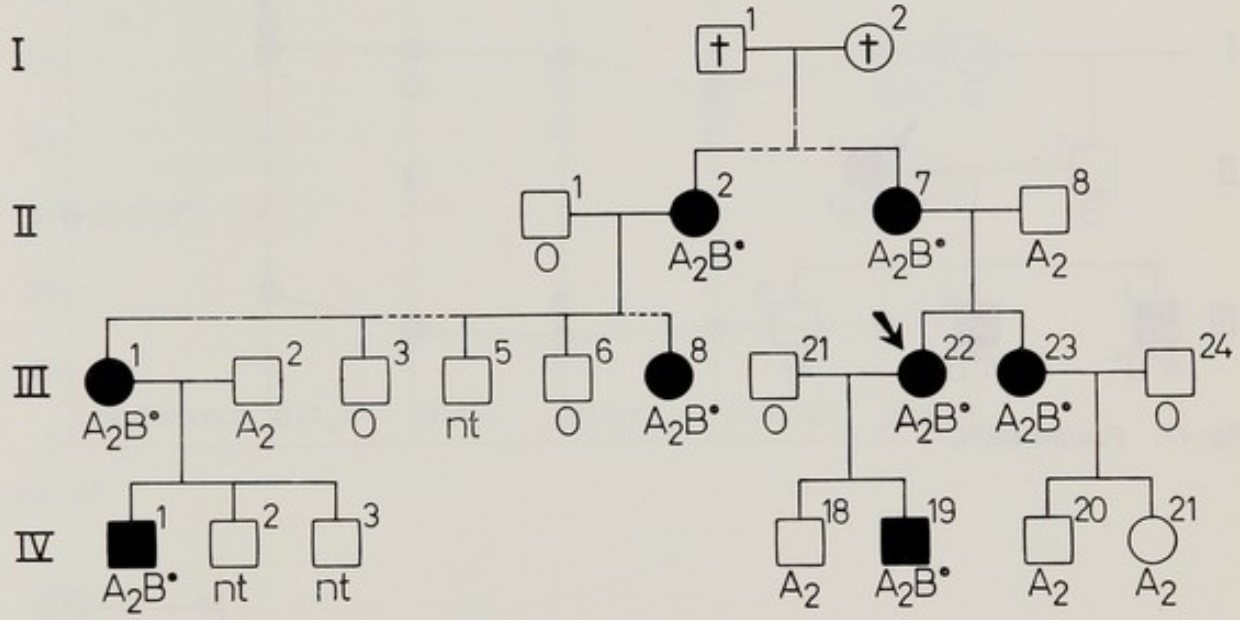


Fig. 15. Famille BRI.

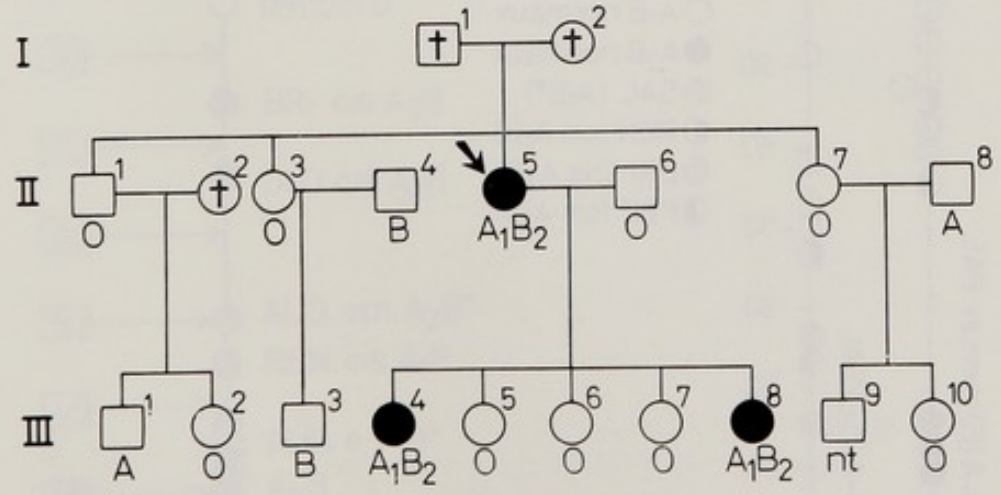


Fig. 16. (D'après Kitahama et Ikemoto, 1966).

Voici maintenant un exemple de Cis A_1B japonais (Kitahama et Ikemoto, 1966; Shikata et coll., 1967) où une mère A_1B_2, mariée à un sujet O, a deux enfants A_1B_2 (Fig. 16). Pour les Cis AB japonais, voir Yamaguchi et coll. (1970).

Voici enfin la famille française REN. (Fig. 17) où les hématies Cis AB sont également reconnues par l'anti-A_1 de Dolichos (Reviron et coll., 1968).

On peut donc grossièrement considérer, dès maintenant, qu'il y a au moins deux variétés de Cis AB: les Cis A_1B et les Cis A_2B.

L'étude immunologique des phénotypes Cis AB à l'aide des méthodes qualitative, quantitative et thermodynamique apporte d'autres informations:

1. L'étude qualitative de l'agglutination montre que les antigènes réactifs des Cis AB sont en quelque sorte dissymétriques. L'antigène A est presque normalement agglutiné, tandis que l'antigène B est considérablement diminué par rapport à un B normal. De plus, on observe un anti-B dans le sérum.

Ce caractère est également reconnu par l'étude des sécrétions. La salive des sujets Cis AB secréteurs inhibe normalement l'agglutination des hématies A par l'anti-A, elle n'inhibe pas

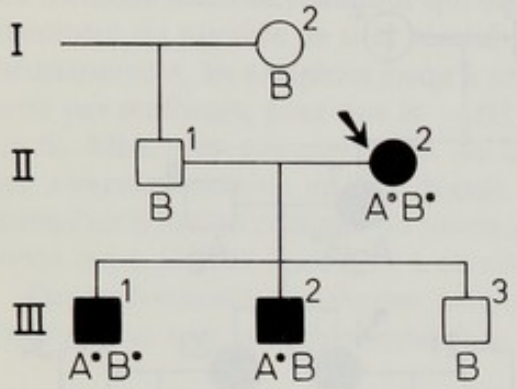


Fig. 17. Famille REN.

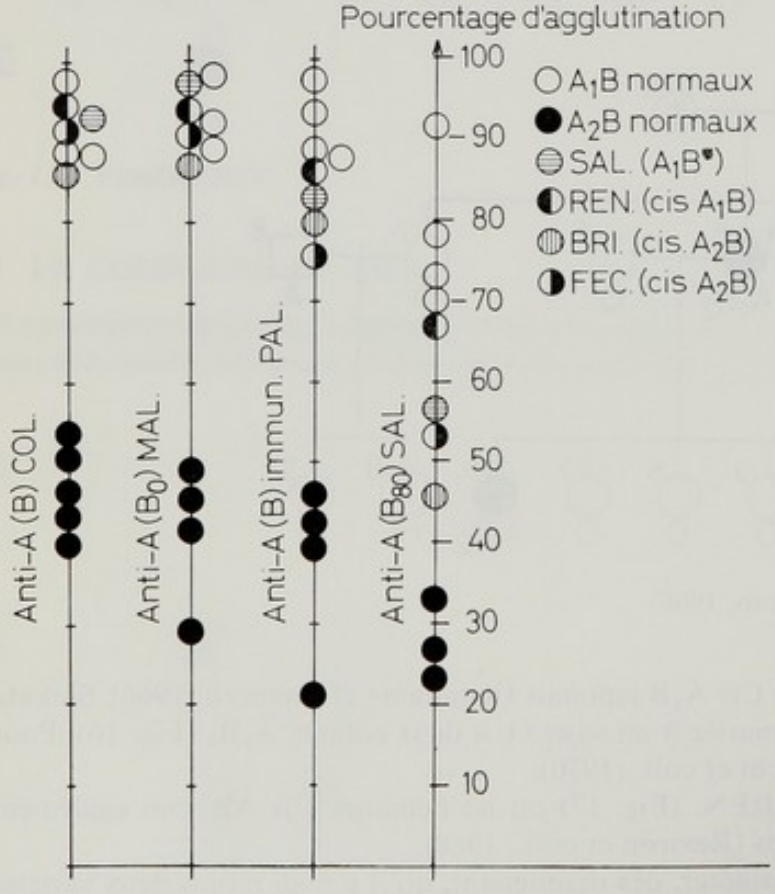


Fig. 18

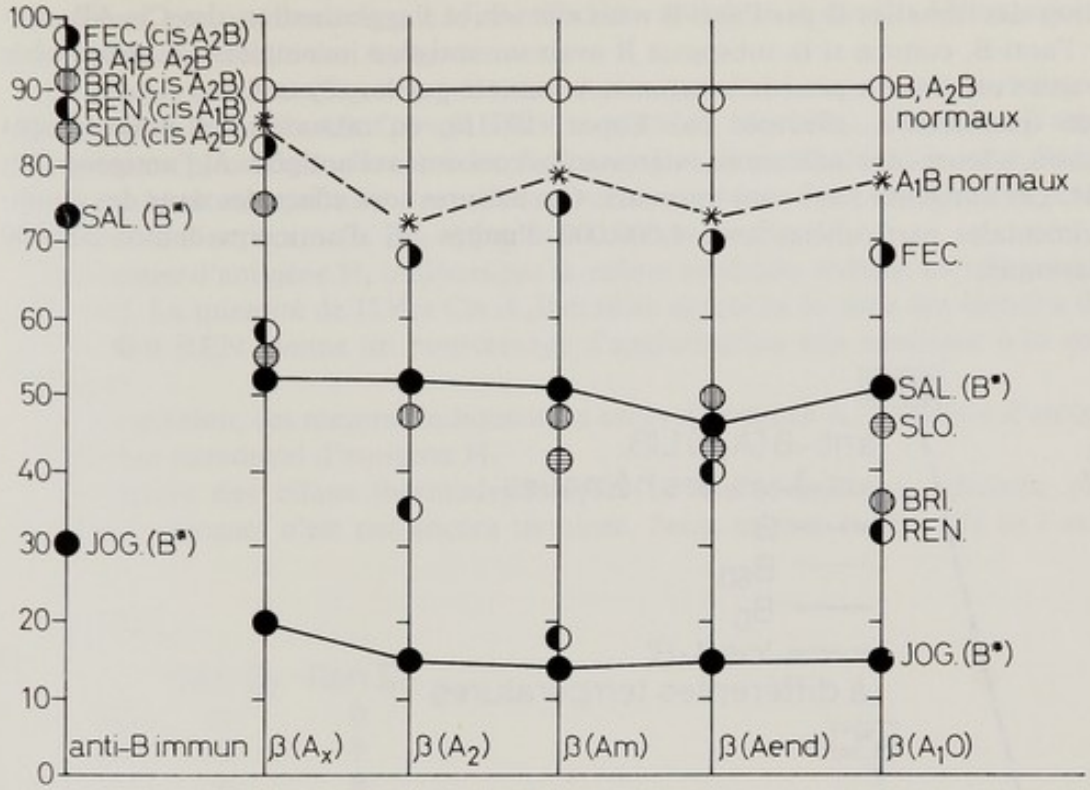


Fig. 19

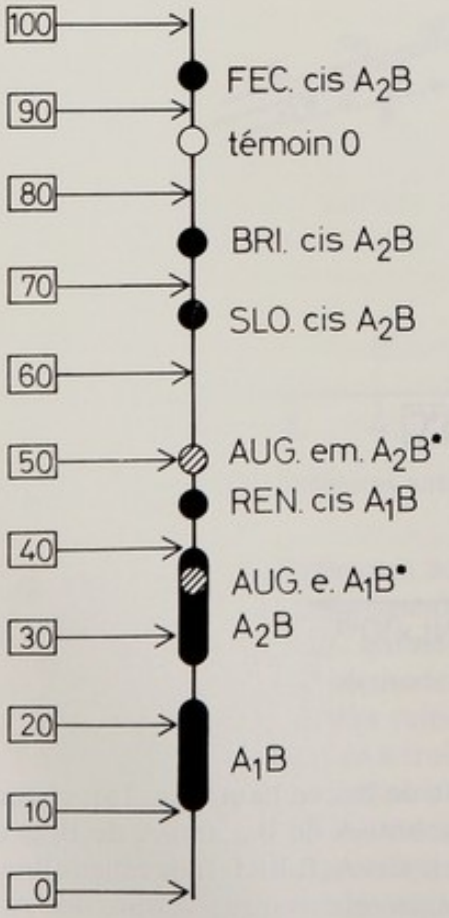


Fig. 20. Pourcentages d'agglutination des hématies de phénotype cis AB, AB* et AB normales par anti-H d'Ulex.

l'agglutination des hématies B par l'anti-B mais elle inhibe l'agglutination des Cis AB eux-mêmes par l'anti-B, comme si la substance B avait un antigène incomplet. Ce phénomène rappelle ce que l'on observe pour la substance A secrétée par les sujets A_x.

2. L'étude quantitative, effectuée par Lopez (1971*b*), en mesurant les pourcentages d'agglutination, a fourni des indications intéressantes concernant l'antigène A, l'antigène B et l'antigène H. Les antigènes I et i sont normaux. Ces mesures sont effectuées dans des conditions expérimentales particulières avec 1.000.000 d'unités N_4 d'anticorps contre 200.000 hématies par mm^3.

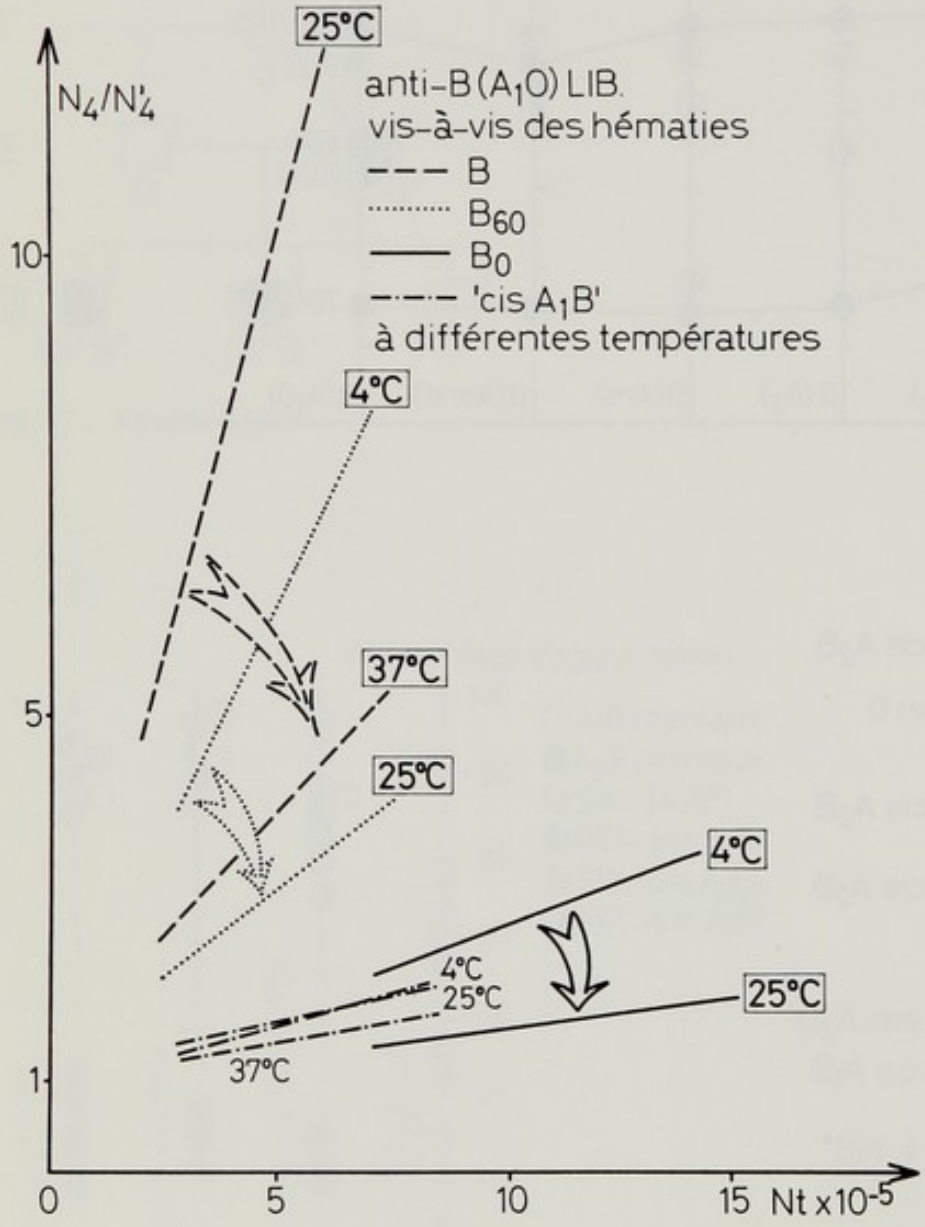


Fig. 21

Voici les pourcentages observés par l'anti-A, de 0 à 100, de bas en haut (Fig. 18); chaque colonne représente des sérums différents: anti-A de B_{100}, anti-A de B_0, anti-A de B_{100} de type immun, anti-A de B_{80}. On voit que le Cis AB REN., le Cis A_2B BRI. (nos échantillons) et le Cis A_2B FEC. (Brocteur, 1971) ont des pourcentages relativement voisins des A_1B normaux et considérablement plus élevés que les témoins A_2B normaux. Seul le sérum anti-A de B_{80} fait apparaître une agglutinabilité des Cis AB inférieure à celle des A_1B. On peut

considérer, par ces mesures, que la réactivité des antigènes A des Cis AB est significativement plus grande que celle des A_2B normaux.

La plaque suivante (Fig. 19) présente les résultats de la mesure, par les mêmes méthodes, de l'antigène B des Cis AB. On voit ici le Cis AB SLO. (Seyfried). L'antigène B apparaît considérablement plus faible que celui des A_2B normaux. Il se situe dans la zone d'agglutinabilité des B_{60}. Ajoutons que nous travaillons, volontairement, dans ces mesures, avec 1.000.000 d'unités N_4 d'anticorps (le B_{60} apparaît ici comme un B_{50}).

La mesure d'antigène H, toujours par la même méthode, indique un très grand excès de H (Fig. 20). La quantité de H des Cis A_2B étudiés approche la zone des témoins 0. De même, le Cis A_1B REN. donne un pourcentage d'agglutination très supérieur à la zone des A_1B normaux.

En conclusion, ces mesures indiquent un excès d'antigène A, un déficit d'antigène B et un excès assez paradoxal d'antigène H.

La mesure des bilans thermodynamiques de ces phénotypes, effectuée par Madame Bouguerra-Jacquet n'est pas encore terminée. Seule la mesure de $-\Delta H$ de l'antigène B du

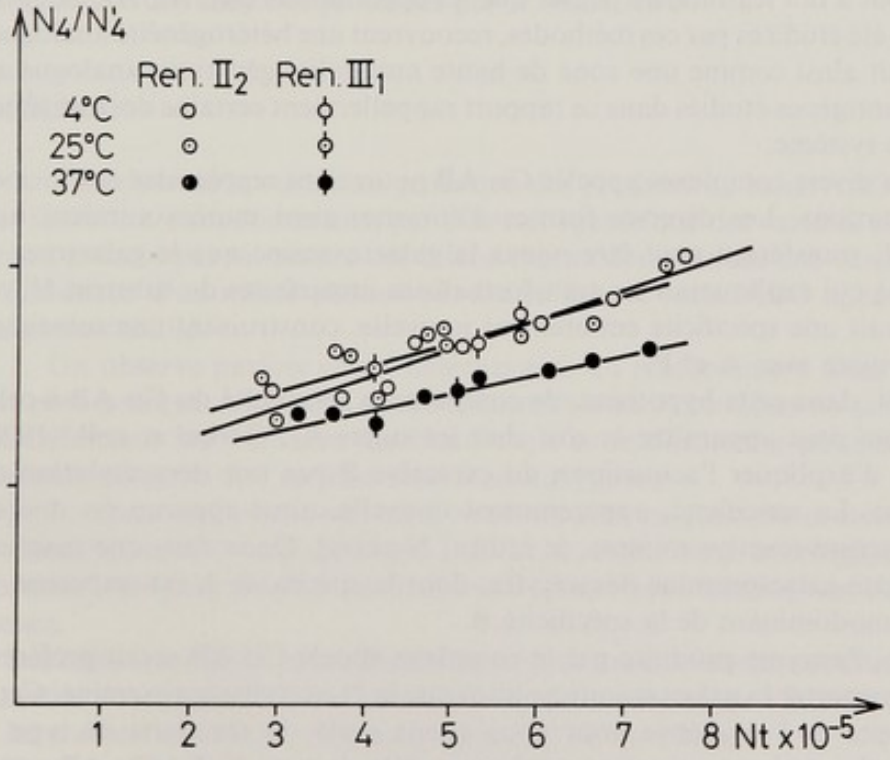


Fig. 22. Affinités de l'anticorps anti-B (A_1O) vis-à-vis des hématies 'cis A_1B'.

Cis AB REN. est actuellement achevée (Bouguerra, 1971): la structure réactive visible sur la Figure 21 est tout à fait originale par rapport au B_0 et au B_{60}. Elle est également différente du B_{60} de la famille GEJ. qui ne figure pas ici. Voici le détail de ces mesures (Fig. 22). On voit ici encore que cette méthode biophysique présente un intérêt de définition proprement génétique puisque les mêmes valeurs sont observées chez la mère et chez l'enfant Cis AB. La variation de pentes mesurée à trois températures est très faible, si bien que le $-\Delta H$ est seulement de l'ordre de 2.000 cal/mole alors que l'antigène B normal, avec le même réactif anti-B dégage une chaleur de l'ordre de 16.000 cal/mole. Nous n'avons pas encore un assez grand nombre de mesures concernant les autres Cis AB étudiés pour calculer leur $-\Delta H$ mais nous pouvons, d'après les premières mesures, conclure que les structures réactives sont différentes entre elles et différentes du Cis A_1B REN. Ce travail se poursuit mais, d'ores et déjà, il apporte une précision précieuse sur l'hétérogénéité structurale des divers Cis AB.

DISCUSSION

Ainsi, trois ordres de faits dominent à notre sens les analyses immunogénétiques actuelles concernant les groupes sanguins ABO: les variations allotypiques multiples, les effets d'interaction chez l'hétérozygote et le complexe Cis AB. Il faudrait trouver une explication donnant une interprétation globale, bien qu'il puisse paraître hasardeux de le faire alors que nous ne possédons aucune donnée sur la structure des transférases produites par le locus ABO.

1. En premier lieu, il apparaît clairement que le locus ABO produit des structures antigéniques dont la variation allotypique paraît considérable et dont les méthodes classiques d'analyse ne donnent qu'une idée très imparfaite. Au contraire, à l'aide des méthodes thermodynamiques, on peut reconnaître des variations à l'intérieur de ce qui paraît être un phénotype homogène. Ces définitions, basées sur les propriétés physiques des structures antigéniques réactives, représentent bien une définition proprement génétique puisque, sauf cas d'interaction, des valeurs identiques sont trouvées à travers les diverses générations chez les membres d'une même famille.

Ainsi, chacun des phénotypes que nous avons appelés B_{60}, B_{20} ou B_0, apparaît comme hétérogène et il paraît tout à fait légitime de penser que les appellations (A_3, A_x, A_m, A_{end} ou A_{el}) qui n'ont pas encore été étudiées par ces méthodes, recouvrent une hétérogénéité analogue.

Le locus ABO apparaît ainsi comme une zone de haute mutabilité génétique analogue au locus MNS et les divers antigènes étudiés dans ce rapport rappelleraient certains des antigènes rares ou familiaux de ce système.

De ce point de vue, les divers complexes appelés Cis AB pourraient représenter des formes particulières de ces mutations. Les diverses formes d'enzymes ainsi mutées auraient une diminution de spécificité, transférant peut être mieux la galactosamine que le galactose, et une diminution d'activité qui expliquerait les transformations imparfaites du substrat H, ou bien l'enzyme mutée aurait une spécificité entièrement nouvelle, construisant une substance donnant une réaction croisée avec A et B.

Il n'est pas sans intérêt, dans cette hypothèse, de comparer la spécificité du Cis AB à celle de l'antigène B acquis qui peut apparaître *in vivo* chez les sujets A_1. Gerbal et coll. (1970) ont récemment proposé d'expliquer l'acquisition du caractère B par une désacétylation de la N-acétylgalactosamine. La spécificité, apparemment nouvelle, ainsi apparue est due en fait à la perte d'une structure réactive minime, le radical N-acétyl. De ce fait, une réaction croisée apparaît entre cette galactosamine désacétylée, dont la spécificité A est respectée, et le galactose, sucre immunodominant de la spécificité B.

Dans cette conception, l'enzyme produite par le complexe appelé Cis AB serait préférentiellement capable de transporter la galactosamine plutôt que la N-acétylgalactosamine. Cette hypothèse rendrait compte de l'asymétrie dont nous avons parlé, la réactivité de type A étant beaucoup plus proche de la réactivité normale que celle de type B. Le Cis AB serait alors une forme particulière d'antigenè A.

Cette explication ne s'écarte pas de la conception classique et ne nécessite aucun élément nouveau concernant la structure du locus mais elle ne repose que sur des arguments théoriques et n'explique pas le renforcement d'un B faible en B normal chez l'hétérozygote.

2. Il reste en effet à expliquer ce renforcement inattendu d'un antigène B faible en un antigène B presque normal chez l'hétérozygote AB: ce phénomène pourrait être interprété comme un exemple de complémentation, l'enzyme B produite chez le trans AB étant de ce fait renforcée. Ceci suppose que le locus est fonctionnellement hétérogène et que l'enzyme doit être polymérique.

Dans ces conditions, si l'on admet la possibilité de crossing-over, les Cis AB apparaissent comme des locus ABO où des crossing-over ont réuni dans une même enzyme à la fois la structure génétique responsable du site actif pour A et la structure génétique responsable du site actif pour B. L'enzyme ainsi complémentée aurait alors, comme c'est le cas général en génétique expérimentale, une activité moindre que l'enzyme sauvage. Mais les structures

réactives des antigènes B des divers Cis AB n'étant probablement pas identiques, on est conduit, dans cette hypothèse, à l'idée que ces points d'échange doivent être nombreux: le locus ABO apparaîtrait alors comme très hétérogène. Ajoutons cependant que, de toute façon, un complexe Cis AO ou Cis BO ne pourrait être différencié d'une mutation rare par aucune analyse immunogénétique. Et inversement, le phénotype A_x et le phénotype B_{20} pourraient représenter ces complexes Cis AO et Cis BO.

En fait, il nous paraît significatif que, dans l'état actuel de nos connaissances, l'on puisse théoriquement expliquer le complexe Cis AB de deux manières: mutation ou crossing-over. Si aucun argument décisif ne permet actuellement de trancher entre ces deux explications, c'est que nous ignorons la structure des enzymes produites. En définitive, ce que nous appelons le locus ABO, pourrait produire – soit une seule enzyme dans tous les cas et le Cis AB serait une mutation particulière mais ceci n'explique pas les phénomènes de renforcement chez l'hétérozygote, – soit une enzyme polymérique, qui peut éventuellement subir une complémentation: si l'on admet que le locus est très hétérogène, les Cis AB et le renforcement des hétérozygotes sont alors explicables, – soit enfin une série d'enzymes génétiquement liées. On voit que la solution viendra, comme souvent en génétique, de l'analyse de l'activité et de la structure des enzymes produites par le locus ABO.

RÉSUMÉ

1. L'analyse de la variation génétique des produits du locus ABO définit, par la biochimie des sucres immunodominants, par l'immunogénétique des variétés plus complexes de phénotypes, et enfin par la mesure des bilans thermodynamiques une variation génétique plus fine à l'intérieur d'un même phénotype. De ce fait, le locus ABO apparaît comme une zone de haute mutabilité.

2. On observe parfois chez l'hétérozygote un renforcement d'un antigène B faible en un antigène presque normal. Aucune mesure thermodynamique ne permet encore de savoir si ces différences sont d'ordre qualitatif mais ce renforcement pourrait être expliqué par une complémentation de l'enzyme chez l'hétérozygote.

3. La découverte du complexe Cis AB a remis en question la structure classique du locus. Il existe plusieurs variétés de Cis AB (A_1B, A_2B, etc.). L'antigène A produit est presque normal, l'antigène B, au contraire, est toujours plus faible et on observe un anti-B dans le sérum.

Les mesures quantitatives confirment ces données démontrant d'autre part une très grande quantité d'antigène H à la surface des hématies des sujets Cis AB.

La mesure des bilans thermodynamiques est en cours.

Deux hypothèses peuvent expliquer le Cis AB:

1. Celle d'une mutation: l'enzyme produite pourrait, par exemple, transférer préférentiellement la galactosamine non acétylée induisant une structure à spécificité A mais avec une réaction croisée pour la spécificité B. Une telle structure serait voisine de celle qui est observée *in vivo* chez les sujets A_1 ayant acquis un antigène B temporaire (B acquis).

2. A l'inverse, on peut supposer que le locus ABO est hétérogène dans sa structure et son fonctionnement. Dans ces conditions, l'enzyme produite pourrait être complémentée chez l'hétérozygote, expliquant le renforcement de l'antigène B faible en antigène B fort. Des crossing-over expliqueraient alors de manière simple la possibilité des complexes Cis AB et éventuellement Cis AO et Cis BO.

REMERCIEMENTS

Nous tenons à remercier Madame le Docteur Seyfried et Monsieur le Docteur Brocteur qui ont

bien voulu nous permettre d'étudier leurs échantillons 'Cis AB' ainsi que Monsieur le Docteur Moullec qui nous a permis de poursuivre l'étude de la famille BRI.

BIBLIOGRAPHIE

Bouguerra, A. (1971): Etude thermodynamique à l'aide de l'anticorps anti-B (A_1O) des antigènes B faibles et de l'antigène B induit par un chromosome 'Cis A_1B'. *Thesis, Paris.*

Brocteur, J. (1971): Communication personnelle.

Filitti-Wurmser, S., Jacquot-Armand, Y. et Wurmser, R. (1950): Sur la combinaison des isohémagglutinines avec des groupes agglutinogènes. *J. Chim. phys., 47*, 419.

Gerbal, A., Liberge, G., Lopez, M. et Salmon, Ch. (1970: Un antigène B acquis chez un sujet de phénotype érythrocytaire A_h^m. *Rev. franç. Transfus., 13/1*, 61.

Hrubisko, M. (1968): Exemple d'une interaction allélique chez l'homme: interaction entre une variante du gène B (B_x ou B_{20}) et A_2. *Nouv. Rev. franç. Hémat., 8/2*, 278.

Kitahama, M. et Ikemoto, S. (1966): Study on a family of unusual inheritance of the ABO groups. *Jap. J. leg. Med., 20/4*, 363.

Lopez, M. (1971*a*): Mesures quantitatives des antigènes de groupes sanguins A, B, H, I, i, des érythrocytes humains normaux. *Thesis, Orsay.*

Lopez, M. (1971*b*): Communication personnelle.

Madsen, G. et Heisto, H. (1968): A Korean family showing inheritance of A and B on the same chromosome. *Vox Sang. (Basel), 14/3*, 211.

Moullec, J. et Le Chevrel, P. (1959): A rare variant of B in a human blood sample belonging to group AB. *Nature (Lond.), 183/4677*, 1733.

Reviron, J., Jacquet, A. et Salmon, Ch. (1968): Un exemple de chromosome 'Cis A_1B'; étude immunologique et génétique du phénotype induit. *Nouv. Rev. franç. Hémat., 8/3*, 323.

Seyfried, H., Walewska, I. et Werblinska, B. (1964): Unusual inheritance of ABO group in a family with weak B antigens. *Vox Sang. (Basel), 9/3*, 268.

Shikata, I., Aihara, M., Okada, H., Maiewa, M. et Takehara, S. (1967): A family of unusual inheritance of the ABO blood group. *Jap. J. leg. Med., 21/3*, 307.

Yamaguchi, H., Okubo, Y. et Hazama, F. (1965): An A_2B_3 phenotype blood showing atypical mode of inheritance. *Proc. Jap. Acad., 41*, 316.

Yamaguchi, H., Okubo, Y. et Tanaka, M. (1970): 'Cis AB' bloods found in Japanese families. *Japan J. hum. Genet., 15/3*, 198.

J. Dausset et L. Legrand

Laboratoire d'Immuno-Hématologie, Institut de Recherches sur les Maladies du Sang, Hôpital Saint Louis, Paris, France

La structure génétique du système HL-A

Le système HL-A, qui est l'équivalent chez l'homme du système H-2 de la souris est probablement le système le plus complexe de l'homme. Quoique son importance fondamentale en transplantation soit démontrée, son véritable rôle biologique, s'il existe, est ignoré, et les forces qui maintiennent son extrême polymorphisme sont inconnues.

Depuis 1958 (Dausset, 1958) de nombreux antigènes ont été décrits (Shulman et coll., 1962; Van Rood et Van Leeuwen, 1963; Payne et coll., 1964; Dausset et coll., 1965; Bodmer et coll., 1966; Ceppellini et coll., 1967; Kissmeyer-Nielsen et coll., 1968; Walford et coll., 1968; Singal et coll., 1968) et deux séries alléliques ont émergées. La première tout d'abord composée des antigènes LA1 ou HL-A1 et LA2 ou HL-A2 (Payne et coll., 1964) comprend maintenant au moins 11 spécificités sérologiquement définies, dont 6 ont reçu des appellations internationales, les autres étant encore à l'étude. Dès maintenant on peut affirmer que cette série se compliquera, par l'addition de nouvelles spécificités (en effet au moins 2 à 3% de gènes de la population caucasienne sont encore non-sérologiquement identifiés) et surtout par la 'sub-division' des antigènes connus dont des variantes ont déjà été décrites pour HL-A9 (Walford et Troup, 1967; Dausset, 1971), HL-A10 (Dausset, 1971; Richiardi et coll., 1971) et Da25 (Dausset, 1971) (Tableau 1).

L'existence d'une deuxième série allélique formée des éléments du système 4^a 4^b (Van Rood et Van Leeuwen, 1963) a été soupçonnée (Dausset et coll., 1965; Van Rood et coll., 1965; Ceppellini et coll., 1967), puis décrite (Dausset et coll., 1968*b*, 1969; Kissmeyer-Nielsen et coll., 1968; Singal et coll., 1968). Elle est à l'heure actuelle composée d'au moins 16 spécificités. Mais sa complexité semble plus grande encore que celle de la première série et à côté des 5 spécificités internationalement reconnues, un grand nombre d'autres existent qui sont à l'étude (Tableau 2). Environ 4% des gènes de la population caucasienne sont encore inconnus.

Dans les Tableaux 1 et 2, les antigènes de la 1ère et de la 2ème série ségrégante ont été disposés de façon à faire apparaître leurs similitudes. Les accolades signifient qu'il existe des anticorps apparemment monospécifiques par absorption qui réagissent avec les spécificités comprises dans l'accolade. Les triangles reliés par une ligne pointillée représentent également des anticorps capables de réagir avec plusieurs spécificités qui n'ont pu être placées côte à côte. Il existe des anticorps réagissant parfois avec 3 ou 4 spécificités, d'autres avec seulement 2 d'entre elles. On dit que les réactivités du 2ème anticorps sont 'incluses' dans celles du 1er anticorps. Les anticorps spécifiques de chaque antigène sont disposés sur la droite des tableaux: leur réactivité est naturellement 'incluse' dans celle des anticorps précédents. La fréquence génique dans la population française est donnée dans la colonne de droite. X_1 et X_2 désignent le ou les gènes non encore décelé(s) sérologiquement. Les autres spécificités,

TABLEAU 1 *H-LA premier locus*

		Fréquence génique
HL-A1	– anti HL-A1	0.130
HL-A3	– anti HL-A3	0.128
HL-A11	– anti HL-A11	0.048
HL-A2	– anti HL-A2	0.259
W28	– anti W28	0.040
HL-A9′ (Da27)	– anti HL-A9′	0.021
HL-A9″	– anti HL-A9″	0.115
HL-A10′ (Da28)	– anti HL-A10′	0.060
HL-A10″ (Da29)	– anti HL-A10″	0.014
Da22, GE33	– anti Da22	0.040
Da25	– anti Da25	0.053
W19 Ao28, To30	– anti Ao28	0.083
Lc26-1 ou 2	– anti Lc26-1 ou 2	
X_1		0.023

Autres spécificités = Lc21, Lc26-1 ou 2, Bt15, Ao6, Ao14, THO, Ao54

HL-A9 a été aussi sub-divisé par Walford et Troup (1967), par Dausset (1971) et par Amos (1971).
HL-A10 par Dausset (1971) et par Richiardi et coll. (1971).
Da22 équivaut à GE33 et probablement à Ao77, Te63.
Da25 équivaut probablement à Lc26-1 ou 2.

TABLEAU 2 *HL-A deuxième locus*

		Fréquence génique
HL-A8	– anti HL-A8	0.081
W14	– anti W14	0.043
W18	– anti W18	0.091
HL-A5	– anti HL-A5	0.074
W5	– anti W5	0.078
W15	– anti W15	0.043
Da24	– anti Da24	0.040
HL-A12	– anti HL-A12	0.161
HL-A13	– anti HL-A13	0.018
HL-A7	– anti HL-A7	0.119
W10	– anti W10	0.046
W27	– anti W27	0.029
W22	– anti W22	0.028
Da30		0.046
W17	– anti W17	0.032
Da31		0.029
X_2		0.040

Autres spécificités 407, TT, U18, 201 KN, ET-SL, ET, CM-SL, CM,
Lc15, 16, 19, 13-14, 25, 27
Ao78, 82
Te53, 56, 64, 70, 71, 72, 73, 74
M2, M3
Sa532, 533
Anth I, Anth II

W10 a été sub-divisé par Albert (1971) en MU68, MU69 et W10*.
W22 a été sub-divisé par Thorsby *et al.* (1971) en AA-AJ et AA* et aussi par Dausset (1971) en Bt22 et Da(9) ou Da30. Da30 équivaut probablement à AA-AJ.
W27 a été sub-divisé par Thorsby en FJH* et FJH-AJ.

dont la liste est portée en bas des tableaux ont été décrites mais n'ont pas encore donné lieu à des comparaisons suffisantes avec notre laboratoire.

Malgré ces incertitudes l'existence de ces deux séries alléliques dans le système HL-A est parfaitement établie, en particulier grâce à l'étude collaborative de plus de 300 familles (Allen et coll., 1970).

Il existe entre les différents produits alléliques de chaque locus de fortes similitudes de structures qui entraînent des réactions croisées multiples (Dausset et coll., 1968*a*; Colombani et coll., 1970; Curtoni et coll., 1969; Svejgaard et Kissmeyer-Nielsen, 1968) dont les mieux identifiées sont représentées sur les Tableaux 1 et 2 par des accolades. On remarque qu'il existe des anticorps 'longs' capables de réagir avec plusieurs spécificités et des anticorps 'courts' ou spécifiques réagissant avec seulement une spécificité: leur réactivité est 'incluse' dans celle de l'anticorps 'long'.

Le produit des deux loci en position cis a été appelé un haplotype. L'étude des fréquences haplotypiques montre tout de suite que l'indépendance des deux loci n'est pas totale puisque certains haplotypes sont retrouvés avec une fréquence plus grande que ne le voudrait le simple hasard. La liste des 15 haplotypes les plus fréquents est donnée dans le Tableau 3. On

TABLEAU 3 *Les 15 haplotypes les plus fréquents dans la population française*

	Nbre d'haplotypes observés	Fréquence haplotypique
HL-A1, 8	29	0.050
2, 12	25	0.044
2, X	25	0.044
9, 12	16	0.027
9, X	15	0.026
3, 7	14	0.024
1, X	14	0.024
Da22, HL-A12	13	0.022
HL-A2, 5	12	0.020
9, 7	12	0.020
10, X	11	0.019
3, X	10	0.019
2, W5	10	0.019
2, W15	10	0.019
X, HL-A12	10	0.019

Population française testée pour HL-A1, 2, 3, 5, 7, 8, 9, 10, 11, 12, 13, W5, 10, 14, 15, 17, 18, 22, 27 et pour Da(6), Da22, 24, 25 et 30.

voit qu'il y a un déséquilibre de linkage entre les deux loci. Néanmoins les crossing-overs entre les deux loci sont relativement fréquents et on estime actuellement que le pourcentage de recombinaisons atteint 0.4% à 1% (Svejgaard et Kissmeyer-Nielsen, 1968).

De nombreuses études de linkage ont été faites et sont restées négatives pour les systèmes ABO, MN, Rh, Duffy, Kidd, Kell, Lewis, Secréteur, ABH, Gm, Inv, Gc, Ag, acide phosphatase, phosphoglucomutase, Lp, Ko et pour le sexe. Par contre certaines publications font état d'un possible linkage avec l'haptoglobine (Amos et coll., 1970) et d'un linkage avec l'enzyme érythrocytaire PGM3 (Lamm et coll., 1970, 1971). Si ces faits étaient confirmés le système HL-A se trouverait sur le chromosome 16, intermédiaire entre ces deux marqueurs.

Enfin l'existence d'un linkage avec le système P a été soupçonnée (Hronkova-Zoulkova et coll., 1968). Dans une étude de linkage faite avec F. Allen (observations non-publiées), nous n'avons pas trouvé dans l'ensemble de nos familles de liaison entre HL-A et P. Cependant

une de nos familles montrait une forte liaison qui a été interprétée comme due à une possible inversion péricentrique rapprochant, chez l'un des parents les deux loci (Tableau 4). Par ailleurs avec Fellous et coll. (1971) nous avons pu par la méthode d'hybridation homme-souris montrer que, parmi les 35 clones étudiés, 16 avaient perdus en même temps les spécificités HL-A et P. Une seule fois la spécificité P est demeurée en l'absence de spécificité HL-A (Tableau 5). La méthode d'hybridation est capable de détecter des linkages beaucoup moins étroits que la méthode familiale classique et les résultats positifs de l'une ne sont pas en contradiction avec les résultats négatifs de l'autre.

TABLEAU 4 *Famille Dupont (Nr. 179)*

	Père			Mère		
a / b	HL-A9′ W18 / X_1 W27	P+		HL-A10 5 / W19 HL-A12	c / d	P+
Enfants	ac	ad		bc	bd	
Fr	P+	Ca	P+	Mi P−	He	P+
→Au	P−	Be	P+	Pi P−		
Br	P+			El P−		
Ch	P+			Ma P−		
→Re	P−			Et P−		
				Vi P−		

Famille exceptionnelle dans laquelle une liaison entre HL-A et P a été trouvée. Pour une fréquence de recombinaisons de 0.1 le Lod Score est de 2.59.

TABLEAU 5 *Possible linkage entre HL-A et P étudié à l'aide d'une hybridation homme-souris*

Homme		Souris
HL-A2, W18 P_1		$H\text{-}2^k$
Ao28, W14 ' P_1		$H\text{-}2^k$
HL-A	P	
HL-A2 ou W14		
+	+	18
−	−	16
+	−	0
−	+	1

(D'après Fellous et coll., 1971.)

Cependant à la satisfaction d'avoir pu, malgré d'énormes difficultés sérologiques, décrire les deux séries ségrégantes doit maintenant faire place la certitude que la complexité du système ne s'arrête pas là.

Cette certitude nous est imposée par les différences de comportement des individus *génotypiquement HL-A identiques* (germains ayant reçu de leurs deux parents les mêmes haplotypes HL-A) et des individus non-apparentés *phénotypiquement HL-A* identiques alors que sous immuno-suppression seul ou pratiquement seul le système HL-A semble intervenir. Les greffes de rein faites entre les premiers persistent dans environ 100% de cas, sous immuno-suppression (Hors et coll., 1971), alors qu'il n'en est pas de même lorsque la greffe est faite

entre les deuxièmes. Les résultats des cultures mixtes de lymphocytes (MLC) sont également frappants. Il n'y a que de très rares cas de stimulation entre les premiers, alors que la stimulation est observée dans 80% des cas entre les deuxièmes (Eijsvoogel et coll., 1971).

Ces faits indiquent que les loci HL-A sont plus complexes que leur description actuelle. En attendant, l'étude bio-chimique des produits des loci HL-A, seule la sérologie peut, à l'heure actuelle nous donner des indications et nous voudrions ici apporter quelques faits nouveaux qui peuvent aider à la compréhension de la structure de ces gènes complexes.

1. EPREUVES D'ABSORPTION-INHIBITION (EN POSITION CIS, TRANS OU ALLÉLIQUE)

Avec Legrand (Legrand et Dausset, 1971) nous avons étudié systématiquement l'influence du blocage d'un des déterminants HL-A sur la possibilité d'absorber sur la même cellule un anticorps dirigé contre un autre déterminant HL-A.

Dans l'exemple de la Figure 1 une cellule possédant HL-A10 et 12 a dans un premier temps été saturée par un anti-HL-A10, puis dans un deuxième temps on a essayé de fixer un anti-

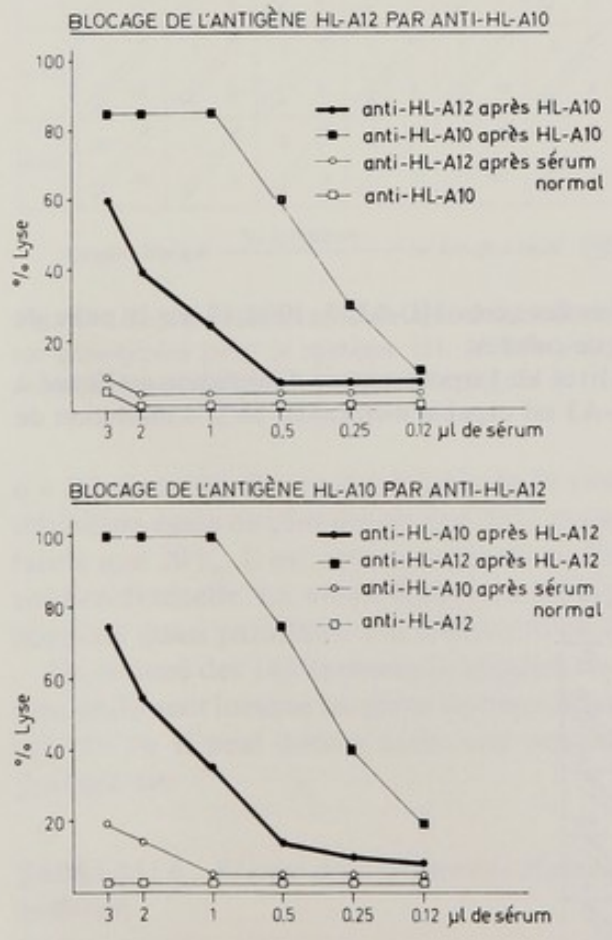


Fig. 1

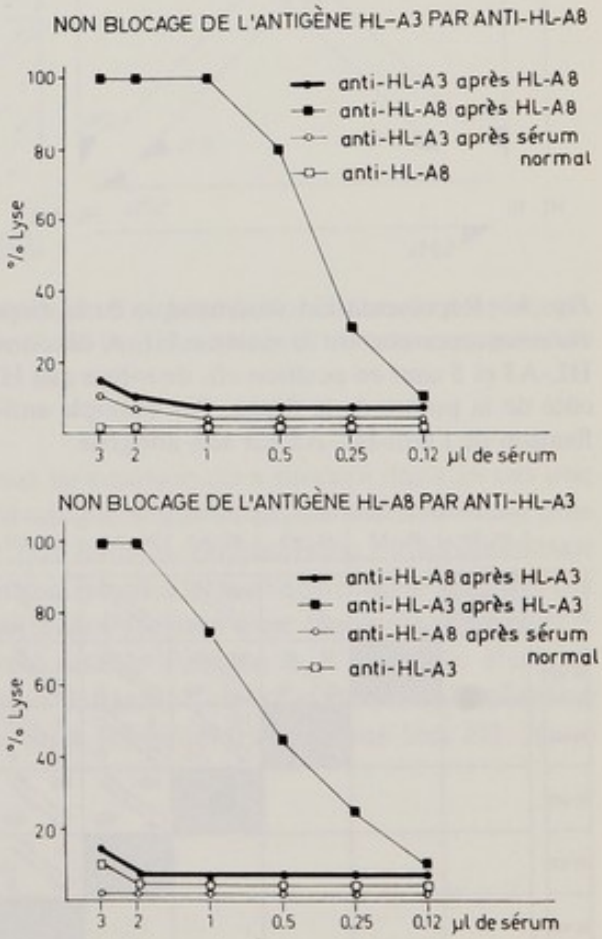


Fig. 2

Fig. 1. En haut: La fixation d'un anticorps anti-HL-A12 sur une cellule préalablement saturée avec un anticorps anti-HL-A10 est partiellement inhibée. Il reste dans le surnageant un anticorps capable à sa plus forte concentration de lyser 60% des cellules correspondantes.
En bas: Epreuve inverse, montrant également une inhibition.

Fig. 2. En haut: La fixation préalable de l'anticorps anti-HL-A8 n'a pas empêché l'absorption totale d'un anti-HL-A3. En bas: Epreuve inverse, également négative.

HL-A12. Cette fixation a été inhibée comme le montre la persistance de l'anticorps dans le surnageant capable encore de donner 60% de lyse de cellules HL-A12 positives.

De même lorsque la même épreuve a été pratiquée en sens inverse, c'est-à-dire lorsque HL-A12 a été bloqué, la fixation de l'anti-HL-A10 a été inhibée à 80%.

Dans d'autres cas au contraire, par exemple entre HL-A3 et HL-A8 aucune inhibition n'a été observée, dans un sens ou dans l'autre (Fig. 2).

Enfin, un 3ème type de réaction a été observée dans d'autres cas: l'inhibition n'est obtenue que dans une seule direction (Fig. 3).

Nous avons voulu étudier toutes les combinaisons possibles sur une cellule génotypée HL-

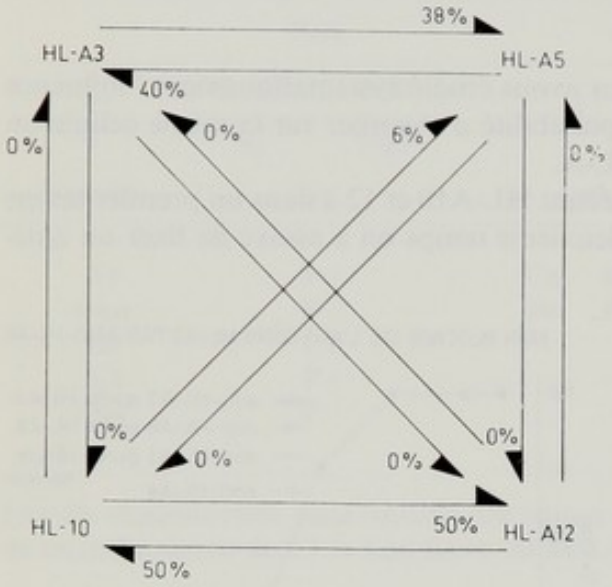


Fig. 3. Représentation schématique de la disposition des gènes HL-A3, 5, 10 et 12 sur la paire de chromosomes portant le système HL-A du donneur de cellules.
HL-A3 et 5 sont en position *cis*, de même que HL-A10 et 12. Le pourcentage d'inhibition est donné à côté de la pointe de la flèche. Par exemple anti-HL-A3 est capable d'entraîner 38% d'inhibition de fixation de l'anti-HL-A5 sur son antigène.

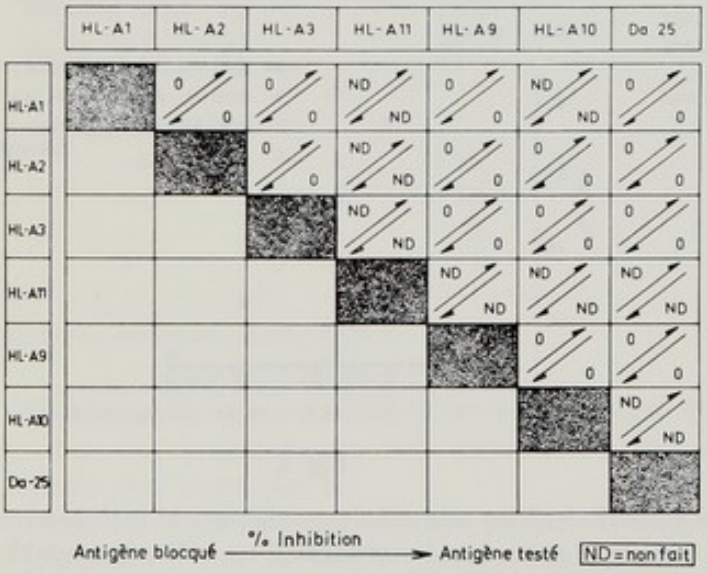


Fig. 4. Représentation schématique des résultats des épreuves d'absorption-inhibition sur des plaquettes génotypées pour le système HL-A, hétérozygotes au ler locus. Le pourcentage d'inhibition est donné au dessus ou au dessous de chaque flèche: l'antigène préalablement bloqué étant à l'arrière de la flèche, l'antigène dont on teste la capacité d'absorption étant à la pointe de la flèche.

A3, 5/10, 12 (Fig. 3). Nous avons été frappés par le fait que l'inhibition n'a été observée exclusivement que dans les quatre combinaisons en position *cis*, c'est-à-dire 3–5 et 10–12 (l'inhibition se produisant dans les deux sens). Par contre aucune inhibition n'a été observée tant en position allélique, qu'en position trans.

Poursuivant ce travail, nous avons voulu savoir si ce phénomène était général et nous avons étudié systématiquement chaque combinaison.

- 27 situations *alléliques* ont été étudiées (Fig. 4) (seules les combinaisons comportant HL-A11 n'ont pu l'être). Aucune inhibition n'a été enregistrée.
- 62 situations en position *trans* ont été étudiées (Fig. 5). Ici encore aucune inhibition

Fig. 5. Représentation schématique des résultats des épreuves d'absorption-inhibition sur des plaquettes génotypées pour le système HL-A. Les antigènes testés sont des produits de gènes en position *trans*. Mêmes symboles que pour la Figure 4.

n'a été observée. Par contre l'étude de 54 situations en position *cis* a montré dans 26 cas une inhibition égale ou plus élevée que 20% et dans 28 cas pas d'inhibition ou une inhibition plus faible que 20%. Il est remarquable de constater que dans 5 combinaisons l'inhibition était unidirectionnelle. La même épreuve a été répétée sur lymphocytes et sur plaquettes avec des résultats quasi parallèles. Par ailleurs la répétabilité de l'épreuve s'est montrée excellente.

Un résumé des 143 épreuves pratiquées est donné dans le Tableau 6. L'existence d'inhibition seulement lorsque les gènes correspondants sont en position cis est hautement significative ($\chi^2 = 51.5$). Il peut donc y avoir une interaction entre les produits des deux loci HL-A, en position cis.

TABLEAU 6 *Résumé des 143 épreuves d'absorption-inhibition*

		Position		
		Cis	Trans	Allélique
Inhibition ⩾20%	+	26	0	0
	−	28	62	27
			$\chi^2 = 51.5$	

2. EPREUVE D'ABSORPTION-INHIBITION SUR LES PRODUITS D'UN MÊME LOCUS

Le pas suivant consistait à savoir si des anticorps dirigés contre un produit d'un même locus étaient capables de s'inhiber l'un l'autre. Pour cette étude nous avons pris avantage des anticorps développés par immunisation volontaire, dans la situation génétique la plus simple c'est-à-dire entre parent et enfant.

Fig. 6. Représentation schématique des résultats des épreuves d'absorption-inhibition sur des lymphocytes et des plaquettes des *mêmes* individus, génotypés pour le système HL-A. Les antigènes testés sont des produits de gènes en position *cis*. Mêmes symboles que pour la Figure 4.

Un père HL-A9 négatif a été immunisé contre les leucocytes de son fils HL-A9 positif. Il est apparu deux anticorps monospécifiques: l'un est apparemment un anti-HL-A9 classique, l'autre, séparé du premier par absorption ne réagit qu'avec un certain nombre d'individus étiquetés HL-A9. Le fils possède donc une *variante* de HL-A9 que nous avons appelée HL-A9′ ou Da27.

Nous avons voulu savoir par les épreuves d'absorption-inhibition sur les cellules de l'immuniseur si la fixation de l'anticorps 'court' anti-HL-A9′ empêchait la fixation de l'anticorps 'long' anti-HL-A9. Les résultats ont été sans équivoque (Fig. 7). Il n'y a aucune inhibition, suggérant fortement l'existence de deux déterminants distincts, sur la cellule de l'immuniseur. La possibilité d'un déplacement d'un anticorps par l'autre plus avide est peu probable, puisque dans le surnageant aucun anticorps n'a été retrouvé. Par contre lorsque nous avons voulu fixer un autre anticorps 'long' anti-HL-A9 provenant d'un autre donneur, nous avons observé un certain degré d'inhibition, montrant bien l'extraordinaire spécificité des anticorps et leur hétérogénéité.

La même épreuve a été répétée dans le cas de HL-A3, avec des résultats tout à fait similaires. Un père HL-A3 négatif avait été immunisé contre son fils HL-A3 positif et avait fabriqué deux anticorps distincts et apparemment monospécifiques par absorption, l'un un anti-HL-A (3+11) (Da12), l'autre dirigé contre HL-A3 seulement (Fig. 8).

Les épreuves d'absorption-inhibition ont montré également qu'il n'était pas possible d'inhiber la fixation de l'anticorps 'long' par le blocage préalable du déterminant spécifique mais que ceci ne s'appliquait qu'aux anticorps autologues et non aux anticorps hétérologues (Fig. 8).

Quelles sont les considérations générales que l'on peut tirer de ces études et pour revenir à notre propos antérieur, quelles sont les différences qui peuvent exister entre deux individus

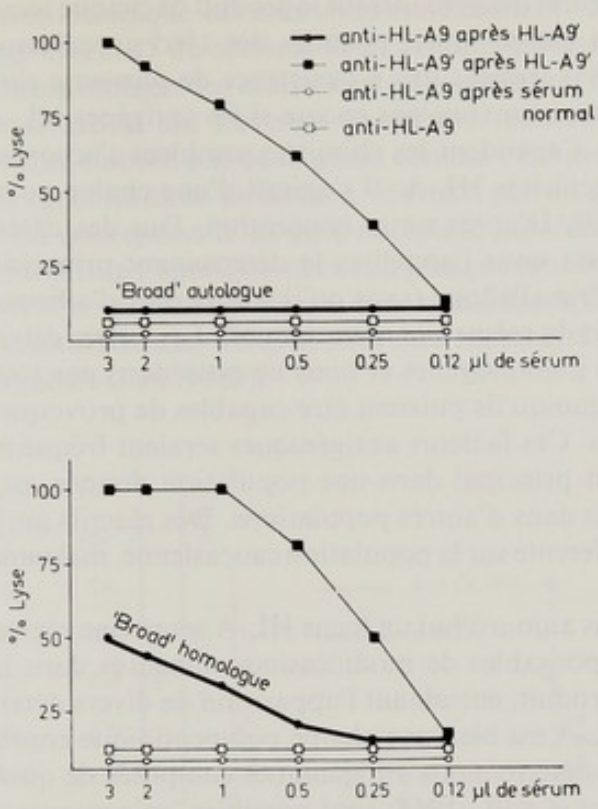


Fig. 7

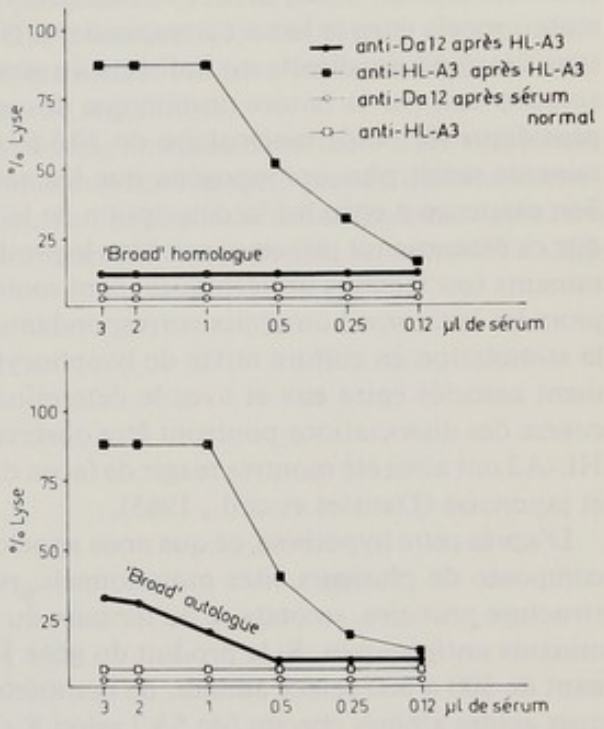


Fig. 8

Fig. 7. En haut : Epreuve d'absorption-inhibition sur les cellules du donneur ayant servi à immuniser le receveur ayant fabriqué les deux anticorps anti-HL-A9 et anti-HL-A9′ (anticorps autologue). La fixation de l'anti-HL-A9′ n'empêche pas l'absorption ultérieure de l'anti-HL-A9.
En bas : La même épreuve est pratiquée sur les mêmes cellules, mais l'anti-HL-A9 utilisé n'a pas été développé chez le même receveur que l'anti-HL-A9′ (anticorps homologue). On observe une inhibition partielle de son absorption.

Fig. 8. Mêmes épreuves que celles de la Figure 7 utilisant un anticorps spécifique anti-HL-A3 et deux anticorps anti-Dal2 (anti-HL-A (3 + 11)) l'un autologue (fabriqué par le même receveur que l'anti-HL-A3), l'autre hétérologue (fabriqué par un autre receveur).

non-apparentés HL-A phénotypiquement identiques qui sont capables de se stimuler l'un l'autre en culture mixte de lymphocytes?

Il y a au moins deux hypothèses qui d'ailleurs ne s'excluent pas mutuellement.

1. L'hétérogénéité des déterminants antigéniques de chaque série allélique constitue la première hypothèse. De nombreuses *variantes* des antigènes HL-A sont déjà décrites, comme HL-A9, 10, Da25, W10, W27 qui sont 'sub-divisés' en deux ou plusieurs variantes, elles-mêmes alléliques. L'antigène HL-A2 avait jusqu'ici résisté à une telle sub-division. Nous avons pu confirmer un sérum N323 trouvé par Hammond (1970) qui réagit avec 50% des cellules HL-A2 positives. Il est probable que ce processus de sub-division se poursuivra mais qu'il aura une limite puisqu'il y a indiscutablement des sujets HL-A phénotypiquement identiques qui ne se stimulent pas. En faveur de cette hypothèse d'hétérogénéité vient s'inscrire, en premier lieu, l'existence de nombreux anticorps réagissant de façon croisée avec ces variantes (Svejgaard et Kissmeyer-Nielsen, 1968; Colombani et coll., 1970). Ces anticorps réagissent avec les différentes variations d'un même déetrminant mais avec des intensités et avidités variables. A l'extrême l'anticorps se fixe sans être capable de provoquer l'agglutination (ANAP) ou la lyse (CYNAP). Néanmoins jusqu'à présent aucune étude d'absorption-inhibition n'a été faite systématiquement avec ces anticorps.

2. *L'existence d'autres déterminants* (ou facteurs) *antigéniques* sur le produit de chaque locus constitue la deuxième hypothèse. Ces facteurs antigéniques, postulés dès 1965 avec Ivanyi (Dausset et coll., 1965; Ivanyi et Dausset, 1966) seraient dûs à l'existence de plusieurs sites mutationnels dans le locus correspondant. Nous ne savons pas encore si les antigènes HL-A sont des produits directs ou indirects du gène. Cependant les chimistes semblent s'accorder actuellement sur la nature protéinique des spécificités HL-A: il s'agirait d'une chaîne polypeptidique de poids moléculaire de 30 à 50.000. D'après notre conception, l'un des déterminants serait plus immunogène que les autres: nous l'appelons le déterminant principal. Son existence a entraîné la description de la série allélique (sans qu'il soit permis d'affirmer que ce déterminant principal soit bien le produit du même site mutationnel). Les autres déterminants (ou facteurs antigéniques) sont moins immunogènes et nous ne possédons pas toujours les anticorps humoraux correspondants quoiqu'ils puissent être capables de provoquer la stimulation en culture mixte de lymphocytes. Ces facteurs antigéniques seraient fréquemment associés entre eux et avec le déterminant principal dans une population donnée, par contre des dissociations pourront être observées dans d'autres populations. Des réactifs anti-HL-A2 ont ainsi été montrés réagir de façon différente sur la population caucasienne, malienne et japonaise (Dausset et coll., 1965).

D'après cette hypothèse, ce que nous appelons aujourd'hui un locus HL-A serait une région composée de plusieurs sites mutationnels, responsables de modifications mineures dans la structure primaire, secondaire ou tertiaire du produit, entraînant l'apparition de divers déterminants antigéniques. Si le produit du gène HL-A est bien une chaîne polypeptidique contenant de 300 à 500 acides aminés, de nombreux déterminants antigéniques composés de quelques acides aminés chacun (de 5 à 7 selon Kabat et coll., 1968) sont possibles, en conservant pourtant à la molécule une structure commune. Il est probable que l'ensemble de la région

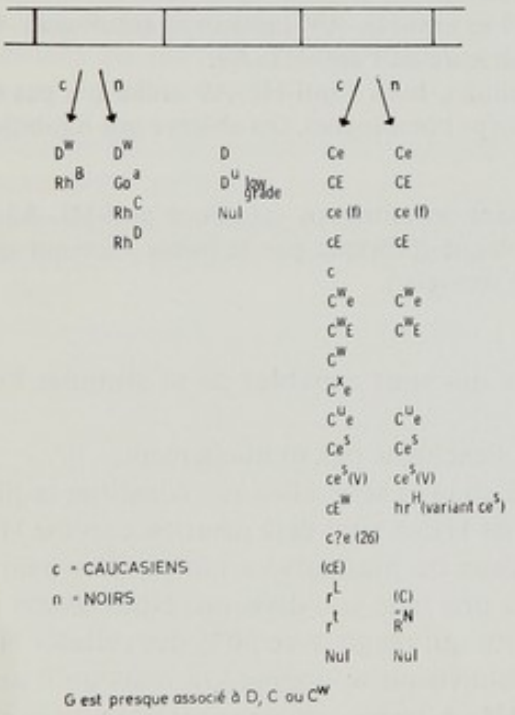


Fig. 9. Représentation schématique du segment de chromosome portant le système Rhésus comportant au moins trois sites mutationnels. La liste des allèles possibles pour chacun de ces sites est donnée verticalement. Des allèles 'nuls' c'est-à-dire sérologiquement muets sont postulés afin de rendre compte de ce que l'on attribue habituellement à des délétions. L'absence de site mutationnel spécifiquement C ou c, E ou e, est justifiée sérologiquement par l'existence de sérums spécifiques dirigés contre les déterminants contenant à la fois C ou c et E ou e. C'est indiscutablement ce site qui possède le plus grand polymorphisme. 7 allèles sont relativement fréquents chez les caucasiens, les autres sont rares ou extrêmement rares.

chromosomique est une unité fonctionnelle. Les deux régions HL-A (par analogie avec la région K et D du système H-2) ne fonctionnent pas indépendamment l'une de l'autre comme semble indiquer les inhibitions obtenues entre les produits de chacune d'elles lorsqu'elles sont en situation cis. Diverses hypothèses peuvent être avancées: (1) l'existence d'un produit unique élaboré par deux gènes comme c'est probablement le cas pour les immunoglobulines; (2) synchronisme de lecture, contrôlé par un gène de régulation.

La conception d'un gène complexe possédant plusieurs sites mutationnels n'est nullement neuve. Elle s'applique en effet à presque tous les systèmes immunogéniques actuellement connus et nous n'en prendrons qu'un seul exemple ici, en l'honneur de notre chairman, celui du système Rhésus. Celui-ci peut être repensé, à la manière du système HL-A, comme l'a déjà suggéré Allen (1969).

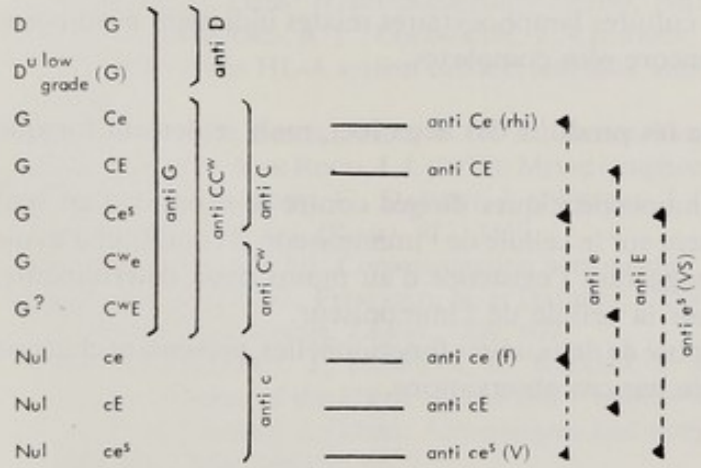


Fig. 10. Réactivité des différents anticorps actuellement décrits dans le système Rhésus contre les produits les plus fréquents du segment chromosomique Rhésus. L'anti-G réagit avec tous les produits contenant D, C, ou C^w. La réactivité de l'anti CC^w est 'incluse' dans celle de l'anti-G. De même anti-C et anti-C^w sont 'inclus' dans anti-CC^w. Enfin les anticorps spécifiques anti-Ce et anti-CE ne réagissent qu'avec une seule spécificité. Il en est de même pour anti-ce, anti-cE et anti-ce^s qui sont 'inclus' dans anti-c. Les anticorps anti-e, anti-E et anti-e^s sont représentés à droite par des triangles qui pointent vers les spécificités avec lesquelles ils réagissent.

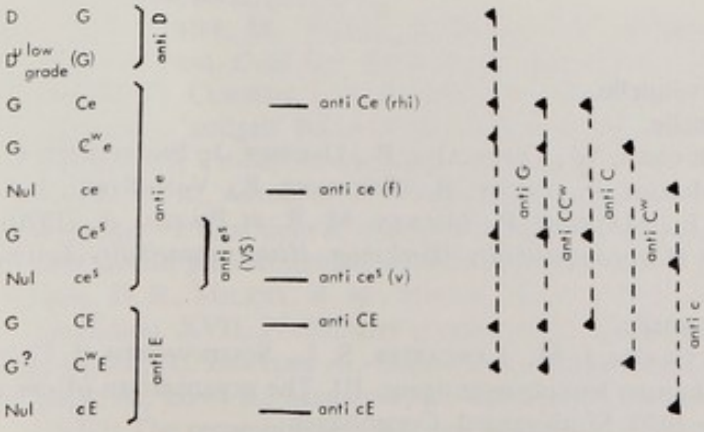


Fig. 11. Même principe que pour la Figure 10 mais ici la classification a été faite *d'abord* en fonction des anticorps anti-E et anti-e. On voit également les 'inclusions' des anticorps spécifiques dans les anticorps réagissant avec plusieurs spécificités. De même, à droite de la figure, apparaissent les anticorps anti-G, anti-CC^w, anti-C, anti-C^w et anti-c dont les triangles pointent vers les spécificités avec lesquelles ils réagissent.

Le locus Rhésus serait lui-même composé de plusieurs sites mutationnels dont les déterminants alléliques des caucasiens sont donnés sur la Figure 9. Au moins quatre sites mutationnels sont connus. D'après la sérologie, il est possible d'associer les séries C et E en une seule série CE avec 7 allèles fréquents chez les caucasiens (et 9 chez les noirs).

Les observations sérologiques peuvent être regroupées, de la même manière que dans le système HL-A. L'anti-G étant le plus 'long' anticorps, inclus l'anti-CC^w, lui-même incluant l'anti-C^w et l'anti-C. Ce dernier inclus à son tour l'anti-Ce et l'anti-CE (Fig. 10). Une classification analogue peut être faite en partant cette fois du 'long' anticorps anti-E et anti-e (Fig. 11).

Les systèmes ABO, Kell, MNSs, P, pourraient également être envisagé de la même manière et nous ne les citons qu'à l'appui de notre thèse en faveur d'un système HL-A plus complexe que l'actuelle conception limitée trop souvent à deux séries alléliques.

En conclusion: La structure génétique du système HL-A a récemment été décrite comme composée de deux loci multialléliques étroitement liés sur un autosome.

Les résultats des greffes de rein et des cultures lymphocytaires mixtes indiquent néanmoins que les loci HL-A sont probablement encore plus complexes.

Des études sérologiques ont montré:

1. Qu'il existait des interactions entre les produits des deux loci, mais seulement lorsque ceux-ci sont en position cis.

2. Que des anticorps apparemment monospécifiques dirigés contre le produit d'un seul locus HL-A peuvent se fixer simultanément sur la cellule de l'immuniseur, à condition d'avoir été développé chez le même receveur suggérant l'existence d'au moins deux déterminants, suffisamment éloignés l'un de l'autre sur la cellule de l'immuniseur.

La conception du système HL-A, composé de deux unités fonctionnelles, présentant chacune plusieurs sites mutationnels est renforcée par ces observations.

REMERCIEMENTS

Nous exprimons notre reconnaissance a Ph. Levine, R. Race, R. Rosenfield et F. H. Allen pour leur précieux conseils et encouragements à dégager les analogies possibles entre les systèmes Rhésus et HL-A, à J. et M. Colombani pour leur importante contribution concernant les réactions croisées, ainsi qui tous les volontaires qui sont venus généreusement donner régulièrement leur sang pour la réalisation de ce travail, ainsi que ceux qui ont accepté d'être immunisé. L'aide technique de Mr. J. P. Roy a été extrêmement précieuse.

BIBLIOGRAPHIE

ALBERT, E. D. (1971): Communication personnelle.

ALLEN, F. (1969): Communication personnelle.

ALLEN, F., AMOS, D. B., BATCHELOR, R., BODMER, W., CEPPELLINI, R., DAUSSET, J., ENGELFRIET, C., JEANNET, M., KISSMEYER-NIELSEN, F., MORRIS, P., PAYNE, R., TERASAKI, P., VAN ROOD, J. J., WALFORD, R., ZMIJEWSKI, C., ALBERT, E., MATTIUZ, P., MICKEY, M. R. et PIAZZA, A. (1970): *Joint Report of the Fourth International Histocompatibility Workshop. Histocompatibility Testing 1970.* Munksgaard, Copenhagen.

AMOS, D. B. (1971): Communication personnelle.

AMOS, D. B., CABRERA, G., BIAS, W. B., MCQUEEN, J. M., LANCASTER, S. L., SOUTHWORTH, J. G. et WARD, F. F. (1970): The inheritance of human leukocyte antigens. III. The organization of specificities. Dans: *Histocompatibility Testing 1970.* Munksgaard, Copenhagen.

BODMER, W., BODMER, J., ADLER, S., PAYNE, R. et BIALEK, J. (1966): Genetics of 4 and LA human leukocyte groups. *Ann. N.Y. Acad. Sci., 129*, 473.

CEPPELLINI, R., CURTONI, E. S., MATTIUZ, P. L., MIGGIANO, V., SCUDELLER, G. et SERRA, A. (1967): Genetics of leukocyte antigens. A family study of segregation and linkage. Dans: *Histocompatibility Testing 1967*, p. 149. Munksgaard, Copenhagen.

COLOMBANI, J., COLOMBANI, M. et DAUSSET, J. (1970): Cross-reactions in the HL-A system with special

reference to Da6 cross-reacting group. Description of HL-A antigens Da22, Da23, Da24 defined by platelet complement fixation. Dans: *Histocompatibility Testing 1970,* pp. 79–92. Munksgaard, Copenhagen.

CURTONI, E. S., RICHIARDI, P., SCUDELLER, G., BERNOCO, D. et CEPPELLINI, R. (1969): Studio delle reazioni croizate fra gli antigeni del sistema HL-A e loro interpretazione immunogenetica. *Riv. Emoter. Immunoemat., 16*, 179.

DAUSSET, J. (1958): Iso-leuco-anticorps. *Acta haemat. (Basel), 20*, 156.

DAUSSET, J. (1971): The genetics of transplantation antigens. *Transplant. Proc., 3*, 8.

DAUSSET, J., COLOMBANI, J., COLOMBANI, M., LEGRAND, L. et FEINGOLD, N. (1968*a*): Un nouvel antigène du système HL-A (Hu-1): l'antigène 15, allèle possible des antigènes 1, 11, 12. *Nouv. Rev. franç. Hémat., 8/3*, 398.

DAUSSET, J., COLOMBANI, J., LEGRAND, L. et FEINGOLD, N. (1968*b*): Le deuxième sub-locus du système HL-A. *Nouv. Rev. franç., Hémat., 8/6*, 841.

DAUSSET, J., IVANYI, P. et IVANYI, D. (1965): Tissue alloantigens in human identification of a complex system (Hu-1). Dans: *Histocompatibility Testing, 1965*, pp. 51–62. Munksgaard, Copenhagen.

DAUSSET, J., WALFORD, R. L., COLOMBANI, J., LEGRAND, L., FEINGOLD, N., BARGE, A. et RAPAPORT, F. T. (1969): The HL-A system sub-loci and their importance in transplantation. *Transplant. Proc., 1*, 331.

EIJSVOOGEL, V. P., SCHELLEKENS, P. T. A., BREUR-VRIESENDORF, B., KONING, L., KOCH, G., VAN LEEUWEN, A. et VAN ROOD, J. J. (1971): Mixed lymphocyte culture and HL-A. *Transplant. Proc., 3*, 85.

FELLOUS, M., BILLARDON, C., DAUSSET, J. et FREZAL, J. (1971): Linkage probable entre les loci HL-A et P. *C. R. Acad. Sci. (Paris), 272*, 3356.

HAMMOND, M. G. (1970): Communication personnelle.

HORS, J., FRADELIZI, D., FEINGOLD, N. et DAUSSET, J. (1971): Critical evaluation of histocompatibility in 179 renal transplants. *Lancet, 7700*, 609.

HRONKOVA-ZOULKOVA, J., IVASKOVA, E., KLOUDA, P. et IVANYI, P. (1968): A possible correlation between antigens of the HL-A system and P system. *Folia biol. (Praha), 14*, 402.

IVANYI, P. et DAUSSET, J. (1966): Allo-antigens and antigenic factors of human leukocytes. An hypothesis. *Vox Sang. (Basel), 11*, 3.

KABAT, E. A. (1968): *Structural Concepts in Immunology and Immunochemistry*. Holt, Rinehart and Winston, New York, N.Y.

KISSMEYER-NIELSEN, F., SVEJGAARD, A. et HAUGE, M. (1968): Genetics of the human HL-A transplantation system. *Nature (Lond.), 219/5159*, 1116.

LAMM, L. U., KISSMEYER-NIELSEN, F. et HENNINGSEN, K. (1970): Linkage and association studies of two phosphoglucomutase loci. *Hum. Hered., 20*, 305.

LAMM, L. U., SVEJGAARD, A. et KISSMEYER-NIELSEN, F. (1971): Further evidence for PGM_3: HL-A linkage. *Nature New Biol., 21*, 109.

LEGRAND, L. et DAUSSET, J. (1971): Cell-surface interaction between HL-A antigen determinants. *Nature New Biol., 234*, 271.

PAYNE, R., TRIPP, M., WEIGLE, J., BODMER, W. et BODMER, J. (1964): A new leucocyte isoantigen system in man. *Cold Spr. Harb. Symp. quant. Biol., 29*, 285.

RICHIARDI, P., CURTONI, E. S., PIAZZA, A. et MATTIUZ, P. L. (1971): Identification of two determinants included in 'antigen' HL-A10 (To 31 and To 40) and of a new family of cross-reacting specificities (HL-A1, 11, To 30, 31, 40). *Tissue Antigens, 1*, 162.

SHULMAN, N. R., ASTER, R. H., PEARSON, H. A. et HILLER, M. C. (1962): Immuno-reactions involving platelets. VI. Reactions of maternal isoantibodies responsible for neonatal purpura. Differentiation of a second platelet antigen system. *J. clin. Invest., 41/5*, 1059.

SINGAL, D. B., MICKEY, R. M., MITTAL, K. K. et TERASAKI, P. I. (1968): Serotyping for homo-transplantation. XVII. Preliminary studies of HL-A sub-unit and alleles. *Transplantation, 6*, 904.

SVEJGAARD, A., BRATLIE, A., HEDIN, P. J., HÖGMAN, C., JERSILD, C., KISSMEYER-NIELSEN, F., LINDBLOM, A., LOW, B., MESSETER, L., MÖLLER, E., SANDBERG, L., STAUB-NIELSEN, L. et THORSBY, E. (1971): The recombination fraction of the HL-A system. *Tissue Antigens, 1*, 81.

SVEJGAARD, A. et KISSMEYER-NIELSEN, F. (1968): Cross-reactive human HL-A isoantibodies. *Nature (Lond.), 219/5156*, 868.

THORSBY, E., BRATLIE, A., MAYR, W., SPARCK, J., SVEJGAARD, A., KJERBYE, K. E. et KISSMEYER-NIELSEN, F. (1971): The HL-A system: antigenic heterogeneity and four new antigens. *Tissus antigens, 1*, 32.

VAN ROOD, J. J. et VAN LEEUWEN, A. (1963): Leukocyte grouping. A method and its application. *J. clin. Invest., 42/9*, 1382.

VAN ROOD, J. J., VAN LEEUWEN, A., SCHIPPERS, A. M. J., VOOYS, W. H., BALNER, E. F. H. and EERNISSE, J. C. (1965): Leukocyte groups, the normal lymphocyte transfer test and homograft sensitivity. Dans: *Histocompatibility Testing 1965*, p. 37. Munksgaard, Copenhagen.

WALFORD, R. L. et TROUP, G. M. (1967): Monospecific lymphocytotoxic antisera, an absorption study. *Vox Sang. (Basel), 12*, 173.

WALFORD, R. L., WALLACE, O., SHANBROM, E. et TROUP, G. M. (1968): Lc-11 (Hunt B, Jones) as a mutually exclusive specificity to Lc-1, 2 and 3 in the main human leukocyte group. *Vox Sang. (Basel), 15*, 338.

Jane S. Schultz and D. C. Shreffler

Department of Human Genetics, University of Michigan, Ann Arbor, Mich., U.S.A.

Genetics and immunochemistry of the Lp antigen of human serum*

Since the discovery in 1956 of immunoglobulin allotypes in human serum by Grubb and Laurell and in rabbit serum by Oudin (1956) many immunologically detected serum protein variants have been described in man and in a number of other species.

The immunoglobulin variants are by far the most extensively studied and the best understood of the immunologically detected variants in human serum. They are easily the subject for a full presentation in themselves. The Gm and Inv systems have been well reviewed elsewhere; no details will be presented here. We will simply point out three key findings with these systems which are of general importance to the immunogenetics of the human serum proteins: (1) the demonstration that individual genetic differences (structural differences) in specific serum proteins can be detected by alloimmune human sera; (2) the description of an extensive serological complexity of the system and the demonstration that the multiple antigenic specificities which segregate as single mendelian units are controlled by a complex of three or more closely linked but distinct genetic loci; (3) the definition of the molecular basis for at least some of these alloantigenic differences as very restricted deviations in amino acid sequences – one or two amino acid substitutions in a specific polypeptide subunit of the immunoglobulin molecule.

There are a number of other aspects of the genetic control of the immunoglobulin molecule which are also extremely interesting, though not yet well understood. Certainly this system is a very important model for the immunologically detected protein variants.

Two antigenic systems which have great potential interest because of their apparent sex-linked mode of inheritance are the Xm and Xh systems, both of which are reported to be polymorphisms of the α_2-macroglobulin fraction and both of which are detectable by Ouchterlony double diffusion test. Xm in limited family studies showed an X-linked mode of inheritance, while Xh could be inherited either as a sex-influenced autosomal trait or a sex-linked trait. Unfortunately, anti-Xm antiserum has been produced only once.

In the case of the Gc system of serum proteins, antigenic properties are of only secondary importance in describing the polymorphism. Gc variants with varying electrophoretic mobilities are detected by immunoelectrophoresis in agar gels. Anti-Gc antibody is used to develop the Gc precipitin bands. It has not been possible to distinguish between Gc1 and Gc2 immunologically. There is one report, however, of immunologically variant forms of Gc1 (Ruoslahti, 1965).

* This research was supported by U.S. Atomic Energy Commission Contract AT (11-1)-1552, by U.S.P.H.S. Research Career Development Award K3-HE-24980 to D. C. Shreffler, and by U.S.P.H.S. Training Grant 2TOI-GM-00071 to J. S. Schultz.

A major interest in our laboratory for the past several years has been the immunologically detected polymorphisms of the β- or low-density lipoproteins. Two variant systems have been reported, the Ag system and the Lp system. The former was identified by Allison and Blumberg (1961). An isoprecipitin was found in the serum of a multi-transfused patient. The specific antigen against which this antibody was directed was termed Ag(a). Since that time a number of additional low-density lipoprotein specificities have been identified through the use of antibodies found in the sera of multi-transfused patients. These include Ag(x), Ag(y), Ag(z) and Ag(t). No entirely clear picture of the inheritance of these antigens has thus far emerged, but Hirschfeld (Hirschfeld and Ritter, 1969) has proposed a model invoking two or three closely linked loci. He found no genetic recombination in 31 critical matings. All specificities seem to be under autosomal dominant control and several anti-Ag antibodies may be present in the serum of one individual. The original anti-Ag donor serum contained anti-(a), (x) and (z).

One study (Rittner and Goenechea, 1969) attempted to localize Ag antigen in subfractions of the β-lipoprotein. Low-density lipoprotein was subjected to equilibrium density gradient centrifugation and each density fraction was tested for ability to react with anti-Ag(t) and anti-Ag(x). Both specificities were found in all β-lipoprotein fractions, indicating that Ag(x) and Ag(t) antigens are heterogeneous in density but have comparable density distributions. Thus far there have been no reports of evidence relating to the structural basis for the antigenic differences. The presence of Ag(x) and Ag(y) on separate β-lipoprotein molecules has been shown by reactions of non-identity between serum of an Ag(x + y +) individual with anti-Ag(x) and anti-Ag(y) reagents (Hirschfeld, 1968). It is not clear, however, whether Ag specificity resides in the protein, carbohydrate or perhaps the lipid portion of the β-lipoprotein molecule.

The second of the β-lipoprotein polymorphic systems is the Lp system, described by Berg (1963). Berg noted that the Ag polymorphism had been found in low-density lipoproteins and that large quantities of low-density lipoprotein could be readily isolated by a number of methods. He therefore began immunizations of rabbits with either pools of human sera or isolated preparations of β-lipoprotein, in an effort to produce anti-Ag sera. When the antisera produced in this manner were absorbed with a panel of individual normal sera and tested by immunodiffusion, absorptions with certain sera left reactions with other sera. Those normal sera which precipitated with reactive absorbed antisera and which themselves absorbed all antibody activity were termed Lp(a+) sera. Those normal sera which failed to precipitate with reactive absorbed antisera or to absorb the antibody activity were termed Lp(a−) sera.

A study of 175 Norwegian families (Berg, 1966*a*), designed to determine the mode of inheritance of the Lp(a) antigen, yielded data consistent with the hypothesis that a single autosomal dominant gene controls the presence of the Lp(a) antigen in serum. The genes controlling presence or absence of Lp(a) antigen were termed Lp^a and Lp^0, respectively, with Lp(a+) individuals of genotype $Lp^a/-$ and Lp(a−) individuals of genotype Lp^0/Lp^0. No linkage or association of the Lp system with other genetic markers was found (Mohr and Berg, 1963).

We initiated a study of the Lp system with the goal of determining the molecular basis for the difference between Lp(a+) and Lp(a−) sera. We, as well as others, have obtained data bearing on this problem. In the course of these studies we have also obtained data which raise questions about the proposed mode of inheritance of Lp lipoprotein differences.

Our method of isolation and partial purification of the Lp antigen involves equilibrium density centrifugation in a model L Spinco preparative ultracentrifuge (Schultz *et al.*, 1968). After an initial precipitation of β-lipoprotein with dextran sulfate, the precipitate is dissolved and fractionated on a sodium bromide gradient. The Lp protein is localized in the fraction of the gradient with the density of approximately 1.085. This density is somewhat higher than the density of the LDL (β) (1.063) but below that of the HDL (1.12). Using this method we find that we can achieve a cleaner separation than that which is accomplished on hydroxyl apatite using the method of Cramer and Brattsten (1961), the original isolation method

employed by Berg. However, ultracentrifugal isolation of Lp(a) lipoprotein is confounded by the similarity in density of Lp(a) lipoprotein and the higher-density end of the β-lipoprotein distribution. The purification problem is further complicated by the fact that Lp protein cross-reacts immunologically with β-lipoprotein (Schultz *et al.*, 1968; Wiegandt *et al.*, 1968). Our results show that all Lp molecules have β-lipoprotein antigenic sites, but not all β-lipoprotein molecules have Lp antigenic sites. Anti-β-lipoprotein will completely remove Lp activity from a serum, but β-lipoprotein activity is only slightly reduced by absorption with anti-Lp. This cross-reactivity makes it impossible to remove contaminating β-lipoprotein from Lp antigen preparations by immunological methods.

Recently, a method of isolation of Lp protein in which accompanying β-lipoprotein was virtually eliminated was reported by Simons *et al.* (1970). This method employs gel filtration on Sepharose 2B as well as preparative ultracentrifugation. Using Lp lipoprotein isolated by this method some of the physical and chemical characteristics of the Lp lipoprotein were elucidated. The lipoprotein was found to have a molecular weight of about 5 million, a high sialic acid content, and an amino acid composition which differed from both LDL and HDL. Lipid compositions of LDL and Lp(a) were found to be quite similar.

Analytical ultracentrifuge sedimentation patterns of the partially purified Lp lipoprotein fraction prepared in our laboratory showed a small peak just ahead of the β-lipoprotein peak. In initial preparations this peak was found to be present only in preparations from sera of those individuals classified as Lp(a+) on Ouchterlony double diffusion tests. The peak was sharply reduced in the serum of individuals classified as weak Lp(a+) and was completely absent in preparations from Lp(a−) sera.

In later experiments employing the same isolation methods the 1.085 fractions from Lp(a−) sera were concentrated approximately 120-fold prior to the analytical ultracentrifuge analysis. Schlieren diagrams of these preparations revealed a very small analytical ultracentrifuge peak in the same position as the Lp peak of strong Lp(a+) preparations.

Wiegandt *et al.* (1968) have shown that the fraction 1.05–1.12 ρ from Lp(a+) individuals yields three bands on polyacrylamide disc gel electrophoresis. The slowest of these bands is absent from unconcentrated Lp(a−) serum. This is therefore considered to be the 'Lp' band. Concentrated 1.05–1.12 ρ fractions from Lp(a−) sera have, however, been shown to contain a band with the same migration on disc gel electrophoresis as the Lp band in Lp(a+) sera (Utermann and Wiegandt, 1970). These data lead us to the conclusion that Lp(a) protein is probably present in at least trace amounts in all human sera. Because the variation therefore appears to be quantitative rather than simple presence-absence, this raised the question whether the genetic control of this trait is as simple as it first appeared to be.

A number of heretofore unreported facts have emerged from several population and family studies undertaken in our laboratory. These findings further underscore the complexity of genetic control of the Lp trait. A higher value than that of other investigators for an American Negro population of 276 individuals was obtained. This value matched the frequency previously found for African tribes (Wendt *et al.*, 1967), but significantly exceeded the value which had previously been reported for an American Negro population (Berg, 1966*b*). A Caucasian population of 3,579 individuals including 360 families was classified for Lp(a) antigen. We found an Lp(a+) frequency in this population of approximately 50% as compared with a 35% frequency obtained by several previous investigators working with comparable populations. Discrepancies in frequency of Lp(a+) individuals were also found in our own laboratory when different antisera were used. This finding could readily explain the differences in frequencies obtained by different investigators. Lp(a+) phenotype frequency was also found to increase with age of the population under study.

Such confounding effects must certainly complicate any genetic analysis, and might lead to doubts as to whether Lp variation is even under genetic control. A strong familial effect was noted; the per cent Lp(a+) offspring decreases from 64% in Lp(a+) strong × Lp(a+) strong matings to 13% in Lp(a−) × Lp(a−) matings. This would indicate a significant

genetic role in the variation. However, the occurrence of 13% Lp(a+) offspring from Lp(a−) × Lp(a−) matings as well as a high frequency of Lp(a−) offspring resulting from Lp(a+) × Lp(a+) matings (36%, when no simple genetic hypothesis predicts more than 25% negatives from such matings) is inconsistent with the single autosomal dominant mode of inheritance previously proposed. There are at least six pieces of evidence which tend to suggest that the Lp variation may reflect a continuous quantitative distribution of antigen rather than simple presence or absence:

1. Family data which can only be explained by postulating quantitative genetic control.
2. The findings of Lp antigen in the serum of all individuals in widely varying amounts.
3. The surprising fact that our original anti-Lp(a) antiserum was prepared against the serum of an individual who was consistently classified Lp(a−) on Ouchterlony double diffusion.
4. The inability of several investigators to find an isoimmune anti-Lp(a) antiserum.
5. Different Lp(a+) frequencies obtained in the same populations with different antisera.
6. Failure to produce an antibody antithetical to anti-Lp(a).

To investigate this question further we therefore undertook the development of a more sensitive quantitative test for Lp antigen in order to determine the distribution of this antigen in the population. A partially purified preparation of Lp antigen was labelled with ^{125}I by

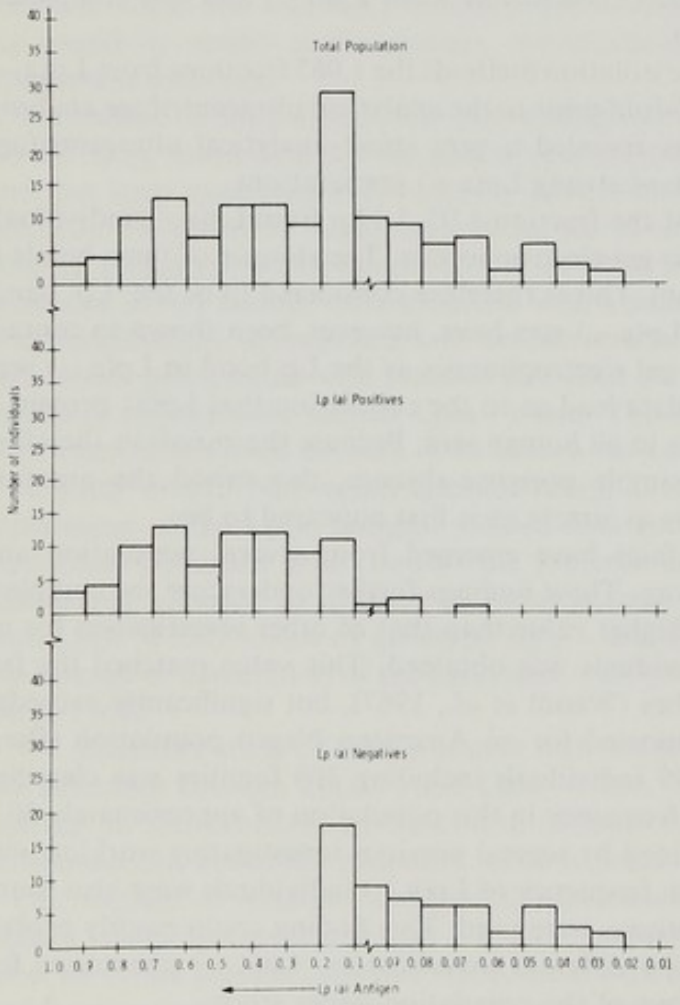


Fig. 1. Histogram of the distribution of quantity of Lp antigen in a sample of 145 random individuals. The units used in this figure are arbitrary units (amount of unknown serum required to inhibit 50% of ^{125}I-labelled Lp antigen uptake by immunoabsorbent/amount of standard serum required to inhibit 50% of ^{125}I-labelled Lp antigen uptake by immunoabsorbent).

the method of Reif (1967). Anti-Lp(a) immunoabsorbent was prepared by the method described by Cuatrecasas (1970). Immunoglobulin from anti-Lp(a) antiserum was precipitated by ammonium sulfate and added to cyanogen bromide-treated Sepharose. To quantitate Lp a measured quantity of unknown serum was added to the immunoabsorbent, followed in 16-20 hours by a measured quantity of ^{125}I-labelled Lp preparation. An Lp level for each serum was then calculated, based on the amount of serum required to inhibit 50% of the uptake of ^{125}I-labelled Lp antigen by immunoabsorbent. The levels obtained for a sample of 145 random sera obtained from the serology laboratories at the University of Michigan Hospital ranged from a value of 1.00 (arbitrary units) for the strongest Lp(a+) serum to 0.02 for a Lp(a−) serum with an extremely low level of antigen. The distribution of values obtained is shown in Figure 1.

The quantitative distribution of Lp antigen in this sample population is continuous. It appears that, for the antiserum used in these tests, the threshold level for distinguishing between positive and negative reactions lies near the peak of the distribution with some overlap of positive and negative reactions in the range of 0.1 to 0.2 (arbitrary units). This overlap is probably a result of errors of measurement in the Ouchterlony immunodiffusion test and could account for discrepancies in Lp(a+) frequencies found for Caucasian populations by different investigators.

It appears, therefore, that Lp antigen levels, contrary to early reports, are continuously distributed, at least in the Caucasian population. Production of Lp(a) antigen is doubtless under genetic control, but the number of genes involved and the influences of environment on its expression are still not fully determined. It seems inescapable that more than one gene is involved. It would be tempting to postulate, on the basis of previous published evidence, that a single major gene is involved in the expression of Lp(a) antigen, but our family data do not support even this. More extensive studies will clearly be required.

TABLE 1 *Inhibition of anti-HL-A with whole sera and serum fractions*

Antisera inhibited with	% Cytotoxicity	
	Anti-HL-A2	Anti-HL-A7
A. —	90	90
B. HL-A2+, Lp(a+) serum	25	—
C. HL-A2+, Lp(a−) serum	30	—
D. HL-A2−, Lp(a+) serum	85	—
E. HL-A2−, Lp(a−) serum	90	—
F. HL-A7+, Lp(a+) serum	—	10
G. HL-A7−, Lp(a−) serum	—	90
H. HL-A7−, Lp(a+) serum	—	90
I. β fraction from 7+, 2+, Lp removed	80	75
J. Lp fraction from 7+, 2+ serum	75	75
K. 7+, 2+, Lp(a)+ serum inhibited with anti-Lp	30	45
L. 7+, 2+, Lp(a)+ serum inhibited with anti-β	20	65
M. Dextran sulfate ppt of 7+, 2+, Lp+	90	90
N. Supernatant from dextran sulfate ppt	10	50
O. Eluent from hydroxyl apatite column, β removed (starting material whole serum)	15	45
P. β from hydroxyl apatite column (starting material whole serum)	90	85
Q. Eluent from hydroxyl apatite column, β removed (starting material dextran sulfate supernatant)	35	40
R. β fraction from hydroxyl apatite column (starting material dextran sulfate supernatant)	80	90

We would like now to turn to another aspect of the immunogenetics of the Lp system. Recently, three separate reports have noted the capacity of serum from donors positive for HL-A7 (Charlton and Zmijewski, 1970), HL-A2 (Van Rood *et al.*, 1970*a*, *b*) and HL-A7b (Van Rood *et al.*, 1970*b*) to specifically absorb the corresponding anti-HL-A antisera. One of these reports (Charlton and Zmijewski, 1970) showed the absorbing activity for anti-HL-A7 to be located in the β-lipoprotein subfraction of serum. Furthermore, a correlation between donor-recipient matching for Lp(a) type and skin graft survival has been reported (Berg *et al.*, 1968), as well as a similarity between amino acid composition of HL-A and the protein part of Lp lipoprotein (Berg, 1971). Therefore, it appeared possible that some relationship might exist between the serum HL-A substances and Lp(a) antigen.

We have examined the relationship between Lp antigen and the soluble inhibitors of anti-HL-A7 and anti-HL-A2 by utilizing partially purified Lp preparations from individuals positive for these HL-A antigens to absorb the corresponding HL-A antisera. The results of these studies are summarized in Table 1. Eight experimental results argue against any relationship between Lp antigen (or β-lipoprotein) and the HL-A active serum inhibitors:

1. A partially purified Lp preparation from a serum which inhibits anti-HL-A2 and anti-HL-A7 does not inhibit these antisera.
2. A β-lipoprotein preparation (Lp-free) from a serum which inhibits anti-HL-A2 and anti-HL-A7 does not inhibit these antisera.
3. Absorption with anti-Lp does not remove or reduce the capacity of a serum to inhibit either anti-HL-A2 or HL-A7.
4. Absorption with anti-β does not remove the capacity of a serum to inhibit anti-HL-A2 or anti-HL-A7.
5. The β-lipoprotein fraction from a hydroxyl apatite column separation of appropriate whole serum (the fraction which contains Lp activity) does not absorb anti-HL-A2 or anti-HL-A7; however, these antisera are inhibited by the column fractions containing the remainder of the serum proteins.
6. When β-lipoproteins are removed from whole serum by dextran sulfate precipitation, the supernatant inhibits anti-HL-A2 and anti-HL-A7 but the β-lipoprotein fraction does not.
7. When the supernatant mentioned in point 6 is fractionated on a hydroxyl apatite column, the fraction which would ordinarily contain the β activity lacks ability to inhibit HL-A2 or HL-A7. The fraction containing the remaining proteins does inhibit both antisera.
8. The Lp phenotype of a serum has no effect on its ability to inhibit anti-HL-A. Only the HL-A phenotype of the serum donor affects its inhibitory capacity. For example, an HL-A2 Lp(a−) serum absorbs anti-HL-A2 as well as an HL-A2 Lp(a+) serum.

Since other data have shown no linkage between the Lp trait and HL-A (Dausset *et al.*, 1968), it would appear at this point that there is neither a genetic nor an antigenetic relationship between Lp protein and HL-A antigens. Thus, both the physiological significance and the genetic control of the Lp protein remain interesting subjects for future investigations.

REFERENCES

Allison, A. C. and Blumberg, B. S. (1961): An isoprecipitin reaction distinguishing human serum-protein types. *Lancet, 1*, 634.

Berg, K. (1963): A new serum type system in man. The Lp system. *Acta path. microbiol. scand., 59*, 369.

Berg, K. (1966*a*): Further studies on the Lp system. *Vox Sang. (Basel), 11*, 419.

Berg, K. (1966*b*): The Lp system as an example of polymorphism in human sera, revealed by antisera from animals. *Internationale Lp Arbeitstagung*.

Berg, K. (1971): Compositional relatedness between histocompatibility antigens and human serum lipoproteins. *Science, 172*, 1136.

Berg, K., Ceppellini, R., Curtoni, E. S., Mattiuz, P. L. and Bearn, A. G. (1968): The genetic

antigenic polymorphisms of human serum β-lipoprotein and survival of skin grafts. In: *Advance in Transplantation*, p. 253. Munksgaard, Copenhagen.

CHARLTON, R. K. and ZMIJEWSKI, C. M. (1970): Soluble HL-A7 antigen: localization in the β-lipoprotein fraction of human serum. *Science, 170*, 636.

CRAMER, K. and BRATTSTEN, K. (1961): Characterization of β-lipoproteins isolated from hydroxylapatite columns. *J. Atheroscler. Res., 1*, 335.

CUATRECASAS, P. (1970): Protein purification by affinity chromatography. Derivatizations of agarose and polyacrylamide beads. *J. biol. Chem., 245*, 3059.

DAUSSET, J., CONTU, L., FEINGOLD, N. and BERGE, A. (1968): Indépendance du système d'histocompatibilité HL-A et des systèmes de groupes des beta-lipoprotéines Lp et Ag. *Nouv. Rev. franç. Hémat., 8*, 868.

GRUBB, R. and LAURELL, A. B. (1956): Hereditary serological human serum groups. *Acta path. microbiol. scand., 39*, 390.

HIRSCHFELD, J. (1968): The Ag system – comparison of different isoprecipitin sera. *Ser. Haematol., 1/1*, 38.

HIRSCHFELD, J. and RITTER, C. (1969): Inheritance of the Ag (x, y and z) antigens. *Vox Sang. (Basel), 14*, 129.

MOHR, J. and BERG, K. (1963): Genetics of the Lp serum types: associations and linkage relationships. *Acta genet. (Basel), 13*, 343.

OUDIN, J. (1956): L'allotypie de certains antigènes protéidiques du sérum. *C. R. Acad. Sci. (Paris), 242*, 2606.

REIF, A. E. (1967): A simple procedure for high-efficiency radioiodination of proteins. *J. nucl. Med., 9*, 148.

RITTNER, CH. and GOENECHEA, S. (1969): Zur Lokalisierung von Ag und Lp durch Dichtegradienten-Ultrazentrifugierung. *Z. Immun.-Forsch., 137*, 390.

RUOSLAHTI, E. (1965): Further polymorphism in the Gc system: evidence for the occurrence of subgroups. *Ann. Med. exp. Biol. Fenn. (Helsinki), 43*, 213.

SCHULTZ, J. S., SHREFFLER, D. C. and HARVIE, N. R. (1968): Genetic and antigen studies and partial purification of a human serum lipoprotein carrying the Lp antigenic determinant. *Proc. nat. Acad. Sci. USA, 61*, 963.

SIMONS, K., EHNHOLM, C., RENKONEN, O. and BLOTH, B. (1970): Characterization of the Lp(a) lipoprotein in human plasma. *Acta path. microbiol. scand., 78B*, 459.

UTERMANN, G. and WIEGANDT, H. (1970): Disk-electrophoretischer Nachweis des Lp(a)-Proteins in Lipoproteinfraktionen. *Humangenetik, 11*, 66.

VAN ROOD, J. J., VAN LEEUWEN, A. and VAN SANTEN, M. C. T. (1970*a*): Anti HL-A2 inhibitor in normal human serum. *Nature (Lond.), 226*, 366.

VAN ROOD, J. J., VAN LEEUWEN, A., KOCH, C. T. and FREDERIKS, E. (1970*b*): HL-A inhibiting activity in serum. In: *Histocompatibility Testing*, p. 483. Munksgaard, Copenhagen.

WENDT, G. G., ERMERT, A., KIRCHBERG, G. and KINDERMANN, I. (1967): ABO Blutgruppen und Serumgruppen bei den Negerstämmen Peulh und Marka. *Humangenetik, 4*, 74.

WIEGANDT, H., LIPP, K. and WENDT, G. G. (1968): Identifizierung eines Lipoproteins mit Antigenwirksamkeit im Lp-system. *Hoppe-Seylers Z. physiol. Chem., 349*, 489.

Kåre Berg

Institute of Medical Genetics, University of Oslo, Blindern, Oslo, Norway

The Lp system - interpretations and views

The study of normal genetic variation of human serum lipoproteins was started by the discovery in 1961 by Allison and Blumberg of the Ag system. In 1963 we were able both to confirm their observation of isoimmunization with genetically controlled antigens belonging to the serum β-lipoprotein, and to demonstrate the Lp system by immune sera produced in rabbits (Berg, 1963, 1964*a*, 1965, 1968*a*,*b*; Berg and Bearn, 1967; Berg and Mohr, 1963). The Lp(a) antigen was revealed by rabbit immune sera after proper absorptions and we found that the antigen was inherited in an autosomal dominant fashion. The Lp(a) antigen was closely related to β-lipoprotein, but it resided in a distinct population of lipoprotein molecules. The reaction of a person's serum with absorbed antiserum was found to be a permanent characteristic of the individual. However, the *in vitro* stability of the antigen was poor, even in the frozen state. In a Norwegian population sample we found a frequency of the Lp(a+) phenotype of approximately 35% and hence a frequency of 0.1948 for the postulated Lp^a gene.

GENETIC STUDIES ON THE Lp SYSTEM

The Lp(a) phenomenon was soon reproduced in several laboratories and a considerable amount of information on the inheritance of the Lp(a) antigen emerged (Wendt, 1966). In 1970 Rittner was able to extract from the literature Lp data from 574 matings with a total of 1461 offspring. He found that the data strongly supported the view that the Lp(a) antigen is governed by autosomal dominant inheritance. Of the matings, 184 were 'critical' inasmuch as both parents were of type Lp(a−). Only one of 547 children from such matings had been reported to be of type Lp(a+).

TABLE 1 *Norwegian families tested for the Lp(a) trait* *

Mating type	Parents		Children				Total
			Lp(a+)		Lp(a−)		
	Obs.	Exp.**	Obs.	Exp.	Obs.	Exp.	
Lp(a+) × Lp(a+)	21	21.6	69	68.9	17	17.1	86
Lp(a+) × Lp(a−)	81	79.8	153	158.9	134	128.1	287
Lp(a−) × Lp(a−)	73	73.6	1	0	272	273.0	273
Total	175	175.0	223		423		646

* From Berg (1968*b*). ** Expected values calculated from the gene frequencies.

Approximately one third of the families collected by Rittner were investigated by our group. Table 1 shows the distribution of the Lp(a) antigen in 175 unselected, presumably healthy Norwegian families with a total of 646 children. For this study each serum was coded so that the investigators did not know the family connection of any sample. High numbers of coded samples were tested each time. Each family serum was tested against the two best antisera available, and all the gel diffusion slides were read independently by two different observers. All our samples were tested in the fresh condition, and although we observed variation in the strength of the precipitin bands, the distinction between positive and negative reactions was quite clear under the conditions of the test and with the antisera used. There was full agreement between antisera and between observers. The code was broken only after the results had been finally recorded. Thus, there can be no doubt that the family data are valid.

Apart from the occurrence of one single Lp(a+) child among 273 from 73 matings where both parents were Lp(a−), the distribution provided solid evidence for autosomal dominant inheritance of the Lp(a) antigen. The data were also submitted to Smith's test for segregation ratios, and the results were in good agreement with the expected values, also in this analysis. There was no evidence that the exceptional Lp(a+) child was illegitimate, and we had to conclude that occasional exceptions to the assumed dominant mode of inheritance might occur.

We also tested sera from members of 30 families with a total of 91 children, from Easter Island. All 72 children from Lp(a−)×Lp(a−) matings were Lp(a−), and also in other respects the distribution was in good agreement with the expected values, assuming autosomal dominant inheritance (Berg, 1968*b*).

We conclude that neither our data, nor those collected by Rittner, suggest that any alternative genetic hypothesis should be considered.

In 1970 a different approach to normal genetic variation in serum lipoproteins was reported and it turned out that the genetic variation observed was the Lp system. Rider *et al.* (1970) noticed that pre-β-lipoprotein occurring in a number of normal serum samples behaved atypically in the ultracentrifuge, inasmuch as it did not float at the density 1.006. From a study of 51 kindreds they concluded that the occurrence of this 'sinking pre-β-lipoprotein' was governed by autosomal dominant inheritance. When they applied immunological techniques they found that their sinking pre-β-lipoprotein was the Lp(a) lipoprotein which, in their electrophoretic test system, migrated in the pre-β-region. This demonstration of the Lp system by a completely different approach yielded an additional confirmation of the autosomal dominant inheritance of the Lp(a) lipoprotein and showed that awareness of the Lp(a) lipoprotein is of practical importance in work with serum lipoproteins, including studies involving phenotyping of hyperlipoproteinaemias.

THE Lp(a) ANTIGEN IN RELATION TO SEX, AGE AND DISEASE

We found no indication of an association between sex and Lp type (Berg, 1966). In Table 2 the sample of unrelated Norwegians has been arranged according to age. There is no suggestion in this material of any age effect as observed by Schultz (1970). The discrepancy is probably not explained by lack of very old people in our material, since Schultz found the largest deviation among persons 50–59 years of age. Apparently, the age effect was observed only with one of the two antisera used (antiserum 56). This antiserum gave a higher frequency of positive reactions than the other antiserum (antiserum 69). A comparison which has been conducted between our two laboratories was performed with antiserum 69.

The different quality of the two antisera used by Schultz makes it particularly difficult to interpret the age effect she observed.

Neither Shreffler's group nor ours found any association between expression of the Lp phenotype and heart disease or diabetes. However, relatively few people with coronary heart

TABLE 2 *Age distribution of 1109 unrelated Norwegians tested for the Lp(a) trait* *

Age group (years)	Lp(a+)		Lp(a−)		Total	χ^2
	Obs.	Exp.	Obs.	Exp.		
<20	113	106.9	191	197.1	304	0.5369
20–30	124	126.3	235	232.7	359	0.0646
30–40	43	41.8	76	77.2	119	0.0531
40–50	64	61.5	111	113.5	175	0.1567
>50	46	53.5	106	98.5	152	1.6225
Total	390	390.0	719	719.0	1109	

* From Berg (1966).

disease have been studied, and more data should be collected. I may mention that Professor Walton (1971) reported at the Bruges Colloquium this year that he had been able to demonstrate β-lipoproteins as well as Lp(a) antigen in human atherosclerotic arterial lesions. He found Lp(a) antigen in such material only from Lp(a+) individuals. If Lp(a) antigen is present in trace amounts also in people who type as Lp(a−), one would perhaps have expected to find the antigen also in lesions in Lp(a−) individuals, since it appears to aggregate in arterial intima.

ON THE IMMUNOLOGY OF THE Lp SYSTEM

The antiserum problem and some of the immunological aspects of the Lp system should be considered in this discussion. It is necessary to observe that a given Lp antiserum has been absorbed for use under certain standardized conditions. Since the antiserum has originally contained large amounts of 'unspecific' antibodies, particularly to 'common' β-lipoprotein antigens, the term 'specific' as well as the 'presence–absence' terminology are operational, meaning that the absorbed antiserum can distinguish between positive and negative human sera under certain conditions. It cannot be assumed that antiserum absorbed for use with a standard procedure will be specific under widely different test conditions. Trace amounts of 'unspecific' antibodies may still be present in the reagent, although they do not affect the testing under standard conditions of sera containing approximately normal amounts of lipoproteins. If the standard reagent and test system are used for analysis of sera with a highly deviating lipoprotein pattern, or for testing of purified and highly concentrated lipoprotein fractions, false positive reactions may occur. Under such circumstances, it may be extremely difficult to decide whether or not a precipitin band represents a specific reaction.

In addition to trace amounts of 'unspecific' antibodies, an absorbed antiserum will quite frequently contain soluble antigen-antibody complexes.

It is important that the reagent to be used has a high specific titre and that unwanted antibodies are easily removed. The antiserum should remain specific over a wide range of absorption ratios so that a 'safe' ratio can be selected for absorption of reagent for routine use. The question of quality of the antisera was considered of great importance at the Lp Workshop in Marburg (Wendt, 1966), and it was decided that only high quality antisera should be used. It was strongly recommended that the quality of the antisera should always be given in reports on the Lp system.

These practical problems are inherent in the use of antisera of animal extraction for studies of this kind, and must always be kept in mind in work with the Lp system.

MOLECULAR CHARACTERISTICS OF THE LP(A) LIPOPROTEIN

From immunochemical experiments we concluded that the Lp(a) antigen resides in a distinct population of lipoprotein molecules (Berg, 1964*b*). This has been confirmed in several laboratories. In addition to Shreffler's group (Schultz *et al.*, 1968), Roelcke *et al.* (1968) in Heidelberg, Utermann and Wiegandt (1971) and Wiegandt *et al.* (1968) in Marburg, and in particular Simons *et al.* (1970) in Helsinki, have studied the biochemistry of the Lp(a) lipoprotein.

Although the Lp(a) lipoprotein is intermediate between the low density lipoprotein and high density lipoprotein as far as flotation characteristics in the ultracentrifuge are concerned, its protein part is apparently related only to the β-lipoprotein. The similarity in amino acid composition between Lp(a) lipoprotein and β-lipoprotein or low density lipoprotein is striking (Berg, 1971*a*). Peptide maps of Lp(a) lipoprotein are very similar but not identical to those of β-lipoprotein (Utermann and Wiegandt, 1971).

The lipid part of the Lp(a) lipoprotein is apparently not of importance for the immunological reaction. On the other hand, it is not certain that the antigen resides in the protein part of the molecule, for treatment with periodate eliminates its capacity to react with antiserum. This may suggest that the antigen resides in the carbohydrate part of the molecule. It is of interest that the reaction to periodate treatment resembles that of HL-A antigen (Sanderson *et al.*, 1971).

In polyacrylamide disc electrophoresis the Lp(a) lipoprotein migrates more slowly than the bulk of the β-lipoprotein. However, we have observed electrophoretic zones corresponding to the area of the protein carrying the Lp(a) antigenic determinant also with sera which type as Lp(a−) immunologically. Similarly, using polyacrylamide gradient electrophoresis a strong protein zone corresponding to the one associated with the Lp(a) antigen was observed with several Lp(a−) sera. Finally, Rittner's work on normal electrophoretic variants of lipoproteins (*vide infra*) appears to demonstrate that lipoproteins other than those carrying the Lp(a) antigen may occur in the region of the Lp(a) lipoprotein, both upon disc electrophoresis and upon ultracentrifugation. Therefore, the mere demonstration of a protein zone in one particular electrophoretic area or corresponding to one particular density class in the ultracentrifuge cannot as such be regarded as evidence that the Lp(a) lipoprotein is present. Such data have, however, been interpreted to indicate the presence of trace amounts of Lp(a) lipoprotein even in Lp(a−) individuals. The data are of course compatible with this view, but it appears that we are back to immunology if we are to test this notion critically. The problem is experimentally not at all easy, because the objections mentioned previously would apply to tests employing highly concentrated fractions of lipoproteins.

QUANTITATIVE VARIATION IN THE LP(A) ANTIGEN

The question of variation in strength of the precipitin reactions has been the subject of much discussion and study. At least two different family studies have indicated that the strength of the reactions also is under genetic control. Thus, control of the amount of antigen is apparently involved in the genetic Lp variation. Two different groups (Rittner, 1970; Wood, 1969) have conducted careful quantitative analyses of the Lp(a) antigen in serum and both found a clearly bimodal distribution. Thus, although there is quantitative variation it is apparently not continuous as would be expected if the mode of inheritance were multifactorial.

Harvie and Schultz (1970) have reported that they could demonstrate trace amounts of Lp(a) lipoprotein in 11 Lp(a−) sera after approximately 120-fold concentration of the lipoprotein by a dextran precipitation technique. Without going into detail about their interesting work, I would like to summarize some of the possible interpretations of reactions

demonstrated under extreme test conditions or after a high degree of concentration of lipoprotein fractions, and of some of the weak reactions obtained with whole serum. The possible interpretations appear to be:

1. Even Lp(a−) individuals have trace amounts of Lp(a) lipoprotein in their serum.
2. Trace amounts of 'unspecific' antibodies in the rabbit immune serum react with serum components other than Lp(a) lipoprotein, particularly under extreme test conditions.
3. Non-immune precipitin reactions involving lipoproteins may occur under certain conditions (particularly with purified lipoproteins).
4. Technical faults, unsuitable antiserum.

Although some workers favour the first interpretation, evidence proving the validity of this concept is apparently still missing (*vide supra*), and I must stress that the immunological problems which one meets in work with the Lp system are considerable.

Harvie and Schultz concluded from their suggestion that trace amounts of Lp(a) lipoprotein may be present in most or all human sera, that the Lp(a) lipoprotein should be regarded as a quantititave genetic trait, rather than one governed by simple Mendelian inheritance. The question whether or not simple Mendelian inheritance would be excluded if the first interpretation were correct must therefore be examined. I believe that this question can clearly be answered in the negative because there are numerous examples of quantitative protein variation governed by simple Mendelian inheritance. The following are some well documented examples:

1. Human serum proteins: People with inherited deficiencies of serum proteins frequently exhibit trace amounts of the protein in question (analbuminaemia, atransferrinaemia).
2. Human serum enzymes: Occurrence of residual enzyme activity in homozygotes for the 'silent' pseudocholinesterase gene.
3. Red cell enzymes: Quantitative variation of human red cell peptidase A activity.
4. Mouse serum proteins: Quantitative variation of the Ss serum protein.
5. Human serum β-lipoprotein: Simple inheritance (in several families) of hyper-β-lipoproteinaemia or hypo-β-lipoproteinaemia.

It appears that the possible occurrence of trace amounts of Lp(a) lipoprotein in several or all Lp(a−) sera would be completely compatible with a simple Mendelian inheritance of the Lp trait. The extensive family data mentioned above provide strong evidence for this concept and could hardly be compatible with polygenic inheritance. The dimorphism observed when the original test method is used and the bimodality obtained with more elaborate quantitative techniques also argue against control of the major part of the variation by more than one locus.

SOME DIFFICULTIES AND PARADOXES ENCOUNTERED IN WORK WITH THE Lp AND Ag SYSTEMS

Some of the difficulties and unsolved paradoxes one encounters in the work with the Lp and Ag systems may be summarized as follows:

1. Exceptional Lp(a+) offspring of Lp(a−) × Lp(a−) parents (Berg, 1968*b*).
2. Exceptions to the postulated, dominant mode of inheritance within the Ag system (Okochi (1967) found 10% Ag(y+) children from Ag(y−) × Ag(y−) matings).
3. Occasional exceptions to the postulated allelism between Ag^x and Ag^y (Okochi, 1967).
4. Scarcity of good antisera from multiply transfused people and poor quality of several Lp(a) antisera from animals.
5. Ag is apparently not antigenic in animals, and Lp not in man. The closest we have come to observe Lp isoimmunization was the production of anti-Lp(a) serum in Lp(a−) baboons (the Lp polymorphism is present in that species).

6. Occurrence of recombinants for Ag antigens thought to be controlled by closely linked genes (family 'B' of Dausset *et al.* (1968) exhibits 3 recombinants between the Ag(a) and Ag(t) components).

I have included some of the observations concerning the Ag system, because it seems appropriate in this discussion to remember that difficulties are encountered in work with both systems. The common denominator for these two systems is that they belong to the serum lipoproteins. Also because of the well known lability of the lipoproteins and the possibility that they may be affected by different nutritional conditions or disease, one may wonder if some of the problems encountered are inherent in any work with such lipid-protein compounds. The lability of the antigens together with the immunological problems could presumably lead to irregularity in the manifestation even of a system controlled by simple Mendelian inheritance.

These, as well as other interpretational problems, make it very difficult to form an opinion as to the exact reasons for differences in test results between laboratories. Such differences could result from differences in antisera, antigen-containing sera or test techniques. For instance, the antiserum most extensively used by Schultz and Shreffler gave a frequency of the positive phenotype which is approximately 20% higher than that most often recorded in laboratories studying comparable populations. This would seem to indicate that the phenomenon studied by these authors is not identical to that analysed in several other laboratories. Schultz (1970) also demonstrated a considerable difference between her own antisera; the probability was less than 0.01 that her antisera 56 and 69 were giving identical results. The discrepancy between her two antisera appears to be considerably more pronounced than that between antisera of different origins observed at the Marburg Workshop.

Also in the part (tested with antiserum 56) of her family data which Schultz considered to be homogeneous there was a considerable deviation from the distribution expected, assuming autosomal dominant inheritance. She found 8 positive children among 82 from 41 matings where both parents were negative, and too many negatives among the offspring of the other two mating types (Schultz, 1970; Table 23).

The distribution of the offspring of different mating types led Schultz to conclude that the Lp trait is governed by a polygenic mode of inheritance (Schultz, 1970). However, the result of a preliminary analysis (Edwards, 1960) seems to suggest that her family data may not fit polygenic inheritance any better than they do simple inheritance. Polygenic inheritance can hardly explain the distribution of phenotypes in the families reported previously. We conclude that the familial behaviour of the Lp(a) antigen cannot readily be explained by polygenic inheritance.

Despite the difference in opinion as to the exact mode of inheritance, there appears to be complete agreement that the evidence for genetic control of the Lp trait is very strong (Schultz, 1970).

OTHER STUDIES WITH RELEVANCE TO THE GENETICS OF THE Lp SYSTEM

In 1970 Rittner reported an attempt to approach the question of genetic variation of serum lipoproteins by means of the technique of disc electrophoresis in polyacrylamide. After purification and concentration of serum lipoproteins of density 1.065–1.10 he observed electrophoretic patterns which from a family analysis appeared to be governed by simple Mendelian inheritance. He found a strong association between Lp types and electrophoretic types. The electrophoretic phenotypes, characterized by one, two or no lipoprotein zones in the area behind β-lipoprotein, were designated C1, C2, C2-1 and C0. The family analysis supported the hypothesis that the patterns are controlled by three alleles, C^1, C^2 and C^0, the former two being co-dominant and C^0 being recessive to C^1 and C^2.

The total frequency of the phenotype Lp(a+) in a sample of blood donors from Bonn was

33.7%. However, the Lp(a+) frequency was around 10% in people with phenotype C0 and around 60% in C1 and C2-1 individuals (Rittner, 1970). This positive association between the phenotype Lp(a+) and presence of the C1 component was highly significant ($P < 0.0005$).

Dr. Rittner's results appear to be compatible with observations made by Dr. Joel Margolis who has developed a method for electrophoresis in a polyacrylamide gradient. By this technique he was able to demonstrate several different electrophoretic patterns of serum β-lipoproteins and these patterns may well be under genetic control (personal communication). The existence of a genetic polymorphism of β-lipoprotein demonstrable by gradient polyacrylamide gel electrophoresis has been suggested again very recently in a report by Wright *et al.* (1971).

If Rittner's results are confirmed, they may significantly improve our understanding of the Lp system and possibly explain some apparent discrepancies. It would appear very promising to explore further the close correlation between the Lp system and these simply inherited electrophoretic variants.

Lp(a) LIPOPROTEIN AND CELL MEMBRANES

The biological significance of inherited variants of serum lipoproteins is not known. We have previously attempted to approach this problem by investigating human donors and recipients of skin grafts. We observed that the grafts survived longer when donors and recipients were of the same Lp type than when they were of different type (Berg *et al.*, 1968). Based on this result, we suggested that a relationship may exist between serum lipoproteins and histocompatibility antigens on cell membranes. Direct evidence for the presence of human transplantation antigen in the β-lipoprotein fraction of serum has been presented recently by Charlton and Zmijewski (1970).

These and other observations prompted an attempt on our part to compare the protein composition of transplantation antigens and serum lipoproteins. A search for amino acid sequence homology would be of particular interest, but unfortunately sequence data are not available for histocompatibility antigens or serum lipoproteins.

Metzger *et al.* (1968) have developed a statistical method which offers the possibility of assessing relationships in composition between proteins even when amino acid sequence data are missing. The analysis results in a difference index (DI) for the two proteins. The method has been constructed such that two proteins with no amino acid in common would have a DI of 100 and two proteins with the same composition would have a DI of 0. Metzger and his co-workers conducted 630 comparisons between pairs of different proteins. The lowest DI

TABLE 3 *Difference indices obtained in comparisons between human serum lipoproteins and different batches of human and murine histocompatibility antigen**

Histocompatibility antigens	Human serum lipoproteins		
	Lp(a) lipoprotein	LDL**	HDL**
Human, RAJI	6.81	8.90	12.62
Human, R-4265	8.96	9.28	14.37
Murine, H-2^d	6.71	13.18	17.48
Murine, H-2^b	8.44	14.43	17.69

* From Berg (1971*c*). ** LDL = low density lipoprotein; HDL = high density lipoprotein.

observed was 9.1. Only about 0.5% of the indices were lower than 10, and about 95% of the indices were higher than 13.5. The mode was 26.

From data on the amino acid composition of the Lp(a) lipoprotein reported by Simons *et al.* (1970) and on papain solubilized HL-A and H-2 alloantigens published by Mann's group (Mann *et al.*, 1970) we have conducted the first comparison of the amino acid composition of serum lipoproteins and histocompatibility antigens. We have reported the results of these studies at this congress and elsewhere (Berg, 1971*a*,*b*,*c*). In a comparison between Lp(a) lipoprotein and β-lipoprotein or low density lipoprotein, we found a DI of the same order as the lowest values observed by Metzger. This confirms the close relationship between these two serum lipoproteins.

Table 3 summarizes the DI's found when the serum lipoproteins were compared with two batches of human HL-A antigen as well as with two batches of murine histocompatibility antigens. The difference indices obtained with the high density lipoprotein did not appear to suggest more than a random relationship between this lipoprotein and transplantation antigens. The figures obtained in the comparison between low density lipoprotein and the two batches of human histocompatibility antigens were, however, of the same order as the lowest values observed in Metzger's extensive study. The DI's obtained in the comparisons between Lp(a) lipoprotein and transplantation antigens were clearly lower than any DI found by Metzger, and to my knowledge such low values have previously been obtained only from comparisons between proteins known to be related. The very low DI from the comparison between Lp(a) lipoprotein and murine H-2^d antigen is particularly striking. The agreement of comparisons between lipoproteins with comparisons between lipoproteins and transplantation antigens, strengthens the impression of a meaningful pattern of difference indices.

Recently, Sanderson *et al.* (1971) reported that they had been able to prepare serologically active HL-A substances with molecular weights only about 8–10,000. It was of course of interest to test if even these small glycopeptides resembled serum lipoproteins with respect to amino acid composition. Figure 1 shows the DI's from comparisons between serum lipoproteins and one of Sanderson's active preparations, designated 'HL-A (Bt4, Pk I)'. The very low DI appears to suggest that the Lp(a) lipoprotein may resemble this small HL-A peptide even more closely than any of the preparations of transplantation antigens described by Mann and his co-workers, with regard to amino acid composition.

Within the limitations of the method, I conclude that the data indicate that there is a relationship in composition between histocompatibility antigens on cell membranes and Lp(a)

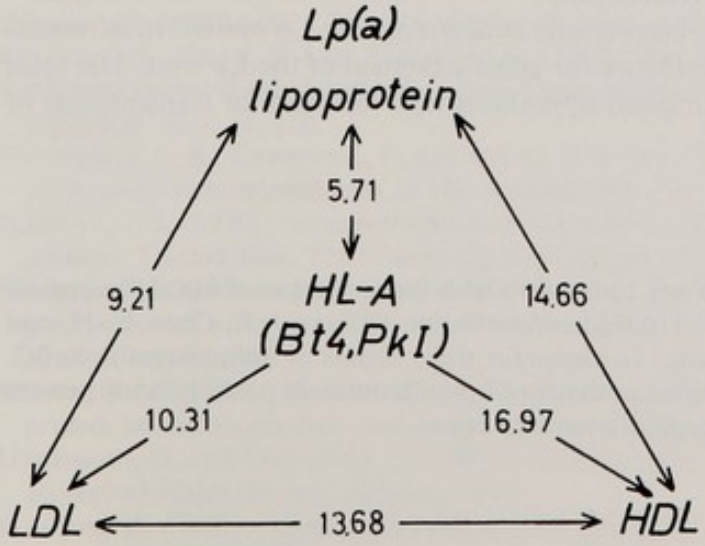


Fig. 1. Difference indices from amino acid contents comparison between a low molecular weight HL-A preparation and serum lipoproteins.

lipoprotein as well as low density lipoprotein or β-lipoprotein in the serum. It is not implied that this similarity is reflected in serological cross-reactivity as revealed by *in vitro* tests, and failure to demonstrate such cross-reactivity would not argue against the existence of a high degree of relationship in composition.

CONCLUDING REMARKS

With the discovery of the Lp(a) antigen, a previously unknown, distinct population of serum lipoproteins was revealed. The evidence for genetic control of the Lp trait is very strong, and originates from several laboratories, including that of Drs. Schultz and Shreffler. There appears to be no disagreement about these two points, despite difficulties in methods of study and differences of opinion as to the exact mode of inheritance. In the discussion of the discrepancies in observations and interpretations between Dr. Shreffler's group and several other workers, immunological and methodological problems (including differences between antisera) as well as the lability of the antigen must be kept in mind. Even if the possibility that trace amounts of Lp(a) lipoprotein may be present in some or all sera which type as Lp(a—) immunologically should be proven, there would be no reason to consider the hypothesis of autosomal dominant inheritance as less valid.

New information which may improve our understanding of the Lp system could possibly bring new insight into some of the unresolved problems. Thus, the demonstration by Rittner of an electrophoretic polymorphism closely related to the Lp(a) phenomenon seems to hold some promise for a finer genetic differentiation. The apparent correlation between the Lp(a) lipoprotein and cell membrane components is also of considerable interest. If the present notions are confirmed, several interesting questions may be raised and new knowledge may be gained about the synthesis of transplantation antigens as well as of serum lipoproteins. If the Lp(a) lipoprotein itself or some component closely resembling it is present in cell membranes, it will be an interesting task to try to find out if the lipoprotein is primarily present in the serum and secondarily becomes attached to the cell membrane, or if the Lp(a) lipoprotein is primarily synthesized as a component of cell membranes. Should the latter turn out to be true, one may ask if the molecular mechanism underlying the genetic Lp variation involves control of the rate of release of this component from cell membranes. At this early stage I shall refrain from further speculations and merely suggest that there may be an important biological relationship between histocompatibility antigens on cell membranes and components in the serum lipoproteins, and that the study of the hereditary Lp trait may be useful in elucidating this interesting biological relationship.

Any interpretation of newer or older observations concerning the Lp system must necessarily take into account the very strong evidence for genetic control of the Lp trait. The total body of evidence strongly supports the original hypothesis that the *mode* of transmission of the Lp property is autosomal dominant.

Note added in proof

Albers and co-workers failed to demonstrate any component with the properties of Lp(a) lipoprotein in the sera of certain individuals even after 110-fold concentration (Albers, J. J., Chen, C.-H. and Aladjem, F. (1972): Human serum lipoproteins. Evidence for three classes of lipoproteins in S_f 0-2. *Biochemistry, 11,* 57). Thus, the question of trace amounts of Lp(a) lipoprotein possibly being present also in sera which type as Lp(a−) immunologically remains open.

REFERENCES

ALLISON, A. C. and BLUMBERG, B. S. (1961): An isoprecipitation reaction distinguishing human serum-protein types. *Lancet, 1,* 634.

BERG, K. (1963): A new serum type system in man – the Lp system. *Acta path. microbiol. scand., 59*, 369.

BERG, K. (1964*a*): Immunhematologi: plasmaproteiner. *T. norske Laegeforen., 84*, 96.

BERG, K. (1964*b*): Immunochemical studies of the Lp(a) factor. *Acta path. microbiol. scand., 62*, 600.

BERG, K. (1965): A new serum type system in man – the Ld system. *Vox Sang. (Basel), 10*, 513.

BERG, K. (1966): Further studies on the Lp system. *Vox Sang. (Basel), 11*, 419.

BERG, K. (1968*a*): Serum lipoproteins. In: *Proceedings, XIth Congress of the International Society of Blood Transfusion, Sydney*, pp. 21–32. Editor: L. Holländer. S. Karger, Basel.

BERG, K. (1968*b*): The Lp system. *Ser. Haematol., 1*, 111.

BERG, K. (1971*a*): Paper presented at *XIX Annual Colloquium Protides of the Biological Fluids, Bruges, Belgium, 1971.*

BERG, K. (1971*b*): Compositional relatedness between histocompatibility antigens and human serum lipoproteins. *Science, 172*, 1136.

BERG, K. (1971*c*): Similarity in amino acid composition between human serum lipoproteins and histocompatibility antigens on cell membranes. *Clin. Genet., 2*, 193.

BERG, K. and BEARN, A. G. (1967): Genetic polymorphisms of human serum β-lipoprotein. *J. clin. Invest., 46*, 1036.

BERG, K., CEPPELLINI, R., CURTONI, E. S., MATTIUZ, P. L. and BEARN, A. G. (1968): The genetic antigenic polymorphisms of human serum β-lipoprotein and survival of skin grafts. In: *Advance in Transplantation*, pp. 253–255. Editors: J. Dausset, J. Hamburger and G. Mathé. Munksgaard, Copenhagen.

BERG, K. and MOHR, J. (1963): Genetics of the Lp system. *Acta genet. (Basel), 13*, 349.

CHARLTON, R. K. and ZMIJEWSKI, C. M. (1970): Soluble HL-A7 antigen: localization in the β-lipoprotein fraction of human serum. *Science, 170*, 636.

DAUSSET, J., CONTU, L., FEINGOLD, N. and BARGE, A. (1968): Indépendance du système d'histocompatibilité HL-A et des systèmes de groupes des bêta-lipoprotéines Lp et Ag. *Nouv. Rev. franç. Hémat., 8*, 868.

EDWARDS, J. H. (1960): The simulation of Mendelism. *Acta genet. (Basel), 10*, 63.

HARVIE, N. R. and SCHULTZ, J. S. (1970): Studies of Lp-lipoprotein as a quantitative genetic trait. *Proc. nat. Acad. Sci. U.S.A., 66*, 99.

MANN, D. L., FAHEY, J. L. and NATHENSON, S. G. (1970): Molecular comparisons of papain solubilized H-2 and HL-A alloantigens. In: *Histocompatibility Testing 1970*, pp. 461–467. Editor: P. I. Terasaki. Munksgaard, Copenhagen.

METZGER, H., SHAPIRO, M. B., MOSIMANN, J. E. and VINTON, J. E. (1968): Assessment of compositional relatedness between proteins. *Nature (Lond.), 219*, 1166.

OKOCHI, K. (1967): Serum lipoprotein allotypes Ag(x) and Ag(y) in Japanese. *Vox Sang. (Basel), 13*, 319.

RIDER, A. K., LEVY, R. I. and FREDRICKSON, D. S. (1970): 'Sinking' pre-beta lipoprotein and the Lp antigen. *Circulation, 41–42/Suppl. 3*, 10.

RITTNER, C. (1970): *Die Lipoprotein-(LDL-)Polymorphismen des menschlichen Serums.* Thesis, Bonn.

ROELCKE, D., KRAH, E. and FAHIMIAN, A. (1968): Die Unterteilbarkeit menschlicher Lipoproteine niederer Dichte (LDL) hinsichtlich ihrer Lp(a)-Eigenschaft durch Ionenaustauscher-Säulenchromatographie. *Blut, 18*, 160.

SANDERSON, A. R., CRESSWELL, P. and WELSH, K. I. (1971): Involvement of carbohydrate in the immunochemical determinant area of HL-A substances. *Nature New Biol. (Lond.), 230*, 8.

SCHULTZ, J. S. (1970): *Immunochemical and Genetic Studies of the Lp System of Human Serum Lipoproteins.* Dissertation, The University of Michigan, Ann Arbor.

SCHULTZ, J. S., SHREFFLER, D. C. and HARVIE, N. R. (1968): Genetic and antigenic studies and partial purification of a human serum lipoprotein carrying the Lp antigenic determinant. *Proc. nat. Acad. Sci. U.S.A., 61*, 963.

SIMONS, K., EHNHOLM, C., RENKONEN, O. and BLOTH, B. (1970): Characterization of the Lp(a) lipoprotein in human plasma. *Acta path. microbiol. scand., B, 78*, 459.

UTERMANN, G. and WIEGANDT, H. (1971): Paper presented at *XIX Annual Colloquium Protides of the Biological Fluids, Bruges, Belgium, 1971.*

WALTON, K. (1971): Paper presented at *XIX Annual Colloquium Protides of the Biological Fluids, Bruges, Belgium, 1971.*

WENDT, G. G. (Ed.) (1966): *Internationale Lp-Arbeitstagung.* Institut für Humangenetik der Philipps-Universistät, Marburg/Lahn.

WIEGANDT, H., LIPP, K. and WENDT, G. G. (1968): Identifizierung eines Lipoproteins mit Antigenwirksamkeit im Lp-System. *Hoppe-Seyler's Z. physiol. Chem., 349*, 489.

WOOD, C. (1969): Genetically determined variant in human β-lipoprotein. *Arch. Dis. Childh., 44*, 544.

WRIGHT, G. L. Jr., FARRELL, K. B. and ROBERTS, D. B. (1971): Gradient polyacrylamide gel electrophoresis of human serum proteins: improved discontinuous gel electrophoretic technique and identification of individual serum components. *Clin. chim. Acta, 32*, 285.

Chapter VIII Cell genetics

G. Pontecorvo

Imperial Cancer Research Fund, Lincoln's Inn Field, London, United Kingdom

Opening remarks

The contributions to this Symposium deal with somatic cell hybridization. They are strictly limited to the uses of it for two purposes: cytological identification of which human chromosome carries a given gene and detection or exclusion of linkage between two or more genes.

The principles so far applied are the same as those developed and routinely used by my colleagues and myself in the early 1950's for formal genetic analysis in fungi *via* vegetative cells (Pontecorvo, 1969). It gives me great satisfaction to quote from my plea at the Ciba Symposium of 1959 for applying similar principles to human somatic cells in culture. I then said that: '... accidental – or perhaps non-accidental – loss of chromosomes and non-disjunction would be quite enough to carry out genetic analysis *via* somatic segregation to a very considerable extent. They permit individual genes to be assigned to individual linkage groups.' And later on: '... I would be extremely happy if within the next ten years we could begin to get somewhere' (Pontecorvo, 1959).

We have got somewhere in just over ten years, though, as often happens, by an unexpected technical roundabout. In 1959 I was thinking of, and making attempts about, chromosome elimination or non-disjunction in diploid cultures from heterozygous individuals. The results – *e.g.* by the use of p-fluorophenylalanine, which acts as magic with diploid fungi – have been unconvincing, to put it mildly.

The solution came from two advances. Ephrussi and his collaborators (1965) showed that hybrids capable of indefinite multiplication could be obtained between cultured cells of different species. Weiss and Green (1967) then made the most useful and unexpected discovery that man × mouse hybrids eliminate successively human chromosomes. Recently Puck's group (Kao and Puck, 1971) found that the same occurs, even more rapidly, in man × chinese hamster hybrids.

The work which is the subject of this Symposium stems from these two basic advances joined with substantial improvements in the cytological identification of individual human chromosomes.

There are already some remarkable successes: the detection of at least one autosomal linkage, the exclusion of linkage between about 15 autosomal other loci and the assignation of at least two genes to their chromosomes. In formal genetics the first steps are notoriously the hardest. We are now probably not far from the phase of exponential growth.

Professor Siniscalco is going to report in another Symposium on another promising advance in the use of somatic cell hybrids: the identification of the linear order of some linked genes based on hybrid clones which have lost *segments* of a relevant chromosome (the X in his first attempt). This is in principle similar to the use of somatic crossing-over in *Drosophila* or *fungi*.

Hybrids of human and mouse (or chinese hamster) cells will continue to be useful for some time for at least two reasons. First, they spontaneously lose human chromosomes. Second, as Ephrussi pointed out long ago, the occurrence of a number of interspecific differences in enzymes makes it possible to use as genetic cell markers human enzymes for which detectable variation between individuals is rare or unknown.

In the former respect, however, there are now new possibilities opened by the technique for directing chromosome elimination (Pontecorvo, 1971). This technique induces elimination of the chromosomes of one 'parent' by irradiating the cells of that 'parent' just before fusion (alternatively, the chromosomes of one 'parent' are labelled by BUdR before fusion and the hybrids exposed to blue light). This means, among others, that we can now use man × man somatic cell hybrids for the detection of linkages. It also means that we can exhumate my old hope of using human diploid heterozygous explants and induce in them segregation, either in the form of monosomy or of non-disjunctional homozygosis.

REFERENCES

Ephrussi, B. and Weiss, M. C. (1965): Interspecific hybridisation of somatic cells. *Proc. nat. Acad. Sci. USA, 53*, 1040.

Kao, F. T. and Puck, T. T. (1971): Genetics of somatic mammalian cells: linkage studies with human-chinese hamster cell hybrids. *Nature (Lond.), 228/5269*, 329.

Pontecorvo, G. (1959): Panel discussion. In: *Biochemistry of Human Genetics*, pp. 279–285. Editors: G. E. W. Wolstenholme and C. O'Connor. J. and A. Churchill Ltd., London.

Pontecorvo, G. (1969): Genetic analysis of 'somatic' cells in filamentous fungi. In: *Heterospecific Genome Interactions, Monograph No. 8*, pp. 1–8. Editor: V. Defendi. The Wistar Institute Press, Philadelphia, Pa.

Pontecorvo, G. (1971): Induction of directional chromosome elimination in somatic cell hybrids. *Nature (Lond.), 230/5293*, 367.

Weiss, M. C. and Green, H. (1967): Human-mouse hybrid cell lines containing partial complements of human chromosomes and functioning human genes. *Proc. nat. Acad. Sci. USA, 58*, 1104.

W. F. Bodmer

Genetics Laboratory, Department of Biochemistry, University of Oxford, Oxford, United Kingdom

Linkage analysis using human-mouse hybrid cells

There are three basic requirements for a successful approach to somatic cell genetics:

1. the availability of appropriate cell culture techniques,
2. genetic markers which can be detected in cells in culture, and
3. methods for genetic analysis using cells in culture.

The techniques of cell culture are now well developed and many cell lines can be cloned with high efficiency. A wide variety of genetic markers can also be detected in cells in culture. These now include drug resistance (especially to 5-bromodeoxyuridine (5BUdR) and 8-azaguanine (8AZG)), electrophoretically distinguishable enzyme differences, cell surface antigens, nutritional mutants and susceptibility to viruses. The sources of variation are induced and selected mutations (for drug resistance, nutritional mutants and antigenic variants) naturally occurring within species, polymorphic differences and differences between species. For some time, the major stumbling block for somatic cell genetics was the lack of a suitable means of genetic analysis. The techniques of cell fusion and hybridization have now provided an approach to genetic analysis, at least at the level of segregation of whole chromosomes. Three important developments have led to the use of hybridization for genetic analysis. The first was the use by Littlefield (1964) of the HAT selective medium based on the work of Szybalski *et al.* (1961) for the isolation of hybrid clones. These techniques have been extended to cases where one of the parent cell lines, though not directly selected against, either grows slowly (Davidson and Ephrussi, 1965) or like the peripheral blood white cell does not actually divide *in vitro* (Miggiano *et al.*, 1969). The second development was the use of inactivated Sendai virus following Okada and Tadokoro (1963) to mediate cell fusion at relatively high frequencies between the cells of widely different specific origins (Harris and Watkins, 1965); and the third the discovery of the loss of human chromosomes in man-mouse hybrids (Weiss and Green, 1967). Man-mouse, and other similar interspecific hybrids are especially useful for genetic analysis because any identifiable gene product that can be distinguished in the two parent species, for example mouse and man, provides a potential genetic marker. Enzyme electrophoretic differences between species have thus provided a most valuable source of markers for genetic analysis using somatic cell hybrids. Between them these three developments have made it possible to produce and select with reasonable frequency man-mouse and other interspecific hybrid cell lines which have segregated to some extent with respect to their content of human chromosomes and so provide an initial basis for somatic cell genetic analysis. In this paper I shall first review some of the genetic data which have been obtained using this approach to somatic cell genetics, with special reference to work done in collaboration with V. Miggiano, M. Nabholz and S. Santachiara which has confirmed the X-linkage of 8AZG resistance in man and

established a new autosomal linkage between human lactate dehydrogenase B and peptidase B, and then discuss some of the potential developments and evolutionary implications of this approach to somatic cell genetics.

LINKAGE WITH THE SELECTED MARKER

The cross made by Weiss and Green (1967) was between a 5BUdR resistant mouse cell line and human fibroblasts. They obtained hybrid products which, after rapid and extensive preferential loss of the human chromosomes, based on their karyotype, appeared to end up with just a single human chromosome. This must presumably be the human chromosome which carries the gene for thymidine kinase, which is the enzyme that is deficient in the 5BUdR resistant line and is required for growth in the HAT selective medium that was used to select out the hybrids. Subsequent studies (Matsuya *et al.*, 1968; Migeon and Miller, 1968) showed that this human thymidine kinase carrying chromosome was a member of the E group of chromosomes, probably number 17. This assignment to chromosome 17 has recently been confirmed using the quinacrine fluorescence technique to identify the human and mouse chromosomes (Miller *et al.*, 1971*a*).

The use of human peripheral blood leukocytes as a source of human cells in a man-mouse hybridization was introduced by Miggiano *et al.* (1969). Partially purified human mixed white blood cells, or lymphocytes, obtained from peripheral blood are fused with 8AZG resistant or 5BUdR resistant mouse cell lines using inactivated Sendai virus and the fusion products are exposed to the HAT selective medium. The white blood cells do not divide and the mouse cells are eliminated by the selective medium, so that only the hybrids containing the human genes either for the enzyme hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) that is deficient in 8AZG resistant cells or for the thymidine kinase that is deficient in 5BUdR resistant cells, are able to grow and form colonies. This technique provides a conveniently available source of cells from any human cell donor, who for example has a particular genotype or abnormal karyotype, that can readily be hybridized with any suitable mouse cell line.

In one such cross between human leukocytes and an 8AZG resistant mouse cell line, 5 primary hybrid clones were picked and analysed with respect to their karyotype and their constitution for a variety of genetic markers (Nabholz *et al.*, 1969). The mean total number of chromosomes in the mouse parent cell line (1R) was 58.2 of which 18 were biarmed. Biarmed chromosomes are generally found in heteroploid mouse cell lines, even though the normal mouse karyotype contains 40 chromosomes all of which are acrocentric. Chromosome rearrangements presumably occurred both during the evolution of the heteroploid line and, where relevant, of the tumour which gave rise to it. The hybrid lines had, on average at 40 to 60 days after fusion, 4 to 14 more chromosomes than 1R,which chromosomes were presumed to be of human origin. Some of them could when originally analysed by overall morphology reasonably be identified as human, while others were indistinguishable from the biarmed and acrocentric chromosomes present in 1R. More recent data on identification of the chromosomes using quinacrine fluorescence and Giemsa differential staining techniques will be discussed briefly later. After about 100 to 150 days, the karyotypes appeared to be reasonably stable, the mean numbers of presumptive human chromosomes per line ranging from 1 to about 5 or 6. All but one of the 5 lines reacted to a specific rabbit anti human antiserum indicating the presence of one or more species specific cell surface antigens and all had retained the mouse H2 histocompatibility antigens corresponding to the parent mouse line 1R.

Human and mouse glucose 6-phosphate dehydrogenase (G6PD) can be readily distinguished by their electrophoretic mobility. All the extracts made from the 5 hybrid lines showed the presence of the human G6PD. There was, in addition, enzyme activity whose mobility was between that of mouse and man and which probably represents 'hybrid' enzyme, formed by the association of one human with one mouse G6PD subunit. Since G6PD is known to be

TABLE 1 *Reversion to 8AZG resistance and joint distribution of human G6PD and LDH-A in clones from a cross of human leukocytes by the mouse 8AZG resistant cell line, 1R*

Medium	Human G6PD	LDH-A		Total clones
		Present	Absent	
HAT	+	7	20	27
Normal*	+	3	12	15
8AZG	−	4	5	9**

* This is a control to check the spontaneous reversion rate to 8AZG resistance in the absence of the HAT selective medium.
** These are the 8AZG resistant, back selected clones.

X-linked in man, the presence of human G6PD in all the hybrids indicates the presence of the human X chromosome or at least a part of it.

A deficiency in HGPRT activity, which is the defect associated with 8AZG resistance, is the primary basis for a human X-linked recessive neurological disease, called the Lesch-Nyhan syndrome (Lesch and Nyhan, 1964; Seegmiller *et al.*, 1968). The simplest explanation, therefore, for the presence of the human X chromosome in all the hybrids is that the normal human HGPRT activity which is required for the outgrowth of the hybrids is controlled by a gene on the X chromosome, which may be the same as that controlling the Lesch-Nyhan syndrome. This explanation was confirmed by the results of a back selection experiment in which 8AZG resistant subclones were selected from the 5 original hybrid lines and tested for their G6PD activity. These clones were also, as a control, tested by electrophoresis for the presence of the human lactate dehydrogenase A subunit (LDH-A). The results of this analysis, as summarized in Table 1, showed an absolute linkage between 8AZG resistance and the absence of human G6PD activity. There was no obvious change in the pattern of occurrence of LDH-A activity, indicating the absence of any unexplained non-specific effects. These data, therefore, provide a direct confirmation in cell culture for the presence of the human HGPRT gene on the X chromosome, based on linkage between the selective marker, HGPRT, and the human G6PD, acting as a presumptive marker for the X chromosome.

LINKAGE BY RANDOM ASSOCIATION OF UNSELECTED MARKERS

Autosomal linkage between the human genes for lactate dehydrogenase B and peptidase B has been established by an analysis of the pairwise association of unselected markers in the products of a human mouse hybridization experiment (Santachiara *et al.*, 1970; Ruddle *et al.*, 1970). Let us take, as an example, the data of Santachiara *et al.* (1970). Nine, presumably independent, hybrid lines were obtained originally as clones from a cross between 1T, a subclone of a 5BUdR resistant subline of the fibroblast-like cell line 3T3 obtained from Howard Green, and peripheral blood white cells from a normal human female. The cross was carried out as already described. Extracts from these hybrids, obtained at between 75 and 120 days after fusion, were assayed electrophoretically for 13 enzymes whose human and mouse forms are distinguishable by their different mobilities. The results of this analysis are shown in Table 2. The variation in the amount of a particular activity found in different lines is mostly due to genetic heterogeneity within the lines resulting from segregation of human chromosomes after the primary clonal isolation of the lines.

The distribution of the enzyme activities indicates a close association between two pairs of human markers; namely LDH-B and Pep-B, and MDH and ADA, consistent with linkage

TABLE 2 *Distribution of human enzyme activities in hybrid lines from a cross of human leukocytes by the mouse 5BUdR resistant cell line, 1T*

Line	LDH-A	LDH-B	Pep-B	Pep-A	Pep-C	Pep-D	MDH	ADA	PGM_1	PGM_2	IPO	G6PD	PHI
3.31	−	±	±	−	−	−	−	−	−	−	−	−	−
4.11	−	+	++	++	−	(Pep-D^1)*	−	−	±	+	++	−	++
4.12	+	+	++	++	+	(Pep-D^2)*	+	++	+	±	+	−	++
4.21	+	+	++	±	−	−	−	−	±	−	++	−	±
4.22	−	−	−	−	−	−	−	−	−	+	−	−	−
4.31	−	+	++	−	−	−	−	−	+	+	−	−	−
4.42	+	−	−	±	−	−	+	±	−	−	++	−	−
4.43	+	+	+	+	−	−	−	−	−	−	−	−	+
4.45	−	+	+	−	−	−	−	−	−	−	+	−	−
No. of positive lines	4	7	7	5	1	2	2	2	4	4	5	0	4

Where possible the amount of human compared to mouse enzyme activity is scored as ±, + or ++.
LDH-A and B are the two subunits of lactate dehydrogenase.
Pep-A, B, C and D are peptidases which are distinguished by the substrates on which they are active.
MDH is the supernatant DPN dependent malate dehydrogenase.
ADA is adenosine deaminase.
PGM_1 and PGM_2 are different forms of phosphoglucomutase.
IPO is an indophenol oxidase.
G6PD is glucose 6-phosphate dehydrogenase.
PHI is phosphohexoseisomerase.
See Santachiara *et al.* (1970) for further technical details.

* Pep-D^1 and Pep-D^2 are allelic forms of human Pep-D, with respect to which the human donor was heterozygous. These results thus show segregation of the members of a pair of homologous human chromosomes into different hybrids.

between the respective pairs of genes. These associations were further investigated by clonal analysis of the lines that carried these markers. All subclones isolated from 4 of the LDH-B+ Pep-B+ lines showed the same consistent association between these two enzymes; their human forms were either present together (29 clones) or both absent (11 clones). This indicates that the two genes for these enzymes are on the same chromosome. The absence of human G6PD and so presumably of the human X chromosome shows that the linkage is autosomal.

The possibility that the linkage between Pep-B and LDH-B involves regulatory loci rather than the structural genes is not ruled out. One explanation for the data, for example, might be that the Pep-B structural gene is linked to a gene required for increased LDH-B expression, which acts equally on the mouse and human LDH-B structural genes. The data of Ruddle *et al.* (1970) which also show association between the human Pep-B and LDH-B activities, come from a cross involving a donor who was a heterozygote for an electrophoretic variant of LDH-B. This enabled them to identify positively the presence of the human LDH-B structural gene in some of their hybrids. Though their data favour the idea that the linkage is between the structural genes, they do not exclude the involvement of a regulatory gene.

The clonal analysis of the two MDH+ ADA+ lines did not confirm the association originally found in the lines. All 13 clones derived from one of the lines (4.12) were still MDH+ ADA+. From the other line (4.42), however, 5 clones were MDH+ ADA+ and 7 clones were MDH+ ADA−. No MDH− ADA+ clones were found. This pattern of association could be explained by linkage between a structural gene for MDH and a regulator gene for ADA, or *vice versa*, with the two structural genes for the enzymes being on different chromosomes. The pattern could also be explained by an early translocation or deletion event in line 4.42 which produced a subline with a chromosome in which the human ADA was lost. Finally, it could be the result of some selective interaction between the two chromosomes carrying the MDH and ADA genes which tended to favour their joint presence in the hybrids. Further work is needed both to confirm the pattern of association between MDH and ADA and to establish its basis. None of the other pairs of enzymes, including in particular the LDH-A and B subunits, showed any evidence of association, indicating that the corresponding genes are probably all on different chromosomes.

GENETIC ANALYSIS OF DIFFERENTIATED FUNCTIONS AND BASIC CELLULAR PROCESSES

Segregating somatic cell hybrids can provide a most valuable tool for the genetic analysis of the control of differentiated functions and basic cellular processes. The work of Klebe *et al.* (1970) on the expression of a kidney specific esterase provides an elegant example of this approach. The esterase is expressed in a cell line (RAG) derived from a mouse renal adenocarcinoma which they have used for many of their human × mouse crosses. Hybrids made with human and other cells that do not express the esterase at significant levels, also do not express the enzyme, as seems to be the case with many differentiated functions. After extensive loss of human chromosomes in such a hybridization, however, a subline was derived which again expressed the kidney esterase. Karyotype analysis suggested that the reappearance of the enzyme activity was associated with the loss of human chromosome C10. This implies that a human gene which regulates negatively the kidney esterase activity is located on chromosome 10 and that the products of this human gene can act on the mouse genome or its products.

The synthesis of ribosomal RNA in hybrids has been studied by Green and his co-workers. Thus, Eliceiri and Green (1969) could detect only mouse 28S ribosomal RNA in the hybrids they studied. This shows directly, incidentally, that the human proteins so far studied in these hybrids can be synthesized using mouse ribosomes. This may not be true for all human proteins, which could complicate the interpretation of data on the genetic control of such pro-

teins obtained from human-mouse hybrids. In a later study, Stanners *et al.* (1971) have shown that hamster-mouse hybrids synthesize both hamster and mouse ribosomal RNA. Further work along these lines, including a study of the ribosomal proteins, should lead to answers to such questions as the linkage relationships among the genes for the ribosomal proteins and their linkage to the r-RNA genes and whether hybrid ribosomes, containing mixtures of proteins or RNA from the two parent species, can be formed.

In our laboratory, we have begun a similar study of the genetic control of mitochondrial function and biogenesis. A preliminary analysis has been made by Clayton *et al.* (1971) of the mitochondrial DNA content of 6 human-mouse hybrid lines. Human and mouse mitochondrial DNA can be distinguished by their buoyant densities. The six lines included two each from our lymphocyte crosses with the mouse 1R and 1T cell lines, one from a Hela × 1T cross (from Howard Green) and another from a cross between a human lymphoid cell line and the mouse L cell derivative A9 (from Henry Harris). Only mouse mitochondrial DNA was found in all the lines, whereas as little as 2% of human mitochondrial DNA could have been detected. It appears that the hybrids studied were not able to propagate the human mitochondria which were presumably present at the time of cell fusion. Conceivably the mouse cytoplasm could, in some way, inhibit the propagation of human mitochondria. However, it seems more likely that the one or more human chromosomes required for human mitochondrial propagation were not present in the hybrids. Even if only one human chromosome were required, this may by chance have been absent from all the hybrids that were studied. If two or more human chromosomes are required, then of course the chance that a hybrid of the requisite genotype was not analysed is greatly increased. Further work with a greater variety of hybrids and studying all components of the mitochondria, should help determine the genetic requirements for the maintenance and functioning of the human mitochondria and their components in the hybrid environment.

EVOLUTION OF THE HYBRID LINES

The major loss of human chromosomes following fusion of human and mouse cells is probably during the first few divisions after nuclear fusion and the formation of the initial hybrid cell. However, further losses do occur, as indicated by the observed segregation, discussed above, for human markers in subclones of hybrid lines which are originally derived from a single hybrid clone. The chromosomal evolution of the hybrids both immediately after and later is probably influenced by quite strong selective pressures. Direct evidence for this comes from apparently systematic changes within a line in the amount of a particular human enzyme activity. Statistical analysis using the data shown in Table 2 of the number of human enzymes (or chromosomes) per line and of the frequency with which a given enzyme is present in any hybrid, indicates that some chromosomes may have a higher probability of being represented in a hybrid than others, but that this probability may vary from line to line according to its particular evolutionary history. These selective effects could lead to association between pairs of markers on different chromosomes, especially in data derived from subclones of a primary clonally derived hybrid, which could be misinterpreted as an indication of linkage. This can best be ruled out by the examination of an adequate number of *independent* isolates of hybrid lines and of their subclones.

A specially interesting case of a possible selective effect associated with a chromosome rearrangement or deletion has been described by Migeon and Miller (1968). They observed, in a cross of human cells by the mouse 1T cell line, which is 5BUdR resistant, that hybrids which had been maintained in culture for longer periods of time tended to lose the chromosome 17, which carries the thymidine kinase gene required for the growth of the hybrid in the HAT selective medium. These have been shown still to express specifically the human thymidine kinase activity (Migeon *et al.*, 1969). The simplest interpretation of these data is that the chromosome 17 had undergone a change, such as the loss of one arm, which made it indistinguish-

able from one of the mouse chromosomes, and that hybrid cells carrying this changed chromosome were at a selective advantage. Genetic evidence for X chromosome breakage based on a breakage of the linkage between the human HGPRT and G6PD genes in human-mouse hybrids has been published by Miller *et al.* (1971b). This, however, does not seem to be such a regular event as the loss of chromosome 17 reported by Migeon and Miller (1968).

The use of quinacrine fluorescence and differential Giemsa staining techniques should greatly facilitate the identification in hybrids of human chromosomes and their possible breakdown products. *In situ* DNA and RNA annealing as described by Gall and Pardue (1969) and John *et al.* (1969) has already been used by Ruddle *et al.* (1970) to help in the identification of the human chromosomes in hybrids. This topic is discussed in detail by Ruddle in this symposium. Preliminary results with the 1R hybrids described above, following a careful characterization of the 1R karyotype (Rowley and Bodmer, 1971), indicate that some chromosomes in the hybrids that were originally classified as human on the basis of their overall morphology clearly are not human (J. Rowley, unpublished observations). These chromosomes were also not present in the mouse 1R parent cell line, and so must have been formed at some time during the evolution of the hybrid. Of the two hybrids studied so far, one, picked for study because it had apparently the largest number of human chromosomes, seems to have retained in at least come cells an identifiable human X chromosome, while the other, which had virtually the same number of chromosomes as 1R, does not. This latter, however, has a 'novel' fragment which may represent the relevant part of the X chromosome maintained in the hybrid. The linkage between HGPRT and G6PD was maintained in both these lines. Careful genetic and karyotypic analysis of subclones of these lines and of 8AZG resistant revertant clones should help identify such an X fragment, which may even have become translocated onto a mouse chromosome. It seems possible that chromosome rearrangements following hybridization may be more common than was at first suspected and may have as yet unexplained selective advantages. In this context, it is interesting to recall the high incidence of biarmed chromosomes in permanent mouse cell lines and in tumours, whereas the normal mouse karyotype includes only acrocentric chromosomes. Many, if not most of these, are probably formed by Robertsonian centromeric fusions, both between homologues to form isochromosomes and between non-homologues (see *e.g.* Rowley and Bodmer, 1971). This apparent advantage of rearranged chromosomes may be due to an interaction between selection and linkage, which has been the subject of much theoretical discussion by population geneticists (see *e.g.* Bodmer and Parsons, 1962; Bodmer and Felsenstein, 1967 for review and further references). Similar mechanisms may, of course, be involved in the evolutionary moulding of the karyotypic differences between species. The karyotypic changes that take place relatively rapidly in cell culture may perhaps be a convenient model for those that occur naturally over much longer periods of time.

DISCUSSION

It is already clear that somatic cell hybridization has provided an opening toward a systematic genetic analysis of cells in culture. Major problems, however, remain to be solved. A most important further development must be the controlled breakage and rejoining of chromosomes to provide a basis for the analysis of recombination between markers on the same chromosome. The results on probable chromosome breakage and rearrangement discussed above provide a start in this direction. Identifiable chromosome fragments produced following hybridization and maintained by selection, should help to at least locate markers to given regions within a chromosome and to determine linear orders, as indicated already by Miller *et al.* (1971*b*). Use can also be made of naturally occurring human chromosomal rearrangements. A good library of genetic markers and techniques for the controlled breakage of chromosomes and the selective recognition of the breakage products will, however, certainly be major requisites for the success of this approach to the analysis of genetic linkage.

Another possible method for linkage analysis in human-mouse hybrids, which does not depend on the human-mouse differences, is to use human cell donors who are potential double heterozygotes for gene products which can be distinguished from those of the mouse. Since many hybrids will include only one or other of the members of a pair of homologues (see the data for Pep-D in Table 2), pairwise association of allelic products from different loci will provide evidence for linkage. This approach may be particularly useful for highly polymorphic antigenic systems such as HL-A, which consists of two closely linked multiple allelic loci. In this case, hybrid analysis could be used to establish an individual's genotype.

Some genetic markers of interest, such as haptoglobin and transferrin, are only synthesized in certain types of cells, in this case from the liver. In order to study these, hybrids must be made with the relevant cell type and ways must be found following the work of Klebe *et al.* (1970) for producing hybrids which express the differentiated functions. If intraspecific hybrids are to be used for genetic analysis, then these must be induced to segregate chromosomes, or else selective techniques for the identification of segregants must be devised. Pontecorvo (1971) has recently reported that in hamster-mouse hybrids he could influence the direction of chromosome loss by damaging the chromosomes of one parent before hybridization. This same technique, or modifications of it, can perhaps also be used with intraspecific hybrids. Before long, mechanisms analogous to viral transduction, or DNA mediated transformation also may well be found in somatic cells in culture.

Cellular hybridization transcends the normal species barriers to the more conventional organismal hybridization, and so provides a novel approach to the study of evolutionary homologies, for example of linkage maps. The formation of heteropolymeric human-mouse bands has been observed for all those enzymes which, from the study of heterozygotes for electrophoretic variants, are dimer molecules (see *e.g.* Santachiara *et al.*, 1970). This is, of course, an indication of evolutionary homology between the corresponding human and mouse polypeptides.

It is tempting to speculate that cell fusion and hybridization accompanied by more or less erratic chromosomal segregation, may have been the precursor of meiosis in primitive unicellular eukaryotes. Such a mechanism could lead to evolutionarily effective recombination of genetic information by segregation of whole chromosomes, before the evolution of precise pairing and recombination within the chromosome (see Bodmer, 1972). In the absence of a precisely controlled mitosis in single celled organisms, extra chromosomes may be much more readily tolerated than in complex multicellular organisms. This is, after all, borne out by experience with tumours, established cell lines and hybrids. Thus cell fusion, which could easily have happened at first by chance, might have led to the advantageous production of increased genetic variability without an immediate need for refinement of the process. Such refinement could easily have been selected for later after the advantage of fusion had already been realized. Meiosis might thus actually have evolved as a regularization of cell division following cell fusion. Perhaps our experiments in the laboratory are now turning back evolution's clock to the times of primitive eukaryotes, and so in the course of our studies in somatic cell genetics we may at the same time learn more about the evolutionary processes that took place at this early stage of development of eukaryotes.

ACKNOWLEDGEMENTS

Experimental work reported on in this paper which was done at Stanford was supported in part by grants from the U.S. Public Health Service and Atomic Energy Commission, and work done in Oxford by a grant from the Medical Research Council.

REFERENCES

BODMER, W. F. (1972): The evolution of recombination mechanisms in bacteria. In: *Proceedings, International Summer Schools on Uptake of the Informative Molecules by Living Cells.* In press.

BODMER, W. F. and FELSENSTEIN, J. (1967): Linkage and selection: theoretical analysis of the deterministic two locus random mating model. *Genetics*, *57*, 237.

BODMER, W. F. and PARSONS, P. A. (1962): Linkage and recombination in evolution. *Advanc. Genet.*, *11*, 1.

CLAYTON, D. A., TEPLITZ, R. L., NABHOLZ, M., DOVEY, H. and BODMER, W. F. (1971): Mitochondrial DNA of human-mouse cell hybrids. *Nature* (*Lond.*), *234*, 560.

DAVIDSON, R. and EPHRUSSI, B. (1965): A selective system for the isolation of hybrids between L cells and normal cells. *Nature* (*Lond.*), *205*, 1170.

ELICEIRI, G. L. and GREEN, H. (1969): Ribosomal RNA synthes is inhuman-mouse hybrids cells. *J. molec. Biol.*, *41*, 253.

GALL, J. G. and PARDUE, M. L. (1969): Formation and detection of RNA-DNA hybrid molecules in cytological preparation. *Proc. nat. Acad. Sci. USA*, *63*, 378.

HARRIS, H. and WATKINS, J. F. (1965): Hybrid cells derived from mouse and man: artificial heterokaryons of mammalian cells from different species. *Nature* (*Lond.*), *205*, 640.

JOHN, H. A., BIRNSTIEL, M. L. and JONES, K. W. (1969): RNA-DNA hybrids at the cytological level. *Nature* (*Lond.*), *223*, 582.

KLEBE, R. J., CHEN, T. and RUDDLE, F. H. (1970): Mapping of a human genetic regulator element by somatic cell genetic analysis. *Proc. nat. Acad. Sci. USA*, *66*, 1220.

LESCH, M. and NYHAN, W. L. (1964): A familial disorder of uric acid metabolism and central nervous system function. *Amer. J. Med.*, *36*, 561.

LITTLEFIELD, J. W. (1964): The selection of hybrid mouse fibroblasts. *Cold Spr. Harb. Symp. quant. Biol.*, *29*, 161.

MATSUYA, U., GREEN, H. and BASILICO, C. (1968): Properties and uses of human-mouse hybrid cell lines. *Nature* (*Lond.*), *220*, 1199.

MIGEON, B. R. and MILLER, C. S. (1968): Human-mouse somatic cell hybrids with single human chromosome (group E) link with thymidine kinase activity. *Science*, *162*, 1005.

MIGEON, B. R., SMITH, S. W. and LEDDY, C. L. (1969): The nature of thymidine kinase in the human-mouse hybrid cell. *Biochem. Genet.*, *3*, 583.

MIGGIANO, V., NABHOLZ, M. and BODMER, W. F. (1969): Hybrids between human leukocytes and a mouse cell line: production and characterization. *Wistar Inst. Symp. Monograph*, *9*, 61.

MILLER, O. J., ALLDERDICE, P. W., MILLER, D. A., BREG, W. R. and MIGEON, B. R. (1971*a*): Human thymidine kinase gene locus: assignment to chromosome 17 in a hybrid of man and mouse cells. *Science*, *173*, 244.

MILLER, O. J., COOK, P. R., MEERA KHAN, P., SHIN, S. and SINISCALCO, M. (1971*b*): Mitotic separation of two human X-linked genes in man-mouse somatic cell hybrids. *Proc. nat. Acad. Sci. USA*, *68*, 116.

NABHOLZ, M., MIGGIANO, V. and BODMER, W. F. (1969): Genetic analysis using human-mouse somatic cell hybrids. *Nature* (*Lond.*), *223*, 358.

OKADA, Y. and TADOKORO, J. (1963): The distribution of cell fusion capacity among several cell strains of cells caused by HVJ. *Exp. Cell Res.*, *32*, 417.

PONTECORVO, G. (1971): Induction of directional chromosome elimination in somatic cell hybrids. *Nature* (*Lond.*), *230*, 367.

ROWLEY, J. D. and BODMER, W. F. (1971): The relationship of centromeric heterochromatin to fluorescent banding patterns of metaphase chromosomes in the mouse. *Nature* (*Lond.*), *231*, 503.

RUDDLE, F. H., CHAPMAN, V. M., CHEN, T. R. and KLEBE, R. J. (1970): Linkage between human lactate dehydrogenase A and B and peptidase B. *Nature* (*Lond.*), *227*, 251.

SANTACHIARA, A. S., NABHOLZ, M., MIGGIANO, V., DARLINGTON, A. J. and BODMER, W. F. (1970): Genetic analysis with man-mouse hybrids: linkage between human lactate dehydrogenase B and peptidase B. *Nature* (*Lond.*), *227*, 248.

SEEGMILLER, J. E., ROSENBLOOM, F. M. and KELLEY, W. N. (1968): Enzyme defect associated with a sex-linked human neurological disorder and excessive purine synthesis. *Science*, *55*, 1682.

STANNERS, C. P., ELICEIRI, G. L. and GREEN, H. (1971): Two types of ribosome in mouse-hamster hybrid cells. *Nature New Biol.*, *230*, 52.

SZYBALSKI, W., SZYBALSKA, E. H. and BROCKMAN, R. W. (1961): Biochemical basis of sequential mutations toward resistance to purine analogs in human cell lines. *Proc. Amer. Ass. Cancer Res.*, *3*, 272 (Abstract).

WEISS, M. C. and GREEN, H. (1967): Human-mouse hybrid cell lines containing partial complements of human chromosomes and functioning human genes. *Proc. nat. Acad. Sci. USA*, *58*, 1104.

F. H. Ruddle[1], *T. R. Chen*[1] *and C. M. Boone*[2]

[1]Department of Biology, Kline Biology Tower, Yale University, New Haven, Conn., U.S.A. and [2]Biology Institute, Hacettepe University, Ankara, Turkey

Assignment of genes to chromosomes using somatic cell hybrids (TK:E-17; LDH-A:C-11)

It is possible to establish gene-gene and gene-chromosome linkage assignments in man using somatic cell hybrids. Mouse-man hybrids are useful materials for this purpose. The human and murine enzymes can be readily discriminated by simple electrophoretic procedures. The human and mouse chromosomes can be identified specifically in the hybrid cells by means of newly devised cytological techniques such as quinacrine mustard staining (Caspersson *et al.*, 1970; Boone *et al.*, 1972) and heterochromatin staining methods (Arrighi and Hsu, 1971; Chen and Ruddle, 1971). Clonally derived hybrid cell populations of independent origins possess complete mouse genomes but reduced human genomes. The human chromosome constitution varies between clones in terms of the number and kinds of human chromosomes present. The 'segregation' of human chromosomes in the clonal lines serves as the basis for linkage analysis. If enzyme markers and chromosomes can be detected concomitantly then one can presume linkage; alternatively, if the expression is discordant then non-linkage is strongly indicated. In some instances drug selection methods permit the 'fixation' of certain human linkage groups, so that each clone retains a particular human chromosome. This greatly facilitates the detection of linkage. Possible difficulties in establishment of linkage relationships may be experienced if chromosomes undergo breakage and rearrangement. These difficulties can be overcome if the clonal populations are of independent origin, and if sufficient clones exist to enable a statistical approach to the ascertainment of linkage. Contrawise, chromosome rearrangement may serve a useful function, since by disturbing the normal linkage arrangements it is possible to make gene assignments to subregions of particular chromosomes.

METHODS

TISSUE CULTURE CELL POPULATIONS

In the work which we shall report many of the clones are of independent origins. Several different hybridizations involving different parental cell lines have been used. These are: (1) WL, a cross between WI-38 and LMTK$^-$ (Boone, 1969; Boone and Ruddle, 1969); (2) J, a cross between Rag and human leucocytes (Ruddle *et al.*, 1970); (3) WR, a cross between Rag and WI-38 (Klebe *et al.*, 1970); and (4) WRD, a second cross between Rag and WI-38. The parental lines were LMTK$^-$, a mouse line deficient in thymidine kinase (TK) originally derived from C3H mice (Kit *et al.*, 1963). Rag is a mouse line deficient in hypoxanthine-

guanine phosphoribosyl transferase (H-G PRT) and derived from a kidney adenoma in BALB mice (Klebe *et al.*, 1970). WI-38 is a human primary fibroblast line derived from fetal lung. The human leucocyte cells were heterozygous for an electrophoretic variant form of lactate dehydrogenase-B (LDH-B) (Ruddle *et al.*, 1970). Methods for the tissue culture maintenance of these stocks have been reported elsewhere (Ruddle *et al.*, 1970).

ENZYME ANALYSIS

The clonal cell lines were prepared for enzyme analysis within several months of their origin. Chromosome analysis was conducted within several passages of the passage used for enzyme analysis. Some twenty gene products could be analysed simultaneously. These enzymes and the methods for their detection are described elsewhere (Shows and Ruddle, 1968; Ruddle *et al.*, 1970; Ruddle and Nichols, 1971).

CHROMOSOME METHODS

The cell populations were prepared according to methods described in detail elsewhere (Moorhead *et al.*, 1960; Chen, 1970). Briefly, each clone was studied with respect to orcein staining, quinacrine mustard (QM) staining, centric heterochromatic (CH) staining, and *in situ* annealing of isotopically labelled mouse sat-DNA or its complementary RNA (Jones, 1970; Pardue and Gall, 1970; Gall and Pardue, 1971). A number of cells (5–30) were studied by each method per clone in many instances. The frequencies of particular human chromosomes were then calculated and compared with the isozyme data.

Comparison of isozyme and chromosome data was facilitated by computer analysis. Computer programs were written which permitted a pair-wise comparison of phenotypes, and phenotypes with specific chromosomes. Details of the computer programs and formats are available on request (Tables 1 and 2).

RESULTS

LINKAGE OF THYMIDINE KINASE (TK) TO CHROMOSOME E-17

The TK linkage was fixed in the WL clones by drug selection. These clones uniformly retained E-17 chromosomes as judged by chromosome analysis (Boone and Ruddle, 1969; Boone *et al.*, 1972) (see also Fig. 1 in the present paper). Similar results have been obtained by others (Migeon and Miller, 1968; Boone and Ruddle, 1969). Miller *et al.* (1971) have recently presented excellent evidence based on QM for the assignment of TK to E-17. In one exceptional clone in our WL series a translocation between E-17 and a mouse chromosome was detected (see Fig. 1 in Boone *et al.*, 1972). The clone was subjected to counter selection using medium containing BUdR. The counter selected cultures lacked the translocated chromosome. This result supports cytological interpretations regarding the nature of the translocation. The translocation of the human chromosome to a mouse chromosome was supported by *in situ* annealing studies using mouse satellite c-RNA. This material reacts only with mouse centric heterochromatin. The centromere of the translocation chromosome was of murine origin by this test. This result was also supported by CH staining which showed large blocks of heterochromatin at the centromere which is characteristic of the mouse biarmed chromosomes. The E-17 contribution to the translocation chromosome was identified by QM staining. The long arm portion of the E-17 together with a segment of human centric heterochromatin was translocated to the mouse chromosome. The presence of human centromeric heterochromatin could be inferred by secondary constriction at the exchange site, presence of heterochromatin at the exchange site which did not react with mouse satellite c-RNA, and the QM morphology. The

observations taken together provide support for the assignment of the TK locus to the long arm of E-17 (Boone *et al.*, 1972) (Table 1).

Table 1 presents data indicating the presence or absence of the E-17 chromosome (Section *a*) and the E-18 chromosome (Section *b*) in the clones tested for enzyme thymidine kinase

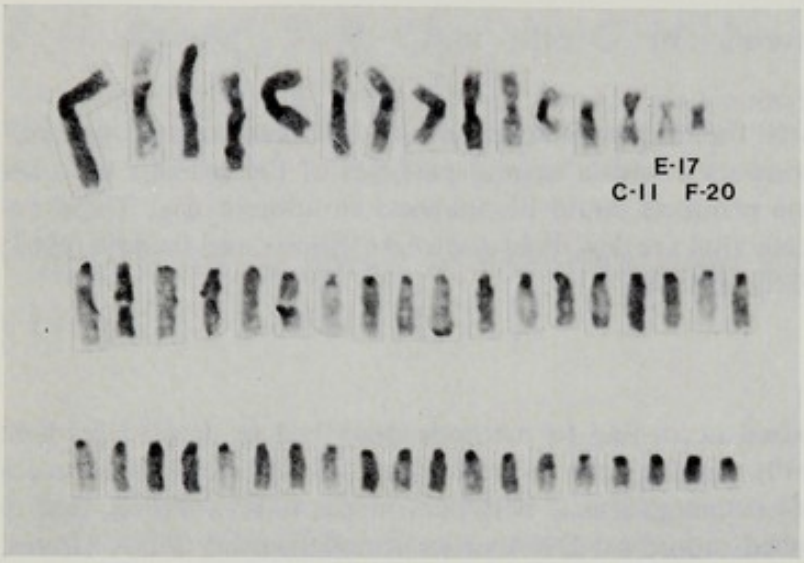


Fig. 1. Karyogram of a centric heterochromatin-stained preparation from a subclone with positive expression of enzymes LDH-A and IDH showing the presumptive C-11 and F-20 chromosomes. The subclone was grown in HAT medium and the E-17 chromosome is present as expected.

TABLE 1

(a) Chromosome E-17 Enzyme TK											
Parent 1	1	1	1	1	1	1	1	1	1	1	1
Parent 2	6	6	6	6	6	6	6	6	6	6	6
Exp. no.	6	6	7	7	7	7	6	7	7	7	7
1st gen.	21	21	11	11	11	11	21	11	11	11	11
2nd gen.	41	41	21	31	41	71	11	21	21	51	51
3rd gen.	51	11	11	11	31	11	11	11	11	31	21
4th gen.	0	0	22	0	0	0	0	0	11	0	0
Per cent	100	83	0	0	0	83	86	0	0	2	88
+ No. of cells	5	6	0	56	18	18	21	52	35	60	17
Per cent	0	0	0	0	0	0	0	0	0	0	0
− No. of cells	0	0	34	0	0	0	0	0	0	0	0
(b) Chromosome E-18 Enzyme TK											
Parent 1	1	1	1	1	1	1	1	1	1	1	1
Parent 2	6	6	6	6	6	6	6	6	6	6	6
Exp. no.	6	6	7	7	7	7	6	7	7	7	7
1st gen.	21	21	11	11	11	11	21	11	11	11	11
2nd gen.	41	41	21	31	41	71	11	21	21	51	51
3rd gen.	51	11	11	11	31	11	11	11	11	31	21
4th gen.	0	0	22	0	0	0	0	0	11	0	0
Per cent	0	0	0	34	0	0	0	0	0	0	6
+ No. of cells	5	6	0	56	18	18	21	52	35	60	17
Per cent	0	0	0	0	0	0	0	0	0	0	0
− No. of cells	0	0	34	0	0	0	0	0	0	0	0

TABLE 2

a. Chromosome C-11	*Enzyme LDH-A*																					
Parent 1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	1	1	1	2	2
Parent 2	6	6	6	6	6	6	6	6	6	6	6	5	5	5	5	5	5	2	2	2	5	5
Exp. no.	6	6	7	7	7	7	6	7	7	7	7	5	5	5	5	5	5	1	1	1	5	5
1st gen.	21	21	11	11	11	11	21	11	11	11	11	31	101	101	101	101	101	11	51	61	31	101
2nd gen.	41	41	21	31	41	71	11	21	21	51	51	71	101	101	101	101	161	151	0	0	91	101
3rd gen.	51	11	11	11	31	11	11	11	11	31	21	0	0	33	193	173	0	0	0	0	0	71
4th gen.	0	0	22	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0
Per cent	60	100	0	68	44	0	76	0	0	7	0	0	95	50	0	0	100	97	0	0	0	0
+ No. of cells	5	6	0	56	18	0	21	0	0	60	0	0	42	6	0	0	13	31	0	0	0	0
Per cent	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
− No. of cells	0	0	34	0	0	18	0	52	35	0	17	15	0	0	14	9	0	0	14	10	14	7
b. Chromosome C-12	*Enzyme LDH-A*																					
Parent 1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	1	1	1	2	2
Parent 2	6	6	6	6	6	6	6	6	6	6	6	5	5	5	5	5	5	2	2	2	5	5
Exp. no.	6	6	7	7	7	7	6	7	7	7	7	5	5	5	5	5	5	1	1	1	5	5
1st gen.	21	21	11	11	11	11	21	11	11	11	11	31	101	101	101	101	101	11	51	61	31	101
2nd gen.	41	41	21	31	41	71	11	21	21	51	51	71	101	101	101	101	161	151	0	0	91	101
3rd gen.	51	11	11	11	31	11	11	11	11	31	21	0	0	33	193	173	0	0	0	0	0	71
4th gen.	0	0	22	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0
Per cent	0	0	0	0	0	0	0	0	0	3	0	0	83	83	0	0	92	84	0	0	0	0
+ No. of cells	5	6	0	56	18	0	21	0	0	60	0	0	42	6	0	0	13	31	0	0	0	0
Per cent	0	0	0	0	0	0	0	6	0	0	0	100	0	0	107	67	0	0	0	0	0	100
− No. of cells	0	0	34	0	0	18	0	52	35	0	17	15	0	0	14	9	0	0	14	10	14	7

This Table presents the data indicating the presence or absence of chromosomes C-11 (Section *a*) and C-12 (Section *b*) in clones tested for enzyme LDH-A. The format is the same as in Table 1.

(TK). The two lines labelled parent 1 and parent 2 give the code numbers of the parent cell lines. The third line gives the experiment code number. The 4th through 7th lines give the clone and subclone code numbers, with the last digit in each code number indicating the media in which the respective clone was grown. The 8th line gives the per cent of cells in each positive clone in which the assigned chromosome is present. The 9th line gives the final number of cells in each clone positive for TK. The 10th line gives the per cent of cells in each negative clone in which the assigned chromosome is present. The last line gives the total number of cells in each clone negative for TK.

For example, in the first column, code numbers 1 (WI-38) and 6 (LMTK$^-$) indicate the parent. The experiment code no. is 6 for hybrid clone 21 and 7 for hybrid clone 24. Lines 4–7 indicate the clone was number 2–4–5, a third subclone, with the terminal 1 indicating the three subsequent clonings were all grown on HAT medium. Lines number 8 and 9 indicate that the E-17 chromosomes were present in 100% of a total of 5 cells, and that this clone was positive for TK. Lines 10 and 11 are zero since this was not a negative clone.

Modified E-17 chromosome (see text) was not incorporated in this Table. Thus, for example in the ninth column, the subclone 24–1–2–1, though positive for TK, has no intact E-17 among metaphase cells analysed. Instead, translocated mouse/E-17 chromosome was detectable in all.

LINKAGE OF LACTATE DEHYDROGENASE A (LDH-A) TO CHROMOSOME C-11

The LDH-A phenotype freely segregated in all clonal series investigated. Therefore, it can be inferred to be unlinked to either E-17 or C-X. In the series of clones examined a strong correlation was observed between LDH-A expression and the occurrence of a C-11 chromosome (Fig. 2). In a total of 23 clones, 12 LDH-A(−) clones were observed and none contained a C-11 chromosome (Table 2).

In no instance have clones been observed with even low frequencies of C-11 chromosomes. It should be emphasized that even one clone of this type (LDH-A −/C-11 +) would provide strong evidence against the assignment of LDH-A to C-11. A total of 11 LDH-A(+) clones were analysed. Of these, 10 possessed recognizable unmodified C-11 chromosomes. A single LDH-A(+) clone was encountered which possessed no intact C-11 chromosome (Table 2). On further analysis it was determined that a translocation chromosome was present which

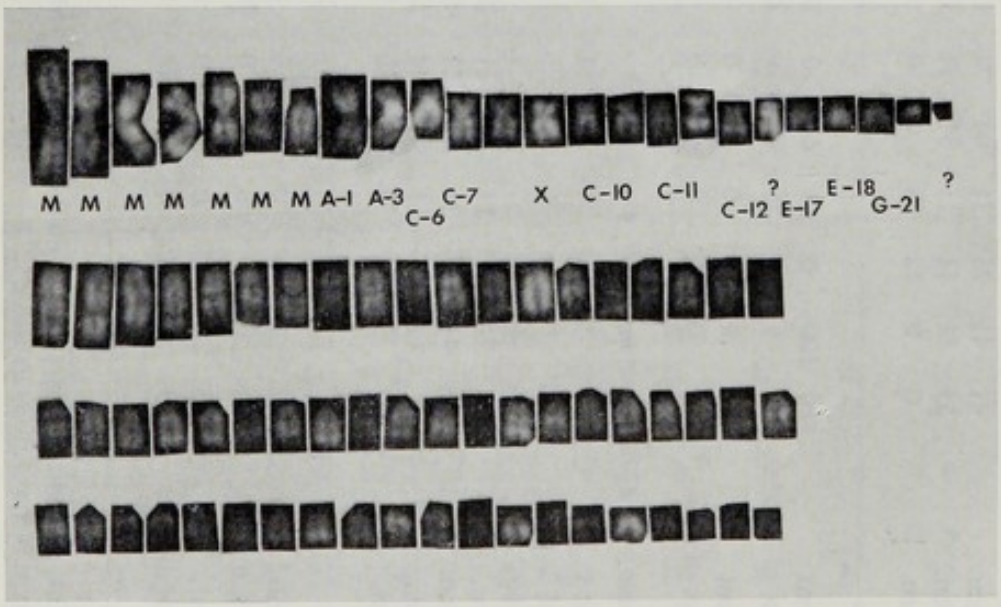


Fig. 2. Karyogram of a QM-stained preparation from an LDH-A positive subclone showing an unmodified C-11 chromosome.

contained a portion of the C-11 chromose (Fig. 3). It appeared on the basis of QM staining that the entire short arm, centromere region, and proximal long arm segment of the C-11 chromosome were present. The origin of the foreign chromosomal segment cannot be determined. The translocation provides presumptive evidence that the locus for LDH-A is restricted to the short arm and proximal long arm regions of the C-11 chromosome. It is also possible that the locus has been translocated elsewhere, and resides in fact on the distal long arm segment. Analysis of other C-11 translocations of independent origins will ultimately permit the establishment of this point.

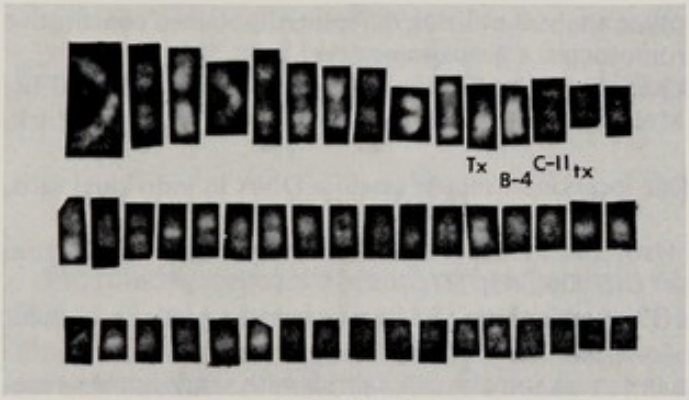


Fig. 3. Karyogram of a QM-stained preparation from an LDH-A positive subclone showing a rearranged C-11 ($C\text{-}11_{tx}$) chromosome. See text for detailed description of the $C\text{-}11_{tx}$.

DISCUSSION

The development of somatic genetics analysis in somatic cell hybrids together with recent advances in chromosome cytology now in combinations make possible a new and powerful approach to human gene mapping. It is obvious that biochemical markers can now be readily assigned to the individual human chromosomes. It is also apparent that use of chromosome translocations and other rearrangements can be used to map genes to specific subregions of chromosomes. The linkage maps which will derive from this work will undoubtedly contribute to many aspects of clinical genetics. Moreover, such linkage information can also be expected to enhance the development of investigations in other areas of somatic cell genetics such as DNA mediated transformation and somatic recombination. Finally, knowledge pertaining to the localization of genes in the genome must in the future contribute to the recognition and analysis of mechanisms which control differentiation and morphogenesis in higher organisms – especially man.

ACKNOWLEDGEMENTS

We are grateful for the excellent technical assistance of Mrs. Iris Kleinman, Miss Elizabeth Nichols, Suzie Chen and Richard Creagan, and Mr. Dixon Yun. We are indebted to Mrs. Mae Reger for her help in the preparation of the manuscript. The work was supported by Grant GM-09966 from the Institute of General Medical Services, The National Institutes of Health, and by Contract PH 43-67-1463 from The Division of Biologic Standards.

REFERENCES

ARRIGHI, F. E. and HSU, T. C. (1971): Localization of heterochromatin in human chromosomes. *Cytogenetics, 10*, 81.

BOONE, C. M. (1969): *Enzyme Expression and Linkage Studies in Somatic Cell Hybrids between Mouse and Human*. Thesis, Yale University.

BOONE, C. M., CHEN, T. R. and RUDDLE, F. H. (1972): Assignment of the lactate dehydrogenase A human gene locus to chromosome C-11 using man-mouse somatic cell hybrids. In preparation.

BOONE C. M. and RUDDLE, F. H. (1969): Interspecific hybridization between human and mouse somatic cells: enzyme and linkage studies. *Biochem. Genet.*, *3*, 119.

CASPERSSON, T., ZECH, L., JOHANSSON, C. and MODEST, E. J. (1970): Identification of human chromosomes by DNA-binding fluorescent agents. *Chromosoma (Berl.)*, *30*, 215.

CHEN, T. R. (1970): Fish chromosome preparations: air-dried displays of cultured ovarian cells in two killifishes (*Fundulus*). *J. Fish. Res. Board Canada*, *27*, 158.

CHEN, T. R. and RUDDLE, F. H. (1971): Karyotype analysis utilizing differentially stained constitutive heterochromatin of human and murine chromosomes. *Chromosoma (Berl.)*, *34*, 57.

GALL, J. G. and PARDUE, M. L. (1971): Nucleic acid hybridization in cytological preparation. In: *Methods in Enzymology, XIIC*. Editors: K. Moldave and L. Grossman. Academic Press, New York, N.Y.

JONES, K. W. (1970): Chromosomal and nuclear location of mouse satellite DNA in individual cells. *Nature (Lond.)*, *255*, 915.

KIT, S., DUBBS, D. R., PIEKARSKI, L. J. and HSU, T. C. (1963): Deletion of thymidine kinase from L-cells resistant to bromodeoxyuridine. *Exp. Cell Res.*, *31*, 297.

KLEBE, R. J., CHEN, T. R. and RUDDLE, F. H. (1970): Mapping of a human genetic regulator element by somatic cell genetic analysis. *Proc. nat. Acad. Sci. USA*, *66*, 1220.

MIGEON, B. R. and MILLER, C. W. (1968): Human-mouse somatic cell hybrids with single human chromosome (Group E): linkage with thymidine kinase activity. *Science*, *162*, 1005.

MILLER, O. J., ALLDERDICE, P. W., MILLER, D. A., BREG, W. R. and MIGEON, B. R. (1971): Human thymidine kinase gene locus: assignment to chromosome 17 in a hybrid of man and mouse cells. *Science*, *173*, 244.

MOORHEAD, P. S., NOWELL, P. C., MELLMAN, W. J., BATTIPS, D. M. and HUNGERFORD, D. A. (1960): Chromosome preparations of leucocytes cultured from human peripheral blood. *Exp. Cell Res.*, *20*, 613.

PARDUE, M. L. and GALL, J. G. (1970): Chromosomal localization of mouse satellite DNA. *Science*, *168*, 1356.

RUDDLE, F. H., CHAPMAN, V. M., CHEN, T. R. and KLEBE, R. J. (1970): Linkage between human lactate dehydrogenase A and B and peptidase B. *Nature (Lond.)*, *227*, 251.

RUDDLE, F. H. and NICHOLS, E. A. (1971): Starch gel electrophoretic phenotypes of mouse × human somatic cell hybrids and mouse isozyme polymorphisms. *In Vitro*, *17*, 120.

SHOWS, T. B. and RUDDLE, F. H. (1968): Function of the lactate dehydrogenase B gene in mouse erythrocytes: evidence for control by a regulatory gene. *Proc. nat. Acad. Sci. USA*, *61*, 574.

Chapter IX Pharmacogenetics

Arno G. Motulsky

Departments of Medicine and Genetics, University of Washington, Seattle, Wash., U.S.A.

History and current status of pharmacogenetics

Pharmacogenetics is a branch of biochemical genetics and its emergence as a field of scientific investigation is coeval with the flowering of human biochemical genetics in the 1950's. The founder of biochemical genetics, Sir Archibald Garrod, with his insistence on the chemical individuality of man, suggested already in the first decade of this century that 'even those idiosyncrasies with regard to drug and articles of food which are summed up in the proverbial saying that what is one man's meat is another man's poison presumably have a chemical basis' (Garrod, 1909). Garrod was far ahead of his times and little attention was paid to his pioneering observations. Another towering figure in the history of genetics, J.B.S. Haldane, took up these ideas some 50 years later. By that time, the one gene-one man enzyme theory had become established through work in microorganisms and Haldane envisioned many of the future developments of biochemical genetics. He stated that 'the future of biochemical genetics applied to medicine is largely in the study of diatheses and idiosyncrasies, differences of innate make-up which do not necessarily lead to disease but may do so' (Haldane, 1954). The time was thus ripe in the 1950's to look for genetically determined enzyme variants in man which might predispose their carriers to untoward or abnormal reactions when exposed to a drug.

Independent from these developments in genetics, a research group at the University of Chicago headed by Dr. Alving was engaged in testing antimalarial drugs for the U.S. Army. Approximately 10% of American Negroes but very few Caucasians developed severe hemolysis when given the new drug primaquine in conventional doses (Hockwald *et al.*, 1952). Other 8 aminoquinoline drugs also caused blood destruction. A series of classical studies demonstrated the intraerythrocytic nature of the defect (Dern *et al.*, 1954) and Beutler worked out that nonprotein glutathione levels of the red cell were affected (Beutler *et al.*, 1955). In 1956 Carson showed that G6PD deficiency appeared to be the primary defect (Carson *et al.*, 1956). An erythrocytic enzyme deficiency which was much more common in Negroes than in other populations strongly suggested a genetic defect and in 1957 a group in Dr. Barton Childs' laboratory in Baltimore first demonstrated that primaquine sensitivity was inherited as an X linked trait (Childs *et al.*, 1958). A new X linked marker gene had thus been found by investigation of a drug reaction.

In 1952 it had been reported that occasional patients developed prolonged apnea when given suxamethonium (Evans *et al.*, 1952). Serum pseudocholinesterase hydrolyzes suxamethonium and lowish pseudocholinesterase levels were found in drug-sensitive but otherwise healthy patients as well as in their family members (Lehmann and Ryan, 1956). Based on these observations, recessive inheritance was suggested by Lehmann's group. However, enzyme levels of putative affected homozygotes and heterozygotes overlapped as did those of heterozygotes with normals. Kalow and his group were able to clarify the issue by demonstrating that pseu-

docholinesterase of affected patients was qualitatively abnormal and could be clearly differentiated from normals by inhibition tests (Kalow and Genest, 1957). Family studies using such inhibition tests clearly established the autosomal recessive mode of inheritance of suxamethonium sensitivity (Kalow and Staron, 1957). All three genetic classes (normal and abnormal homozygotes as well as heterozygotes) could be differentiated without any overlap. Thus, another genetic marker had become defined by a study of a drug idiosyncrasy.

When Lehmann presented his data of genetically low pseudocholinesterase levels in Copenhagen at the 1st Congress of Human Genetics in 1956, I discussed these findings comparing them with primaquine sensitivity and pointed out the importance of these observations as models for other types of drug reactions. At that time, I served on the subcommittee on Blood Dyscrasias of the Committee on Research of the American Medical Association and at the invitation of that committee prepared an article '*Drug Reactions, Enzymes and Biochemical Genetics*' on these and related matters. It is of interest that a senior pharmacologist who had to pass on the report objected to these new ideas on the grounds that physicians might use the existence of genetic differences as an excuse for careless use of drugs. However, the article was published in *JAMA* in October, 1957 (Motulsky, 1957), but the word pharmacogenetics was not yet used. The concepts relating drug idiosyncrasy to genetic variation were mentioned in a lecture at the Free University of Berlin in late 1957 attended by Vogel. In a 1959 review paper on human genetics Vogel included the relation of drugs to enzymes and first used the term pharmacogenetics (Vogel, 1959).

In 1959 and 1960 the genetic determination of acetylation of isoniazid was demonstrated by Knight *et al.* (1959) and by Evans *et al.* (1960). Monogenically determined variation in acetyl transferase activity was shown to be the cause of the discontinuous distribution of isoniazid blood levels. In all these examples, biochemical studies on the nature of drug reaction preceded genetic studies.

The year 1962 saw the publication of Kalow's monograph on '*Pharmacogenetics*' (Kalow, 1962), and in 1963 a book on experimental pharmacogenetics by Meier appeared (Meier, 1963). The first symposium on pharmacogenetics at Titisee, Germany in 1964 was followed by the New York Academy of Sciences symposium on pharmacogenetics in 1967 and by another symposium in 1970 in Titisee. Full publications of the 1967 New York (LaDu and Kalow, 1968) and Titisee 1970 (Goedde *et al.*, 1970) symposia are available. A New York Academy of Sciences symposium on drug metabolism in 1970 devoted many papers to various aspects of pharmacogenetics (Vesell, 1971).

DEFINITION OF PHARMACOGENETICS

Pharmacogenetics may be defined as the field dealing with the effects of genetic variations on response to drugs. Vesell (1969) narrows the definition on the study of clinically significant consequences of hereditary variation in handling of drugs. While the emphasis in the early days of pharmacogenetics was on idiosyncratic drug reactions such as primaquine sensitivity and suxamethonium apnea, later work has dealt with various types of genetic variation and includes the effect of genetic factors on drug metabolism. Kalow (1971) distinguishes between studies on the genetics of drug metabolism and genetically determined susceptibility of tissues and organs to drugs. A number of studies have dealt with genetic diseases which may render their carriers differentially susceptible to the action of certain drugs. Similarly, the differential reaction of embryos to various drugs if dependent upon the genotype of the fetus or upon aberrant metabolic biotransformation of the drug by mother and/or fetus belongs to pharmacogenetics.

Untoward drug reactions caused by antibody mediated mechanisms are usually subsumed under the term drug allergy and are not usually included in pharmacogenetics. However, excellent evidence is accumulating that the ability to form antibodies is under genetic control

(McDevitt and Chinitz, 1969), and thus drug allergies probably fall into the field although no critical human studies have been published yet.

The role of drugs in the production of point mutations or of chromosomal aberrations in somatic or germinal cells is probably best assigned to the area of chemical mutagenesis rather than to pharmacogenetics. However, possible differential susceptibility to mutagenic agents would be part of pharmacogenetics. Some investigators would consider any study at the interface of pharmacology and genetics as part of the field of pharmacogenetics. With a new field, strict boundaries are not desirable and the future will show the exact delineation of this area.

CURRENT STATUS OF PHARMACOGENETICS

REASONS FOR FAILURE TO FIND NEW POLYMORPHISMS BY DRUG STUDIES

The hope that many new genetic markers such as G6PD deficiency and atypical cholinesterase could be found by study of drug reactions has not materialized. Nor have many drug reactions turned out to be caused by transmission of a single Mendelizing gene. Only a relatively small number of pharmacogenetic phenomena are mediated by such mechanisms. Besides G6PD deficiency and pseudocholinesterase deficiency, these include polymorphisms in acetylation of INH and related drugs (Evans *et al.*, 1960), drug induced hemolytic anemia due to Hb Zurich and some other unstable hemoglobins (Motulsky and Stamatoyannopoulos, 1968), resistance to anticoagulants unrelated to metabolic breakdown (O'Reilly, 1970), pseudocholinesterase increase causing suxamethonium resistance (Neitlich, 1966; Yoshida and Motulsky, 1969), and methemoglobinemia due to mild methemoglobin reductase deficiency (Cohen *et al.*, 1968). In other instances such as glaucoma precipitated by cortisone (Armaly, 1968) the mode of inheritance is not yet fully clarified but may be monogenic. Several reasons for the relatively small number of such examples may be cited. When a drug moves through the mammalian organism, several enzyme mediated reactions occur such as absorption, plasma protein binding, interaction with cell membranes and cell organelles, liver detoxification, and renal excretion. Genetic variation may be found at several or all of these steps. Minor variation at any one site studied against the pattern of variation at many steps will not give the type of discontinuous response curve seen with hemolysis in G6PD deficiency or prolonged apnea in cholinesterase deficiency. Otherwise stated, the genetic background of all the other phenomena involved in biotransformation will contribute to the total variation in drug response. The effect of variation at any one site may not be apparent unless the enzyme affected can be tested directly. Only major effects or those where a threshold mediates for all or no phenomena (such as hemolysis) can be readily detected. Since many of the enzymes involved in drug metabolism may be tissue-specific and not readily testable in blood or serum, their effects remain poorly defined. Future attention to studies of drug metabolizing enzymes in tissue culture cells may circumvent some of these difficulties. A series of papers in the 1970 New York Academy of Sciences Symposium on Drug Metabolism illustrates the beginnings of such an approach (Krooth, 1971; Nebert and Bausserman, 1971; Neufeld and Cantz, 1971; Kelley *et al.*, 1971; Cox, 1971).

The nature of genetic variation is such that relatively few biochemical variants will have a dramatic all-or-none effect. Most enzyme variants are associated with only relatively small increases or elevations in enzyme activity so that the majority of the expected effects of genetic variation are unlikely to be gross deviations from normal.

Pharmacologists working with lower species usually lack training in genetics and rarely use genetic techniques in their experimental design. Basic geneticists have become interested in chemical mutagenesis but few have worked with pharmacogenetic phenomena as defined here. Studies in human pharmacogenetics require family studies which most clinical pharmaco-

logists are usually not organized to do. Such studies are often difficult and take a great deal of time, effort, and personnel. Most clinical pharmacologists do not have the training nor the time to execute them. Most medical geneticists have many other problems and will not necessarily limit their efforts to research on drug related phenomena. We need more clinical pharmacologists trained in medical genetics to do such work.

TWIN STUDIES AND POLYGENIC INHERITANCE

An interesting development in pharmacogenetics has been the use of twin studies in attempting to define genetic factors in drug metabolism. It is somewhat paradoxical that, as twin studies have somewhat gone out of fashion in medical genetics in general, they have become popular in pharmacogenetics. Twin studies may be highly useful as the first approach to genetic investigations. If, in their response to a drug, identical twins are just as variable as nonidentical twins or even as unrelated persons, family studies will shed little additional light on the problem and are unlikely to be helpful. Since it may sometimes be easier to perform twin studies rather than family investigations, the twin approach may be very useful. Considering the existence of many potentially variant enzymes in drug metabolism, most biochemical geneticists would have predicted high heritability in twin studies. As we will hear in detail from Dr. Vesell, the half-lives of all drugs examined so far by twin studies – alcohol, dicumarol, phenylbutazone, antipyrine (Vesell *et al.*, 1971), halothane (Cascorbi *et al.*, 1971), nortriptyline (Alexanderson *et al.*, 1969), isoniazid (Bönike and Lisboa, 1957) – in fact have shown greater concordance for identical twins than fraternal twins. Many pharmacologists knowing the effect of a variety of external factors on drug disposal would have predicted differently. As in all other phenomena, *both* heredity and environment are important and the demonstration of high heritability for drug biotransformation does not mean that environmental factors are unimportant. The geneticist must further stress that twin studies alone do not give any (or very little) information on the mode of inheritance. Family studies are always required to dissect further the nature of genetic factors in drug metabolism.

The results of family studies have recently been published for both nortriptyline (Åsberg *et al.*, 1971) and phenylbutazone disposal (Whittaker and Evans, 1970) and at an earlier date for dicumarol metabolism (Motulsky, 1964). In all these instances no clearcut monogenic pattern was found; the data (particularly extensive for phenylbutazone) were compatible with polygenic inheritance of drug metabolism. These findings imply that genetic variation at several rather than at a single step of drug metabolism causes the observed variation between individuals. Many geneticists feel vaguely dissatisfied when family studies show the effect of polygenic inheritance. Biologic intuition and more and more data tell us that most, if not all, biologic phenomena involve the action of many genes. Scientific proof of polygenic inheritance is considered by some geneticists analogous to the scientific demonstration by social scientists of many common sense phenomena in the sociological world. The conclusion that polygenic inheritance is operative comes from resemblance of relatives and is based on statistical approaches. The ultimate analysis of polygenic inheritance in pharmacogenetics and elsewhere needs to be laboratory based rather than biometrical. Hopefully, future studies will be able to come to grips with the individual steps of drug metabolism mentioned earlier and analyze the effect of individual genes by family studies. Data accumulated in recent years have demonstrated that a relatively small number of interacting genes may give the bell shaped distribution curve in a population consistent with polygenic inheritance. In many pharmacogenetic phenomena a restricted number of variant genes may be involved so that their action may become amenable to direct analysis.

While analysis of polygenic phenomena ultimately will thrive with full pathophysiologic and biochemical understanding of the action of genes on drug response, empirical attempts to associate common genes of unknown physiologic function to polygenically determined

phenomena may sometimes be successful. A recent example is the strong association of blood group A with thrombosis induced by steroid contraceptive pills. Women of blood group A are three times more likely to develop thrombosis than women of other ABO blood groups (Mourant *et al.*, 1971). Genetically expressed, blood group A represents one among an undefined number of genes predisposing to thrombosis. The mechanism is not known, but it is interesting that blood group A is associated with increased antihemophilic globulin levels (Preston and Barr, 1964). This association is one of the strongest when considering the effects of ABO blood groups on disease (Mourant *et al.*, 1971). In general, most attempts to relate a given polymorphism to a given disease in the absence of any biologic relationship will be fruitless. With computers, such studies, however, may be relatively easy to carry out and occasionally interesting results may be obtained.

POLYMORPHISMS

Developments in human biochemical genetics have given future work in pharmacogenetics a solid theoretical foundation. Enzyme polymorphisms or the existence of structurally variant enzyme types are quite common in the population. When unselected enzymes are examined for electrophoretic differences a significant proportion of individuals in a given population carries a different enzyme than the standard type. Often the variant enzyme is associated with somewhat higher or lower activity so that carriers of the enzyme variant may react differently, particularly when exposed to a drug which depends upon the particular variant enzyme for its metabolism (Motulsky, 1970). As many as 30% of randomly selected enzymes may show polymorphic variation of this type, *i.e.* more than 1–2% of persons carry the gene specifying the variant enzyme (Harris, 1969). Allelic genes which specify gene products with gene action (*i.e.* enzyme levels) within the normal range are known as isoalleles. As an example, isoallelic variation exists at the G6PD locus where the electrophoretically detectable common Gd A+ gene product is associated with 15% less enzyme activity (Motulsky, 1970). If not electrophoretically detectable, the existence of isoalleles may be difficult to demonstrate although the task is easier with X linked inheritance where a given gene in males with a single X chromosome is unopposed by allelic action.

A small fraction of polymorphisms in man have been defined to date. If at least 15% of proteins are polymorphic and if the total number of genes specifying proteins in man is estimated to be 50,000, a minimum of about 7,500 proteins should be polymorphic. We have only scratched the surface with our existing knowledge of polymorphism. As more polymorphisms are being discovered, the study of pharmacogenetics will become more sophisticated.

HETEROGENEITY

The classic pharmacogenetic samples of G6PD deficiency and pseudocholinesterase abnormality have brilliantly demonstrated the existence of considerable genetic heterogeneity at the loci involved in these abnormalities. Thus, G6PD deficiency can be caused by many different mutations at the X linked G6PD locus (Motulsky *et al.*, 1971), the African and Mediterranean types of G6PD deficiency being the important ones. These principles are likely to hold true for many other enzyme deficiencies and may have important practical consequences. Thus, the African type of G6PD deficiency causes milder hemolysis with drugs and fewer drugs cause blood destruction than is observed with the Mediterranean type of G6PD deficiency which may cause more severe and potentially fatal hemolysis (Motulsky *et al.*, 1971).

Pharmacogenetic principles are becoming important in drug treatment of genetically and biochemically heterogeneous disease which may appear homogeneous clinically. A person with hypercholesterolemia may owe his high cholesterol to a polygenic mechanism since

cholesterol level has a significant polygenically determined heritability (Schaefer *et al.*, 1958). Some may be affected with the autosomal dominant type II hypercholesterolemia (Levy and Langer, 1971) and others with a more poorly defined separate genetic disorder associated with elevation in both triglycerides and cholesterol. Other diseases such as nephrosis and hypothyroidism may also be the cause of cholesterol elevation. It appears that different drugs such as cholestyramine (for type II) and clofibrate may be indicated for treatment of the different genetically determined hypercholesterolemic states (Levy and Langer, 1971). Large scale family studies and drug trials are in progress.

Pharmacogenetics has contributed to better understanding of drug enzymology. Since both PAS and INH are inactivated by acetylation, one would expect that the existence of the genetically determined rapid and slow inactivation phenotypes would apply to both drugs. However, acetylation of PAS follows a unimodal distribution curve in a population and there is no correlation between PAS and INH activation (Motulsky, 1964). A different enzyme therefore appears operative for INH and PAS acetylation. More biochemical work is being carried out on the various acetylating enzymes and the interaction with different substrates such as serotonin needs definition.

The specific curative effect of a drug may give insight into the genetic mechanisms of certain diseases. Acrodermatitis enteropathica is an autosomal recessive disease associated with severe skin rash and intractable diarrhea. The disease was fatal until it was empirically discovered that the drug Diodoquin specifically cured patients with the disease (Lever, 1959). The drug is not absorbed and presumably prevents absorption of toxic metabolites allowing a better investigation of the basic defect.

RACIAL DIFFERENCE IN DRUG SENSITIVITY

A genetic mechanism was suspected when blacks and Mediterraneans developed drug hemolysis or when Japanese populations had a much higher number of rapid inactivators of isoniazid than white populations. Recently, clioquinol or Enterovioform, a common antidiarrheal agent, was withdrawn from the market in Japan when it was found that the drug produced subacute myelo-opticoneuropathy in many of its users in that country (Editorial, 1971). Dysesthesia, ataxia, visual impairment and muscular weakness were common symptoms. Since the drug is widely used in Europe and the U.S. without apparent toxicity, it is possible that Japanese react differently to the drug than European populations. Further studies are urgently needed.

HETEROZYGOSITY FOR INBORN ERRORS AS A CAUSE OF DRUG REACTIONS

A conceptually important type of drug reaction is represented by methemoglobinemia induced by a variety of antimalarial drugs. Methemoglobinemia in those instances is associated with mild methemoglobin reductase deficiency (Cohen *et al.*, 1968). The data suggest that patients with this deficiency are heterozygote carriers for the autosomal recessive type of methemoglobinemia. This is a rare defect which causes cyanosis early in life. Parents of patients are obligatory carriers and have normal or almost normal methemoglobin levels under usual living conditions. When challenged with a methemoglobin inducing drug, such carriers in contrast to normals apparently have insufficient enzyme to reduce methemoglobin and methemoglobinemic cyanosis results. Simple genetic arithmetic indicates that even for a rare autosomal recessive disease, there are many carriers in the population. With a disease frequency of 1/10,000, about 2% of the population would be carriers. The example that carriers for the methemoglobinemia gene are sensitive to the action of methemoglobin forming drugs is important. There are many different inborn errors of metabolism and other autosomal recessive diseases in the population. Each one is usually quite rare, but since the heterozygote frequency for each disease is considerably higher than the disease incidence there are many

heterozygotes for one or another disease in the population. It is likely that such heterozygotes may be susceptible or resistant to certain drugs or environmental agents. In the elucidation of the pathogenesis of drug reactions and various common diseases, special attention should be given to this phenomenon, particularly in instances where the enzyme defect is known and can be assayed.

PRACTICAL USES OF PHARMACOGENETICS

Screening of populations with a high frequency of traits predisposing to untoward drug reactions has been recommended but rarely implemented. Screening for G6PD deficiency of hospital patients and various other groups exposed to antimalarial therapy would avoid some hemolytic episodes. For instance, the U.S. Army does not screen but repatriates soldiers with primaquine induced hemolysis from Indochina (Conrad, 1971). In hospitals where many drugs are given, screening could be quite useful in the interpretation of anemias. Screening for pseudocholinesterase abnormality is usually not done, yet 1/3000 Caucasians will have prolonged apnea when given suxamethonium. With good anesthesiologic care, artificial respiration can be given with no untoward results to the patient. However, if such care is not available patients may die. Unfortunately, hospitals without anesthetists will be last to introduce screening although suxamethonium probably is less likely to be used in such institutions.

The differential racial distribution of some traits raises problems. G6PD deficiency is very rare among Northern populations, quite common in blacks and not infrequent in Mediterranean populations (Motulsky *et al.*, 1971). Pseudocholinesterase abnormality is almost absent in blacks and quite rare in Oriental populations such as the Japanese (Motulsky and Morrow, 1968). In multiracial populations, universal screening is expensive and many individuals would be tested who are not at risk. Automated equipment already exists for pseudocholinesterase screening and together with the development of notation of population origin in medical records (or even without such special identification) may solve some of these problems.

Screening can be potentially misused. Thus, recommendations have been made to remove G6PD deficient workers from not very well specified industrial environments which might cause hemolysis (Stokinger *et al.*, 1968). It would appear unfair to deprive G6PD deficient persons of their jobs until careful studies have shown that inhaled or skin absorbed material is in fact hemolytic in the G6PD deficiency variant which is common in the population potentially at risk (*e.g.* African type of G6PD deficiency in U.S. blacks).

Testing of donor blood for transfusion poses thorny issues. Red cells with the mild African type of G6PD deficiency can be transfused with no harm in most situations, while a good case can be made not to use blood with the Mediterranean type of G6PD deficiency. A more detailed discussion regarding these points is given elsewhere (Motulsky, 1971).

Tests for the half life of a drug such as dicumarol may be helpful prior to institution of longterm anticoagulant therapy (Vesell and Page, 1968). Since prothrombin time determinations will almost do the same job in alerting physicians to possible dangers, such tests may not usually be required. However, such an approach may become useful for other potentially toxic drugs before longterm therapy is started. Thus, Dilantin toxicity in patients who also take INH will only occur in patients with acetyl transferase deficiency (*i.e.* slow INH inactivators) (Kutt, 1971). Acetyl transferase status can be readily determined.

PHARMACOGENETICS AS A FIELD OF ECOGENETICS

Drugs are only a small fraction of environmental chemical agents to which mankind is exposed. Various other potentially toxic agents exist in our environment and may cause dam-

age to some genetic constitutions and not to others. The term 'ecogenetics' has been used to refer to the study of genetic aspects of ecology (Brewer, 1971). Homozygotes for a trypsin inhibitor deficiency develop emphysema relatively early in life and even heterozygotes for the trait (1–3% of the population) may have an increased frequency of chronic pulmonary insufficiency (Lieberman, 1969). Testing is relatively easy and affected persons should be particularly cautioned against smoking and exposure to other chemical irritants.

It has been known for many years that alcoholic cirrhosis of the liver only affects some heavy drinkers but not all chronic alcoholics. It has been suggested many years ago that individuals with a relatively small amount of body hair (in populations where there is considerable variation in amount of body hair) are most susceptible to toxic damage by alcohol than are hairy individuals (Chvostek, 1922). Data to support these contentions have been published (Muller, 1952). The genetic and biochemical relationships are not quite clear. Amount of body hair in males has strong genetic determinants and may have some relationship to testosterone metabolism or genetic variation in testosterone receptors. Testosterone has been used in mice to induce alcoholic dehydrogenase (Ohno *et al.*, 1970). More work on these possible relationships is required. The need for this type of information is obvious. Identification of susceptible individuals to any kind of drug or chemical damage renders preventive medicine more rational and manageable.

CONCLUSIONS

The concept of pharmacogenetics has elicited considerable interest in some medical and pharmacologic circles. Much of the work done in the area has often been peripheral and not directed to the central core of the field, *i.e.* the influence of genetic factors on drug handling. Better understanding of biochemica pharmacology and enzymology use use of mammalian tissue fibroblast culture is likely to provide new insights and tools for genetically oriented studies. Considerably more attention needs to be given to studies of human families. Tests for the prevention of untoward drug reactions already exist, but their general introduction into public health and medicine has not been accomplished yet. The pharmacogenetic approach can be extended and applied to differential response to a variety of potentially toxic environmental chemicals.

REFERENCES

Alexanderson, B., Evans, D. A. P. and Sjöqvist, F. (1969): Steady-state plasma levels of nortriptyline in twins: influence of genetic factors and drug therapy. *Brit. med. J.*, *4*, 764.

Armaly, M. F. (1968): Genetic factors related to glaucoma. *Ann. N.Y. Acad. Sci.*, *151*, 861.

Åsberg, M., Evans, D. A. P. and Sjöqvist, F. (1971): Genetic control of nortriptyline kinetics in man. *J. med. Genet.*, *8*, 129.

Beutler, E., Dern, R. J., Flanagan, C. L. and Alving, A. S. (1955): The hemolytic effect of primaquine. VII. Biochemical studies of drug-sensitive erythrocytes. *J. Lab. clin. Med.*, *45*, 286.

Bönicke, R. and Lisboa, B. P. (1957): Über die Erbbedingtheit der intraindividuellen Konstanz der Isoniazidausscheidung beim Menschen. *Naturwissenschaften*, *44*, 314.

Brewer, G. (1971): Human ecology, an expanding role for the human geneticist. *Amer. J. hum. Genet.*, *23*, 92.

Carson, P. E., Flanagan, C. L., Ickes, C. E. and Alving, A. S. (1956): Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science*, *124*, 484.

Cascorbi, H. F., Vesell, E. S., Blake, D. A. and Helrich, M. (1971): Genetic and environmental influence on halothane metabolism in twins. *Clin. Pharmacol. Ther.*, *12*, 50.

Childs, B., Zinkham, W., Browne, E. A., Kimbro, E. L. and Torbert, J. V. (1958): A genetic study of a defect in glutathione metabolism of the erythrocyte. *Bull. Johns Hopk. Hosp.*, *102*, 21.

Chvostek, V. F. (1922): Klinische Vortrage. *Wien. klin. Wschr.*, *35*, 381 and 408.

COHEN, R. J., SACHS, J. R., WICKER, D. J. and CONRAD, M. E. (1968): Methemoglobinemia provoked by malarial chemoprophylaxis in Viet Nam. *New Engl. J. Med., 279*, 1127.

CONRAD, M. E. (1971): Personal communication.

COX, R. P. (1971): Early events in hormonal induction of alkaline phosphatase in human cell culture: hormone-cell interaction and its dependence in DNA replication. *Ann. N.Y. Acad. Sci., 179*, 596.

DERN, R. J., WEINSTEIN, I. M., LEROY, G. V., TALMAGE, D. W. and ALVING, A. S. (1954): The hemolytic effect of primaquine. I. The localization of the drug-induced hemolytic defect in primaquine-sensitive individuals. *J. Lab. clin. Med., 43*, 303.

EDITORIAL (1971): Clioquinol and neurological disease. *Brit. med. J., 2*, 291.

EVANS, D. A. P., MANLEY, K. and MCKUSICK, V. A. (1960): Genetic control of isoniazid metabolism in man. *Brit. med. J., 2*, 485.

EVANS, F. T., GRAY, P. W. S., LEHMANN, H. and SILK, E. (1952): Sensitivity to succinylcholine in relation to serum-cholinesterase. *Lancet, 1*, 1229.

GARROD, A. E. (1909): *Inborn Errors of Metabolism.* Henry Frowde, London. Reprinted by Oxford University Press, London, 1963.

GOEDDE, H. W., ALTLAND, K. and SCHLOOT, W. (Eds) (1970): Pharmacogenetics. (2nd International Titisee Workshop, March 13–14, 1970). *Humangenetik, 9*, 197.

HALDANE, J. B. S. (1954): *The Biochemistry of Genetics.* George Allan and Unwin Ltd., London.

HARRIS, H. (1969): Enzyme and protein polymorphism in human populations. *Brit. med. Bull., 25*, 1.

HOCKWALD, R. S., ARNOLD, J., CLAYMAN, C. B. and ALVING, A. S. (1952): Status of primaquine: toxicity of primaquine in Negroes, report to the Council on Pharmacy and Chemistry. *J. Amer. med. Ass., 149*, 1568.

KALOW, W. (1962): *Pharmacogenetics; Heredity and the Response to Drugs.* W. B. Saunders, Philadelphia, Pa.

KALOW, W. (1971): Topics in pharmacogenetics. *Ann. N.Y. Acad. Sci., 179*, 654.

KALOW, W. and GENEST, K. (1957): A method for the detection of atypical forms of human serum cholinesterase. Determination of dibucaine numbers. *Canad. J. Biochem., 35*, 339.

KALOW, W. and STARON, N. (1957): On distribution and inheritance of human serum cholinesterase, as indicated by dibucaine numbers. *Canad. J. Biochem., 35*, 1305.

KELLEY, W. N., FOX, I. H., BEARDMORE, T. D. and MEADE, J. C. (1971): Allopurinol and oxipurinol: alteration of purine and pyrimidine metabolism in cell culture. *Ann. N.Y. Acad. Sci., 179*, 588.

KNIGHT, R. A., SELIN, J. and HARRIS, H. W. (1959): Genetic factors influencing isoniazid blood levels in humans. *Transactions of Conference on Chemotherapy of Tuberculosis, 18th Conference: 52.*

KROOTH, R. (1971): Molecular models for pharmacological tolerance and addiction. *Ann. N.Y. Acad. Sci., 179*, 548.

KUTT, H. (1971): Biochemical and genetic factors regulating dilantin metabolism in man. *Ann. N.Y. Acad. Sci., 179*, 704.

LADU, B. N. and KALOW, W. (Eds) (1968): Pharmacogenetics. *Ann. N.Y. Acad. Sci., 151/2*, 691.

LEHMANN, H. and RYAN, E. (1956): The familial incidence of low pseudocholinesterase level. *Lancet, 2*, 124.

LEVER, W. F. (1959): Acrodermatitis enteropathica with familial incidence of dermatitis and moniliasis. *Arch. Derm. Syph. (Chic.), 80*, 621.

LEVY, R. I. and LANGER, T. (1971): Hypolipidemic drugs and hyperlipoproteinemia. *Ann. N.Y. Acad. Sci., 179*, 475.

LIEBERMAN, J. (1969): Heterozygous and homozygous alpha-1-antitrypsin deficiency in patients with pulmonary emphysema. *New Engl. J. Med., 281*, 279.

MCDEVITT, H. O. and CHINITZ, A. (1969): Genetic control of antibody response: relationship between immune response and histocompatibility (H-2) type. *Science, 163*, 1207.

MEIER, H. (1963): *Experimental Pharmacogenetics; Physiopathology of Heredity and Pharmacologic Responses.* Academic Press, New York, N.Y.

MOTULSKY, A. G. (1957): Drug reactions, enzymes and biochemical genetics. *J. Amer. med. Ass., 165*, 835.

MOTULSKY, A. G. (1964): Pharmacogenetics. In: *Progress in Medical Genetics, Vol. 3*, pp. 49–74. Editors: A.G. Steinberg and A.G. Bearn. Grune and Stratton, New York, N.Y.

MOTULSKY, A. G. (1970): General remarks on genetic factors in anesthesia. *Humangenetik, 9*, 246.

MOTULSKY, A. G. (1972): Hemolysis in G6PD deficiency. *Fed. Proc.*, in press.

MOTULSKY, A. G. and MORROW, A. (1968): Atypical cholinesterase gene E_1^a: rarity in Negroes and most Orientals. *Science, 159*, 202.

MOTULSKY, A. G. and STAMATOYANNOPOULOS, G. (1968): Drugs, anesthesia and abnormal hemoglobins. *Ann. N.Y. Acad. Sci., 151*, 807.

MOTULSKY, A. G., YOSHIDA, A. and STAMATOYANNOPOULOS, G. (1971): Variants of glucose-6-phosphate dehydrogenase. *Ann. N.Y. Acad. Sci., 179*, 636.

MOURANT, A. E., KOPEĆ, A. C. and DOMANIEWSKA-SOBCZAK, K. (1971): Blood-groups and blood-clotting. *Lancet, 1*, 223.

MULLER, G. (1952): Der erbkonstitutionelle Hypogenitalismus des Mannes als Dispositionsfaktor der Lebercirrhose. *Med. Klin., 47*, 71.

NEBERT, D. W. and BAUSSERMAN, L. L. (1971): Aryl hydrocarbon hydroxylase induction in cell culture as a function of gene expression. *Ann. N.Y. Acad. Sci., 179*, 561.

NEITLICH, H. W. (1966): Increased plasma cholinesterase activity and succinyl choline resistance: a genetic variant. *J. clin. Invest., 45*, 380.

NEUFELD, E. F. and CANTZ, M. J. (1971): Corrective factors for inborn errors of mucopolysaccharide metabolism. *Ann. N.Y. Acad. Sci., 179*, 580.

OHNO, S., STENNIS, C. and CHRISTIAN, L. C. (1970): Sex difference in alcohol metabolism; androgenic steroid as an inducer of kidney alcohol dehydrogenase. *Clin. Genet., 1/1*, 35.

O'REILLY, R. A. (1970): The second reported kindred with hereditary resistance to oral anticoagulant drugs. *New Engl. J. Med., 282*, 1448.

PRESTON, A. E. and BARR, A. (1964): The plasma concentration of factor VIII in the normal population. II. The effects of age, sex and blood groups. *Brit. J. Haemat., 10*, 238.

SCHAEFER, L. E., ADLERSBERG, D. and STEINBERG, A. G. (1958): Heredity, environment, and serum cholesterol: a study of 201 healthy families. *Circulation, 17*, 537.

STOKINGER, H. E., MOUNTAIN, J. T. and SCHEEL, L. D. (1968): Pharmacogenetics in the detection of the hypersusceptible worker. *Ann. N.Y. Acad. Sci., 151*, 968.

VESELL, E. S. (1969): Recent progress in pharmacogenetics. *Advanc. Pharmacol. Chemother., 7*, 1.

VESELL, E. S. (Ed.) (1971): Drug metabolism in man. *Ann. N.Y. Acad. Sci., 179*, 1.

VESELL, E. S. and PAGE, J. G. (1968): Genetic control of Dicumarol levels in man. *J. clin. Invest., 47*, 2657.

VESELL, E. S., PASSANANTI, G. T., GREENE, F. E. and PAGE, J. G. (1971): Genetic control of drug levels and of the induction of drug-metabolizing enzymes in man: individual variability in the extent of allopurinol and nortriptyline inhibition of drug metabolism. *Ann. N.Y. Acad. Sci., 179*, 752.

VOGEL, F. (1959): Moderne Probleme der Humangenetik. In: *Ergebnisse der Inneren Medizin in der Kinderheilkunde, Vol. 12*, pp. 52–125. Editors: L. Heilmeyer, R. Schoen and B. de Rudder. Springer-Verlag, Berlin.

WHITTAKER, J. A. and EVANS, D. A. P. (1970): Genetic control of metabolism of phenylbutazone. *Brit. med. J., 4*, 323.

YOSHIDA, A. and MOTULSKY, A. G. (1969): A pseudocholinesterase variant (E Cynthiana) associated with elevated plasma enzyme activity. *Amer. J. hum. Genet., 21*, 486.

Elliot S. Vesell

Department of Pharmacology, The Milton S. Hershey Medical Center,
The Pennsylvania State University College of Medicine, Hershey, Pa., U.S.A.

Genetic and environmental factors affecting drug metabolism in man

One important adverse effect of the administration of many new potent drugs to large populations is the selection from the exposed population of individuals with a mutant, catalytically less efficient form of the enzyme required to metabolize the drug. After such individuals take the 'normal' dose of the drug, their aberrant enzyme is often evident by the appearance of some form of drug toxicity. Motulsky (1957), Vogel (1959) and Kalow (1962) laid the conceptual framework of the new field of pharmacogenetics, which emerged as an attempt to understand and deal with this situation. An international conference on pharmacogenetics was held in 1967 (La Du and Kalow, 1968), and its proceedings as well as those of several other symposia and reviews on pharmacogenetics have been published (La Du, 1965; Vesell, 1969, 1971). The field of pharmacogenetics has grown significantly since the mid-1950s when less than a dozen entities classifiable under this heading were identified. Progress has consisted less in the description of entirely new conditions than in the recognition of variant forms of the old entities and in further biochemical, genetic and clinical characterization of these disorders. A new area in pharmacogenetics that has stimulated much current activity was opened by the recognition that large differences among healthy, non-medicated individuals in their rates of metabolism of commonly used drugs are primarily under genetic control and are negligibly affected by environmental factors (Vesell and Page, 1968*a*, *b*, *c*).

Each of the ten pharmacogenetic conditions shown in Table 1 behaves as a single factor probably resulting from a single point mutation transmitted in classical Mendelian fashion. At some of these loci multiple mutant alleles have been detected. These pharmacogenetic entities are in fact inborn errors of metabolism affecting drug-metabolizing enzymes. Several are extremely rare, like most inborn errors of metabolism; bishydroxycoumarin sensitivity, deficient parahydroxylation of diphenylhydantoin and warfarin resistance have been reported thus far in only one or two families. However, more extensive population screening could reveal that these pharmacogenetic conditions are much more common than currently believed. In any event, other conditions listed in Table 1, such as glucose-6-phosphate dehydrogenase (G-6-PD) deficiency and slow inactivation of isoniazid, occur commonly; and in certain populations the gene frequency of the 'aberrant' allele is higher than that of the 'normal' allele (Vesell, 1969). With the exception of G-6-PD deficiency, problems of drug toxicity from these pharmacogenetic conditions arise when the individual with the mutant enzyme receives only a single type of drug. The most notable exception, G-6-PD deficiency, produces hemolysis after administration of many commonly used drugs, although in the mutant form encountered most frequently in the United States, the resultant hemolysis is mild and self-limited, even in the face of continued drug administration. The point is that the conditions listed in Table 1

TABLE 1 *Ten pharmacogenetic conditions with putative aberrant enzyme, mode of inheritance and drugs that can elicit the signs and symptoms of the disorder*

Name of condition	Aberrant enzyme and location	Mode of inheritance	Drugs that produce the abnormal response
1. Acatalasia	Catalase in erythrocytes	Autosomal recessive	Hydrogen peroxide
2. Slow inactivation of isoniazid	Isoniazid acetylase in liver	Autosomal recessive	Isoniazid, sulfamethazine, sulfamaprine, phenelzine, dapsone, hydralazine
3. Suxamethonium sensitivity or atypical pseudocholinesterase	Pseudocholinesterase in plasma	Autosomal recessive	Suxamethonium or succinylcholine
4. Diphenylhydantoin toxicity due to deficient para-hydroxylation	?Mixed function oxidase in liver microsomes that parahydroxylates diphenylhydantoin	Autosomal dominant	Diphenylhydantoin
5. Warfarin resistance	?Altered receptor or enzyme in liver with increased affinity for vitamin K	Autosomal dominant	Warfarin
6. Bishydroxycoumarin sensitivity	?Mixed function oxidase in liver microsomes that hydroxylates bishydroxycoumarin	Unknown	Bishydroxycoumarin
7. Glucose-6-phosphate dehydrogenase deficiency, favism or drug-induced hemolytic anemia	Glucose-6-phosphate dehydrogenase	X-linked incomplete codominant	A variety of analgesics [acetanilide, acetylsalicylic acid, acetophenetidin (phenacetin), antipyrine, aminopyrine (Pyramidon)], sulfonamides and sulfones [sulfanilamide, sulfapyridine, N_2-acetyl-sulfanilamide, sulfacetamide, sulfisoxazole (Gantrisin), thiazolsulfone, salicylazosulfapyridine (Azulfadine), sulfoxone, sulfamethoxypyridazine (Kynex)], antimalarials [primaquine, pamaquine, pentaquine, quinacrine (Atabrine)], non-sulfonamide antibacterial agents [furazolidone, nitrofurantoin (Furadantin), chloramphenicol, p-aminosalicylic acid], and miscellaneous drugs [naphthalene, vitamin K, probenecid, trinitrotoluene, methylene blue, dimercaprol (BAL), phenylhydrazine, quinine, quinidine]
8. Drug sensitive hemoglobins *a*) Hemoglobin Zurich *b*) Hemoglobin M	Arginine substitution for histidine at the 63rd position of the β-chain of hemoglobin Hemoglobin composed of 4 β-chains	Autosomal dominant Autosomal dominant	Sulfonamides Sulfisoxazole
9. Acetophenetidin-induced methemoglobinemia	?Mixed function oxidase in liver microsomes that de-ethylates acetophenetidin	Autosomal recessive	Acetophenetidin
10. Inability to taste phenylthiourea or phenylthiocarbamide	Unknown	Autosomal recessive	Drugs containing the N–C=S group such as phenylthiourea, methyl and propylthiouracil

do not constitute a major threat of drug toxicity to many individuals. However, since the early 1950s it has been recognized that large differences exist among individuals in rates of plasma decay of commonly used drugs. For example, the decay from plasma of such commonly used drugs as phenylbutazone (Burns *et al.*, 1953), antipyrine (Brodie and Axelrod, 1950) and bishydroxycoumarin (Weiner *et al.*, 1950) exhibits 3- to 10-fold variations among individuals. These differences among individuals constitute a major therapeutic danger to the population receiving drugs according to the currently employed method based almost exclusively on body weight. The patient who clears a drug rapidly from the body would require more of the drug to achieve a therapeutic effect than the patient of intermediate clearing capacity. However, toxicity could result from administration of the usual drug dosage to a patient with a very low ability to remove the drug. In this individual with low clearing capacity, drug accumulation resulting from chronic drug administration could produce toxicity. To avoid these extremes of drug toxicity and failure to derive therapeutic benefit, abandonment of drug administration solely according to body weight and individualization of drug doses based on measurements of drug concentrations in blood are advocated (Vesell and Passananti, 1971). By no currently available method other than measurement of drug concentrations in blood can the physician distinguish among his patients with respect to their drug clearing capacities and administer drugs according to their individual needs.

Investigation of the responsiveness of the general population to a drug in terms of the amount of a drug required to produce a given effect may take the form of a continuous unimodal distribution curve or of a discontinuous polymodal curve (Fig. 1). Until recently, studies of

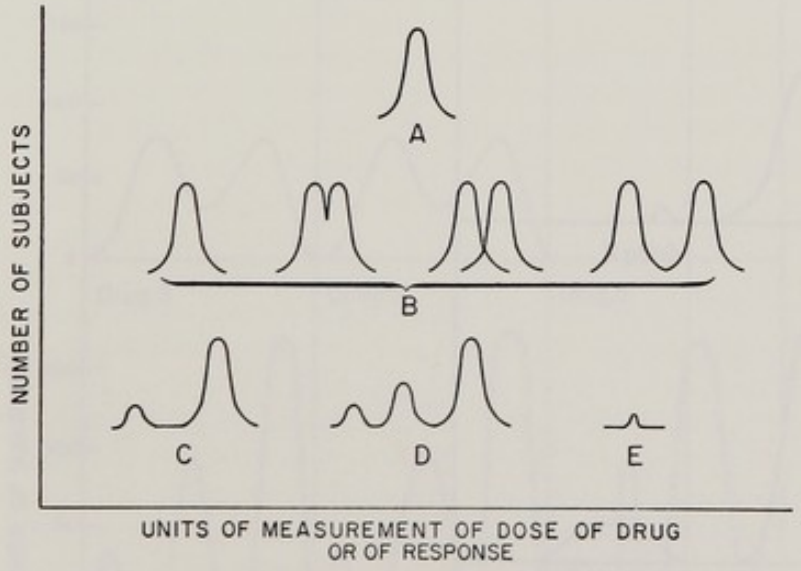


Fig. 1. Types of frequency distribution for the responsiveness of individuals to various drugs. (Modified from Kalow, 1962.)

drug responses that yield a normal or continuous distribution curve have been almost entirely ignored. To construct unimodal, Gaussian distribution curves large populations are required. Furthermore, genotypes are hard to deduce from such curves, although theoretically for polygenically controlled traits children should exhibit values midway between those of their parents. In contrast, discontinuous curves of response obtained from disorders transmitted as Mendelian dominants or recessives are more easily analyzed because each discrete curve generally corresponds to a different genotype. In other words, the mutant genes and their corresponding phenotypes segregate both in pedigrees and in the distribution curves. Figure 1 adapted from Kalow (1962) compares the normal or Gaussian distribution curve obtained for metrical characters, typically under polygenic control (curve A), with the discontinuous multimodal curves for traits transmitted as Mendelian recessives and dominants. Curve E, though

unimodal and continuous, actually indicates a response by individuals possessing a single point mutation for a genetically transmitted polymorphic trait, a response which normal individuals do not exhibit. Examples include hemolysis in some individuals with glucose-6-phosphate dehydrogenase deficiency after administration of antimalarials, or acute arthritic attacks in certain individuals with the gene for gout after receiving thiazide diuretics. Figure 1 includes under group B a unimodal curve, although all other examples under category B are multimodal. Because it may actually conceal genetic heterogeneity, this unimodal curve is included in group B. After more sensitive biochemical tests are performed, this unimodal curve may be shown to be composed of several genetically and phenotypically distinguishable components. For example, the broad overlapping curve obtained on plotting the serum pseudocholinesterase activity of a large population is separable into 3 discrete curves with the use of the enzyme inhibitor dibucaine.

The situation depicted in Figure 1 is oversimplified because it suggests that drug responsiveness is a single trait or factor determined by alleles at one genetic locus or even, in the case of polygenic inheritance, by alleles at a few separate loci. While true in some cases, the situation can be more complex because the intensity and duration of the physiologic responses produced by many drugs are affected by at least four independent processes: absorption, distribution, biotransformation and excretion of the drug. Typically, these processes occur at different sites in the body and are controlled by many different genetic loci. The information

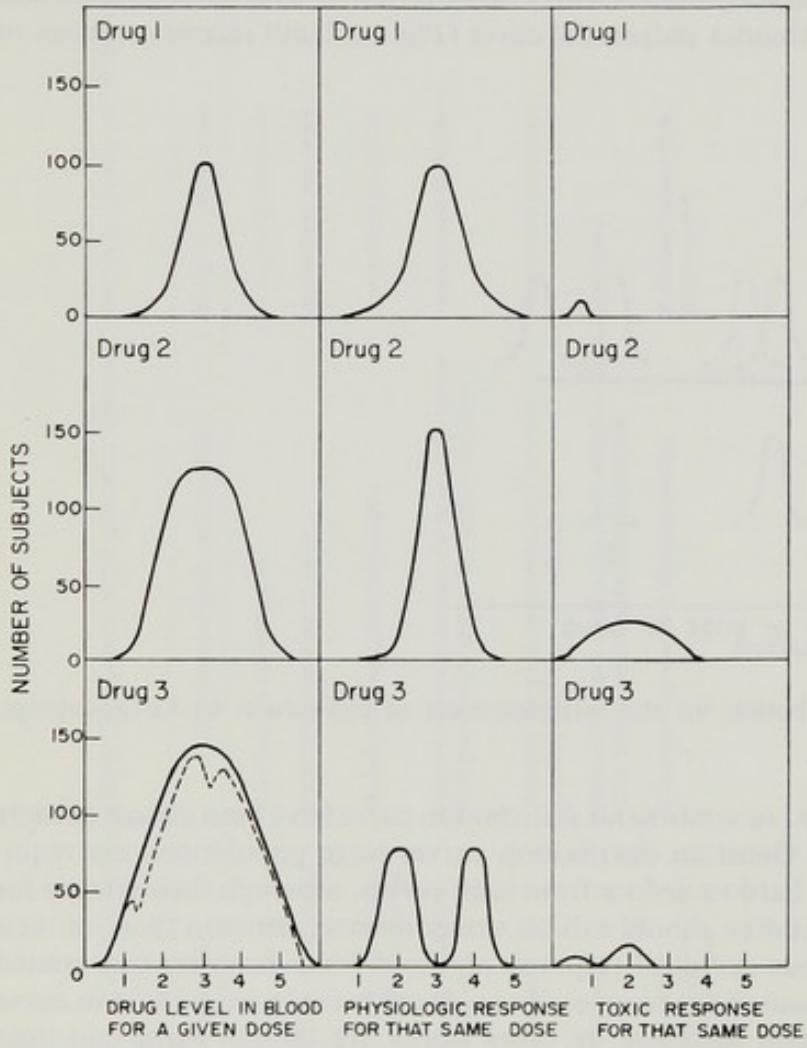


Fig. 2. Relationship for 3 different hypothetical drugs between drug concentration, physiologic response, and toxic response all for the same dose of drug tested in a large number of subjects. These 3 different drugs illustrate theoretical situations in which the subjects exhibit different relationships between drug concentration, physiologic reaction and toxicity.

concerning the genetic control of these processes is currently unavailable even for a single drug. Furthermore, several drugs produce toxicity at intracellular sites different in genetic control and tissue location from the receptor sites on which these drugs act to produce their therapeutic effects. Thus, genetic control of drug toxicity may be quite independent of genetic control of the therapeutic, physiologic responsiveness to drugs. To emphasize that the blood concentration of drug, therapeutic responsiveness and toxicity may represent three genetically independent events for certain drugs, the following theoretical possibilities are offered.

Figures 2 and 3 illustrate for each of six different hypothetical drugs the relationship that could theoretically exist for these three separate parameters of drug action. For drugs 1 and 4 in Figures 2 and 3, the curves for blood concentration and physiologic response are identical, so that the physician could predict the extent of the body's therapeutic reaction to these drugs simply from a knowledge of their concentrations in the blood. This is the usual situation for most drugs, but there are exceptions. Drugs 2, 3, 5 and 6 depict hypothetical cases of various extents of dissociation between drug concentrations in blood and pharmacologic responsiveness, a situation in which it might seem unwise for a physician to use only measurements of the drug in the blood to predict the patient's responsiveness to that drug. However, if the physician knew just how the curves for blood concentrations and responsiveness differed, he might still be able to use such measurements to achieve a given therapeutic response. From drug 3 on, the curves become more complex and instead of all three parameters being uni-

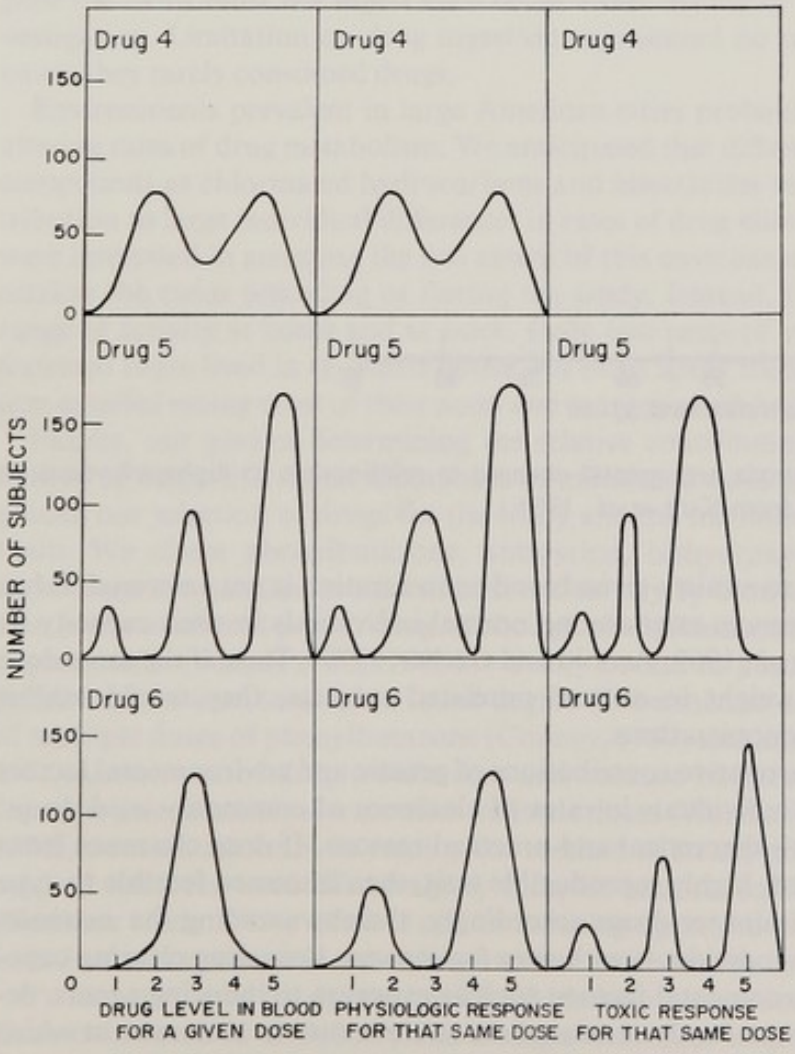


Fig. 3. Other possible relationships among the 3 pharmacologic parameters illustrated in Fig. 2 for 3 additional hypothetical drugs. Here the relationships are more complex than in Fig. 2 and polymodal curves of response are shown.

modally distributed, at least one becomes multimodal. As shown for the blood concentrations of drug 3, a very broad unimodal distribution may conceal heterogeneity, which can be occasionally unmasked by application of more sophisticated assay systems.

For many drugs, a direct relationship exists between the blood concentration of the therapeutic agent and its toxicity. Figure 4 shows this situation to be the case for diphenylhydantoin (DPH) (Kutt *et al.*, 1964). A commonly used anticonvulsant, DPH produces multiple toxic reactions including nystagmus, ataxia, dysarthria and drowsiness. Kutt *et al.* (1964) demonstrated that these toxic reactions are dose related; as the blood concentration of DPH rises, the severity of the toxic reaction increases progressively from nystagmus to ataxia to mental changes. To generalize from this and other similar examples, variation among individuals in drug responsiveness seems to arise not from differences among individuals in the nature of their physiologic or toxic receptor sites for drugs, but rather from differences among individuals in rates of drug clearance from the blood. Uniformity in the type of DPH toxicity produced by a given blood concentration is impressive in Figure 4, but the question of how

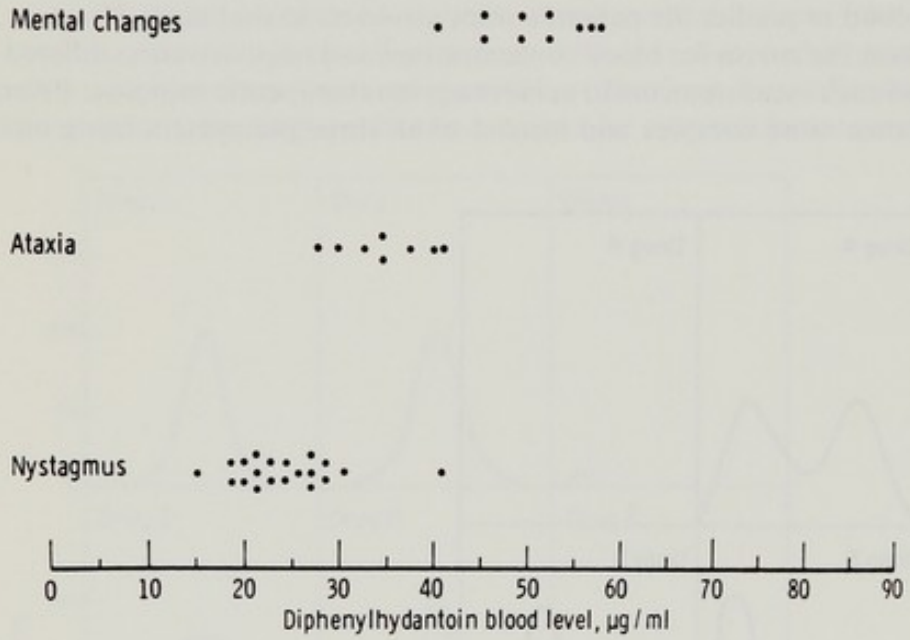


Fig. 4. The onset of nystagmus, ataxia and mental changes in relationship to diphenylhydantoin blood concentrations. (Reproduced from Kutt *et al.*, 1964.)

much DPH each patient required to attain a given blood concentration is not answered. It has been shown that significant differences exist among normal individuals in their capacity to clear DPH from blood (Glazko *et al.*, 1969; Arnold and Gerber, 1970). Thus, if the same dose of DPH were administered by weight to normal unrelated subjects, they would exhibit markedly different DPH blood concentrations.

We have attempted to assess the relative contributions of genetic and environmental factors to these large differences among individuals in rates of clearance of commonly used drugs. The results are of interest for both theoretical and practical reasons. If drug clearance from blood were a genetically controlled, highly reproducible trait, then it seemed feasible to type subjects for these rates and to administer drugs accordingly, thereby avoiding the extremes of undertreatment and toxicity in individuals with very fast or very slow drug clearing capacity, respectively. Numerous environmental factors, such as exposure to inducing agents, degree of health or illness, and hormonal and nutritional status are known to alter rates at which humans metabolize drugs. Several drugs such as phenylbutazone enhance their own metabolism (Conney, 1967). In mice, responsiveness to a drug such as hexobarbital differs according to age, sex, litter, painful stimuli, ambient temperature, degree of crowding, time of day of

drug administration, and type of bedding (Vesell, 1968). Such experiments would imply that in man a large component in the causation of variations among individuals in drug metabolism would be environmental.

Causes of variations among individuals can be separated into their environmental and heritable components through the use of human twins. This approach, first introduced by Francis Galton in 1875, permits determination of the extent to which such individual variations are genetically controlled, hence reproducible and thereby of predictive value in the determination of individually optimum doses of drugs.

Over the past six years we investigated normal, adult, Caucasian twins living in the Washington, D.C. area to quantitate the genetic and environmental components of large individual variations in rates of drug elimination from plasma (Vesell and Page, 1968*a*,*b*,*c*; Cascorbi *et al.*, 1971; Vesell *et al.*, 1971*b*). The study attempted to elucidate the mechanisms whereby large individual differences in rates of drug elimination are maintained in man.

During the period of the investigation we attempted to maintain the environments of the volunteer twins unchanged from their usual patterns. However, no therapeutic agents were administered for one month preceding the study. This single limitation was imposed because our goal was to determine in the uninduced state the extent of and mechanisms responsible for individual variations in rates of drug elimination. Since several hundred commonly encountered therapeutic agents can enhance rates of drug elimination by inducing hepatic microsomal drug-metabolizing enzymes (Conney, 1967), we recognized that to accomplish our purpose the twins must not ingest such drugs either during or for a short period before our investigation. Limitation on drug ingestion represented no radical change for these twins, because they rarely consumed drugs.

Environments prevalent in large American cities probably contain compounds capable of altering rates of drug metabolism. We anticipated that differential individual exposure to such compounds as chlorinated hydrocarbons and insecticides might produce an appreciable contribution to large individual differences in rates of drug elimination from plasma. Because we were interested in assessing the full extent of this environmental component, we did not hospitalize the twins preceding or during the study. Instead, they were allowed their customary range of activity at home and at work. Only two pairs of our identical and two pairs of our fraternal twins lived in the same house. All twins spent their working day apart. This separation entailed eating most of their noon and evening meals in different places.

Finally, our goal of determining the relative contribution of environmental and genetic factors to large individual differences in uninduced rates of drug elimination from plasma, guided our selection of drugs for the study and the methods we employed for administering them. We chose phenylbutazone, antipyrine, bishydroxycoumarin, and ethanol because these drugs are handled in man almost exclusively by biotransformation rather than by excretion of the unaltered parent drug. Although phenylbutazone (Burns *et al.*, 1953) and bishydroxycoumarin (Weiner *et al.*, 1950) are avidly bound to plasma proteins, albumin binds antipyrine and ethanol to a much lower degree (Soberman *et al.*, 1949). Because administration of multiple doses of phenylbutazone (Conney, 1967) and antipyrine (Breckenridge and Orme, 1971) alters rates of drug metabolism and because bishydroxycoumarin is poorly absorbed from the gastrointestinal tract after multiple doses (Weiner *et al.*, 1950; O'Reilly and Aggeler, 1970), we were reluctant to employ the method of steady-state blood levels. The steady-state method has been used effectively by Sjöqvist and his colleagues in studies on nortriptyline (Hammer *et al.*, 1969), which is not an inducing agent, but which presents problems in gastrointestinal absorption and redistribution that make the steady-state method more suitable.

Twins were typed for approximately 30 blood groups to document the nature of the twinship. Each twin received at 9:00 A. M. a single oral dose of phenylbutazone (6 mg/kg), several months later a single oral dose of antipyrine (18 mg/kg) and several months later again a single oral dose of bishydroxycoumarin (4 mg/kg). Blood specimens were drawn at regular intervals after drug ingestion and the concentration of drug in plasma was plotted as shown in

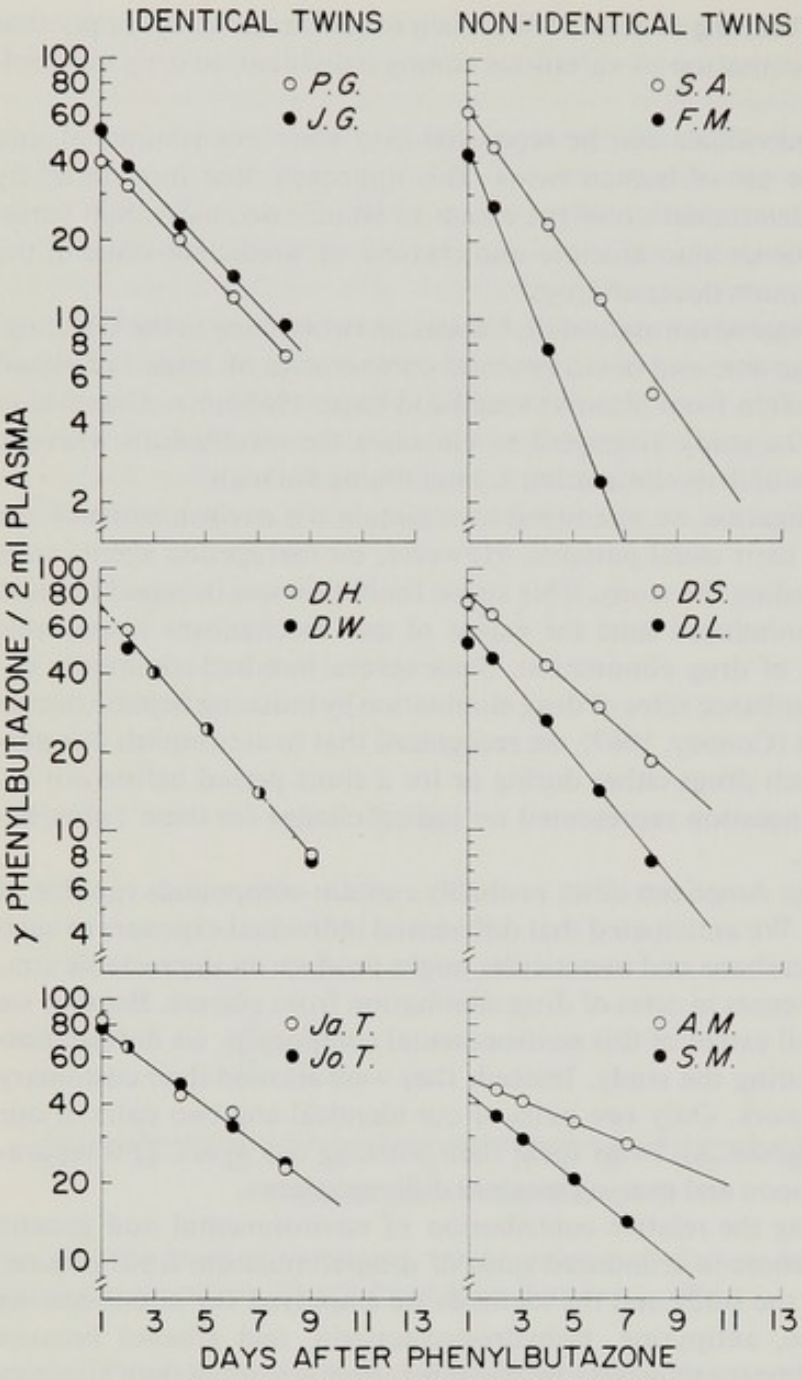


Fig. 5. Decline of phenylbutazone in the plasma of 3 sets of identical twins (left) and 3 sets of fraternal twins (right). The log of the phenylbutazone concentration in 2 ml of plasma is shown at intervals after a single oral dose of 6 mg/kg. (Reproduced from Vesell and Page, 1968*a*.)

Figures 5, 6 and 7 (Vesell and Page, 1968*a*,*b*,*c*). These curves illustrate for each of the three drugs tested typical examples of rates of elimination from the plasma of identical and fraternal twins. Half-lives of the three drugs, determined from these curves, appear in Table 2.

Table 3 shows the results of experiments (Vesell *et al.*, 1971*b*) in which the half-life of ethanol was determined in the plasma of these twins after a single oral dose of 95% ethanol (1 ml/kg) at 9:00 A.M. Plasma levels were estimated by gas chromatography (Goldbaum *et al.*, 1964).

For these drugs intratwin differences in half-life are appreciably greater in fraternal than identical twins. Therefore, the major mechanisms responsible for individual differences in rates of elimination of phenylbutazone, antipyrine, bishydroxycoumarin, and ethanol from plasma are genetic rather than environmental. The contribution of heredity to large individual variations in the plasma half-lives of each of these drugs was estimated from the for-

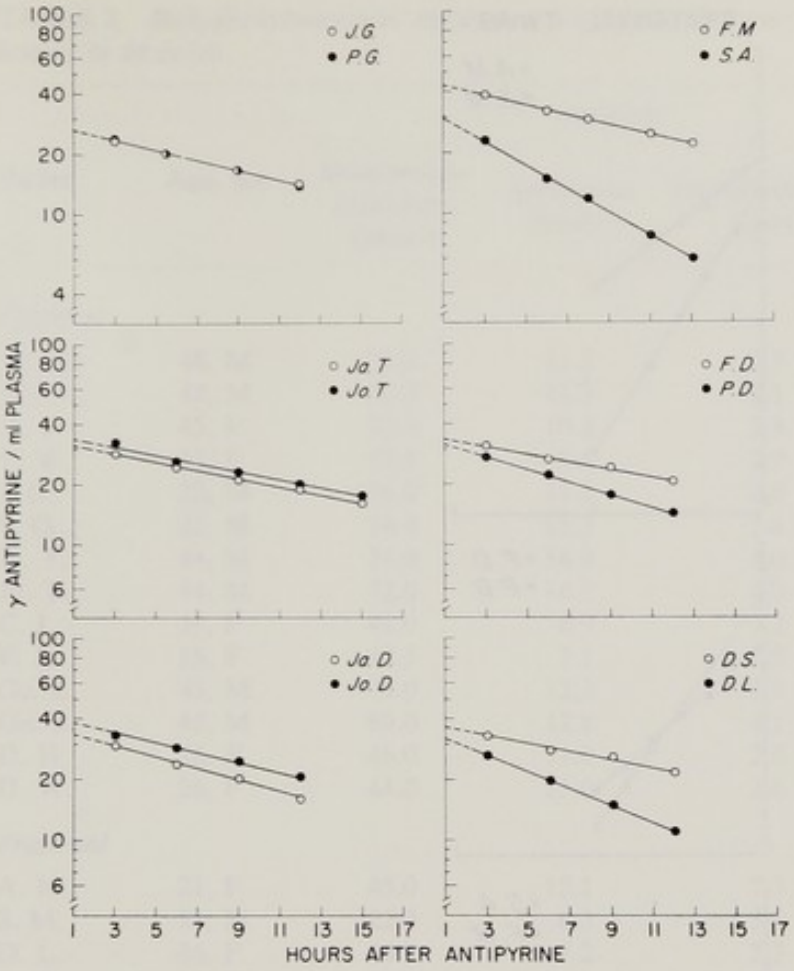


Fig. 6. Decline of antipyrine in the plasma of 3 sets of identical twins (left) and 3 sets of fraternal twins (right). The log of the antipyrine concentration in 1 ml of plasma is shown at intervals after a single oral dose of 18 mg/kg. (Reproduced from Vesell and Page, 1968*b*.)

mula described by Neel and Schull (1954) and Osborne and DeGeorge (1959):

$$\frac{\text{(variance within pairs of fraternal twins)} - \text{(variance within pairs of identical twins)}}{\text{(variance within pairs of fraternal twins)}}$$

Theoretically, values derived from this formula could range from 0, indicating negligible hereditary and complete environmental control, to 1, indicating virtually complete hereditary influence. For phenylbutazone, antipyrine, bishydroxycoumarin and ethanol, values for the contribution of heredity were 0.99, 0.98, 0.97, and 0.99, respectively. Our studies on twins yielded intraclass correlation coefficients close to theoretical expectation solely on the basis of genetic control, according to which fraternal twins, having in common approximately half of their total number of genes, should have a value of 0.5, whereas identical twins should have a value of 1. For rates of metabolism of phenylbutazone, antipyrine, bishydroxycoumarin and ethanol, the intraclass correlation coefficients of identical twins were 0.83, 0.85, 0.85, and 0.82, respectively, and for fraternal twins, 0.33, 0.47, 0.66, and 0.38, respectively (Vesell and Page, 1968*a*,*b*,*c*; Vesell *et al.*, 1971*b*). Evidently for these drugs, large individual differences in rates of elimination from plasma in normal subjects not receiving other therapeutic agents are surprisingly free of environmental influence. As shown by repeated half-life determinations, non-medicated, normal subjects have a reproducible plasma half-life for these drugs. Since phenylbutazone (Burns *et al.*, 1953) and bishydroxycoumarin (Weiner *et al.*, 1950) are 98% bound to plasma proteins, differences among individuals in plasma elimination rates of these drugs might possibly involve binding of the drugs to albumin. However, antipyrine and

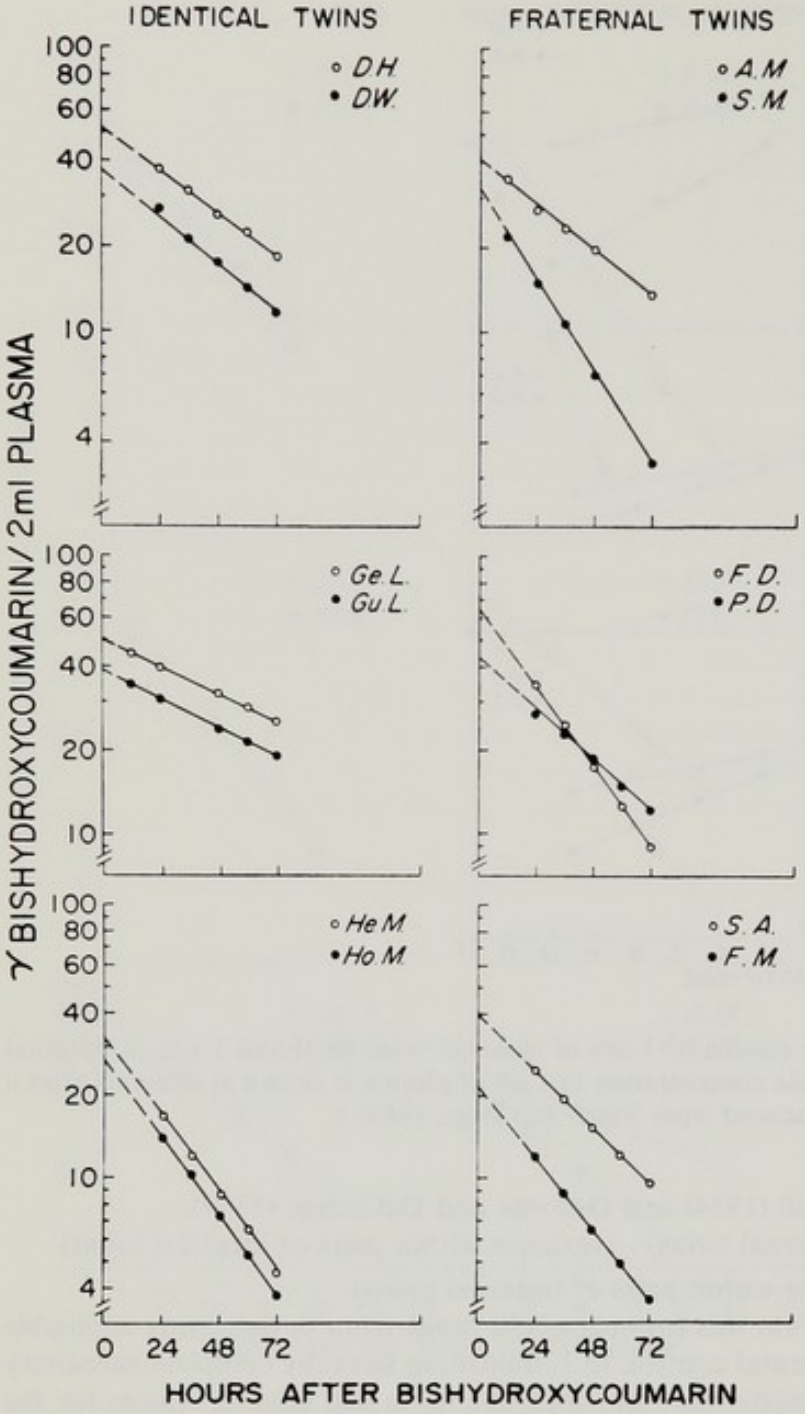


Fig. 7. Decline of bishydroxycoumarin in the plasma of 3 sets of identical twins (left) and 3 sets of fraternal twins (right). The log of the bishydroxycoumarin concentration in 2 ml of plasma is shown at intervals after a single oral dose of 4 mg/kg. (Reproduced from Vesell and Page, 1968*c*.)

ethanol are not appreciably bound to plasma proteins (Soberman *et al.*, 1949). Therefore, it seems reasonable to conclude that for antipyrine and ethanol, if not also for phenylbutazone and bishydroxycoumarin, variations in plasma half-life arise from genetic differences in rates of metabolism rather than in distribution. Appreciable variations do exist in rates of metabolism of these drugs, as indicated by ranges for the plasma half-lives of ethanol, antipyrine, phenylbutazone and bishydroxycoumarin of 2-fold, 3-fold, 6-fold and 10-fold, respectively, among the 28 individuals in our study (Tables 2 and 3).

In another study these same twins (Table 4) exhibited almost 4-fold variations in the metabolism of a single intravenous dose of 3.4 mg of radioactive halothane (Cascorbi *et al.*, 1971).

TABLE 2 *Bishydroxycoumarin, antipyrine, and phenylbutazone half-lives with smoking and coffee history in 28 twins*

Twins	Age, sex	Half-life			Smoking (packs/day)	Coffee (cups/day)
		Bishydroxy-coumarin (hours)	Antipyrine (hours)	Phenylbutazone (days)		
Identical						
Ho. M	48, M	25.0	11.3	1.9	0.5	2
He. M.	48, M	25.0	11.3	2.1	1	3
D. T.	43, F	55.5	10.3	2.8	0	5–6
V. W.	43, F	55.5	9.6	2.9	2	8–10
J. G.	22, M	36.0	11.5	2.8	1	1–2
P. G.	22, M	34.0	11.5	2.8	1	1–2
Ja. T.	44, M	74.0	14.9	4.0	0	6
Jo. T.	44, M	72.0	14.9	4.0	0	2–3
C. J.	55, F	41.0	6.9	3.2	0	2
F. J.	55, F	42.5	7.1	2.9	0	2
Ge. L.	45, M	72.0	12.3	3.9	0	4
Gu. L.	45, M	69.0	12.8	4.1	0	4
D. H.	26, F	46.0	11.0	2.6	0	0–1
D. W.	26, F	44.0	11.0	2.6	0	3–4
Fraternal						
A. M.	21, F	45.0	15.1	7.3	1.5	2
S. M.	21, M	22.0	6.3	3.6	0	0
D. L.	36, F	46.5	7.2	2.3	0	2–3
D. S.	36, F	51.0	15.0	3.3	2	3–4
S. A.	33, F	34.5	5.1	2.1	1	2
F. M.	33, F	27.5	12.5	1.2	0.5	2
Ja. H.	24, F	7.0	12.0	2.6	0	10–15
Je. H.	24, F	19.0	6.0	2.3	1.5	10
F. D.	48, M	24.5	14.7	2.8	0	1
P. D.	48, M	38.0	9.3	3.5	1.5	8
L. D.	21, F	67.0	8.2	2.9	1	6
L. W.	21, F	72.0	6.9	3.0	1	2–3
E. K.	31, F	40.5	7.7	1.9	0	0
R. K.	31, M	35.0	7.3	2.1	1	0

The difference between identical and fraternal twins in intrapair variance is significant: $P<0.005$ ($F = 36.0$, $N_1 = N_0 = 7$).

These large individual differences in halothane metabolism are under predominantly genetic control (Cascorbi *et al.*, 1971). However, the corrected value of 0.88 and the uncorrected value of 0.63 for the contribution of heredity to individual differences in rates of halothane metabolism indicate a larger environmental component for this drug than observed for ethanol, phenylbutazone, antipyrine, or bishydroxycoumarin. Two reasons that could account for the greater environmental influence over variations in halothane than in phenylbutazone, antipyrine or bishydroxycoumarin metabolism include the possibility of its control by a different enzyme system more susceptible to induction by commonly encountered environmental substances or alternatively the fact that halothane is metabolized to a much smaller extent than the other drugs investigated. Table 4 shows that the percent of the administered dose of halothane metabolized ranges in these 20 subjects from only 2.7 to 11.4. When such a small frac-

TABLE 3 *Ethanol metabolism in identical and fraternal twins after a single oral dose of 1 ml/kg of 95% ethanol*

Twins	Age, sex	Wt (kg)	Rate of ethanol metabolism (mg/ml/hr)	Intrapair difference
Identical				
Ba. J.	23, F	52	0.32	0
Ba. J.	23, F	47	0.32	
B.	61, F	62	0.38	0.02
S.	61, F	50	0.40	
W. V.	22, M	77	0.30	0
J. V.	22, M	67	0.30	
Dan. E.	22, M	65	0.22	0
Dav. E.	22, M	72	0.22	
Ge. L.	47, M	62	0.27	0.04
Gu. L.	47, M	59	0.31	
A. M.	35, F	67	0.36	0.03
B.Z.	35, F	68	0.39	
C. J.	56, F	54	0.36	0
F. J.	56, F	54	0.36	
Fraternal				
Cl. H.	57, F	61	0.35	0.01
Chs. H.	57, M	74	0.34	
H. H.	47, F	43	0.42	0.14
P. M.	47, F	43	0.28	
F. D.	49, M	76	0.48	0.26
P. D.	49, M	76	0.22	
D. L.	36, F	55	0.38	0.04
D. S.	36, F	56	0.34	
E. E.	54, F	58	0.32	0.08
E. W.	54, F	58	0.24	
E. K.	32, F	58	0.26	0.04
R. K.	32, M	116	0.30	
S. A.	36, F	45	0.22	0.10
F. M.	36, F	80	0.32	

tion of an administered dose is metabolized, environmental contributions to variations in biotransformation could play a proportionately larger role. For halothane, the intraclass correlation coefficient for identical twins was 0.52 and for fraternal twins 0.36, respectively. Table 5 shows heritability of variations in the metabolism of these drugs as calculated by several other methods. Other methods were employed because the technique utilized in the preceding estimates assumes that environmental differences between twinships are negligible. Therefore, these calculations are too high to the extent that such differential environmental factors operate. Falconer (1960) approached the problem by partitioning variance into several genetic and environmental components; although this approach can be utilized in family studies (Burt and Howard, 1956), it cannot be applied to twin data. Halothane shows a very small genetic component of control by the Holzinger (1929) index $(r_I - r_F)/(1 - r_F)$ or by the method of Falconer (1960), modified by using values of V_A/V_P (variance due to additive and phenotypic factors) provided from the family study of Whittaker and Price Evans (1970). The estimation of hereditary control of a trait according to Falconer (1960) is based on the proportion of the phenotypic variance contributed by the two genetic components of variance, the

TABLE 4 *Cumulative excretion of halothane metabolite in twins in per cent of dose injected*

Twins*	Age	Sex	Weight (lbs)	Hours after injection					Intrapair differences %	
				2	4	6	8	24	Absolute	Normalized
Identical										
Gu. L.	47	M	133	0.9	1.6	2.3	2.6	7.4	1.4	17.3
Ge. L.	47	M	135	0.9	1.8	2.7	3.7	8.8		
He. M.	50	M	152	0.5	1.1	1.8	2.2	7.3	0.6	8.6
Ho. M.	50	M	165	0.7	1.2	2.0	2.8	6.7		
Dav. E.	22	M	165	1.2	2.3	3.5	4.1	9.7	1.7	16.2
Dan. E.	22	M	150	1.4	2.2	3.4	4.3	11.4		
A. M.	36	F	150	1.7	3.5	4.7	5.6	10.7	1.4	14.0
B. Z.	36	F	150	1.1	2.3	2.9	3.7	9.3		
Bar. J.	24	F	115	2.2	3.5	4.5	5.5	8.3	0.2	2.4
Bev. J.	24	F	104	2.0	2.6	3.8	4.6	8.1		
							Average	8.8	Average	11.7
Fraternal										
E. E.	55	F	130	0.2	0.5	1.0	1.3	2.7	2.9	70.0
E. W.	55	F	127	0.6	1.2	2.0	2.5	5.6		
H. H.	48	F	96	0.4	0.7	1.3	1.7	6.0	1.0	18.2
P. M.	48	F	90	0.3	1.1	1.8	2.5	5.0		
F. D.	49	M	165	0.2	0.5	0.8	1.2	3.4	0.1	2.9
P. D.	49	M	165	0.3	0.6	0.9	1.2	3.5		
D. L.	39	F	112	0.8	1.9	3.0	3.8	9.1	2.2	27.5
D. S.	39	F	128	0.8	1.4	2.0	2.7	6.9		
J. R.	41	F	144	0.6	1.2	2.6	3.5	7.5	2.3	36.2
N. M.	41	F	154	0.9	1.4	2.3	3.0	5.2		
							Average	5.5	Average	31.0

*Identical = monozygotic; Fraternal = dizygotic.

so-called additive and dominance components of variance:

$$H = 2(r_I - r_F) = \frac{V_A + 1.5\,V_D}{V_P}$$

A twin study performed in Stockholm on variations in steady-state blood concentrations of nortriptyline in otherwise non-medicated, non-hospitalized twins showed appreciably smaller intratwin differences in identical than in fraternal twins (Alexanderson *et al.*, 1969). These marked differences between identical and fraternal twins in steady-state blood levels

TABLE 5 *Heritability of variations in drug metabolism of twins utilizing different methods of data analysis*

	Antipyrine	Phenyl-butazone	Bishydroxy-coumarin	Ethanol	Halothane
$(V_F - V_I)/V_F$	0.98	0.99	0.97	0.98	0.88
r_I	0.85	0.83	0.85	0.82	0.52
r_F	0.47	0.33	0.66	0.38	0.36
$(r_I - r_F)/(1 - r_F)$	0.72	0.75	0.56	0.71	0.25
$2(r_I - r_F)$	0.76	1.00	0.38	0.88	0.32

r = intraclass correlation coefficient.

of nortriptyline confirmed our conclusions from twin studies with phenylbutazone, antipyrine, bishydroxycoumarin, ethanol and halothane.

Recently, Whittaker and Price Evans (1970) performed a family study to assess the genetic contribution to variability among individuals in phenylbutazone metabolism. They concluded that variability among individuals in phenylbutazone metabolism was under polygenic control. Previously, a similar conclusion was reached by Motulsky (1964) from a family study on variability in the plasma half-lives of bishydroxycoumarin after a single oral dose. Whittaker and Price Evans (1970) obtained a normal distribution of phenylbutazone half-lives in plasma after correcting for height and also after administering a 3-day course of phenobarbital to 'render the environment more uniform'. A significant regression of mean offspring value on mid-parent value indicated that approximately 65% of the observed phenotypic variance was caused by the additive effects of genes. These results agree closely with those from our earlier study on phenylbutazone metabolism in twins if V_D (variance due to the effect of dominance) is not neglected in the following formula derived from Falconer (1960):

$$H = \frac{\frac{1}{2}V_A + \frac{3}{4}V_D}{\frac{1}{2}V_A + \frac{3}{4}V_D + V_{Ew}} = \frac{V_F - V_I}{V_F}$$

Whittaker and Price Evans (1970) state that V_D is too small to be significant, although they admit that V_{Ec} is probably large. ($V_E = V_{Ew} + V_{Ec}$. V_{Ew} and V_{Ec} are the within twin pair variance and the common variance between twin pairs, respectively.) Neither V_D nor V_{Ec} was measured in their family study. In other studies of polygenically controlled traits in man, V_D is small but not negligible. In their classic study of height and intelligence, Burt and Howard (1956) reported a value of 0.16 and 0.17 for the contributions of V_D to height and intelligence, respectively. If their value for V_D of 0.16 is utilized as an estimate of V_D in calculating the family data of Whittaker and Price Evans (1970) – and it seems more reasonable to use this hypothetical value than to disregard V_D completely – there is good agreement between the results of the family study of Whittaker and Price Evans ($H = 0.88$) and our twin data on phenylbutazone ($H = 0.75$ or 1.00) (Table 5). Since these values are close to the estimate (0.99) based on the formula for heritability that we employed ($H = (V_F - V_I)/V_F$), we may conclude that differential environmental factors operating between twinships in our investigation were small.

Another recent study in two extensive Swedish pedigrees with high steady-state plasma concentrations of nortriptyline suggested that the appreciable individual differences in the steady-state plasma concentrations of this drug were polygenically controlled (Åsberg *et al.*, 1971). Thus far, both the family studies and the twin data have agreed in their conclusions that large differences among healthy, non-medicated volunteers in rates of drug metabolism are primarily controlled by genetic factors.

The use of twins has lost favor in human genetics; while the defects inherent in twin studies have been repeatedly emphasized, their advantages in investigating variations among individuals in rates of drug metabolism have not previously been described. Even the assumption that 'monozygotic human twins have identical inheritance' has been challenged by Storrs and Williams (1968) on the basis of large differences in 20 parameters among newborn monozygotic quadruplet armadillos. The twin method does suffer from several disadvantages including its inability to establish the mode of inheritance of a genetically controlled trait and its assumption of an environmental equality in all subjects, identical as well as fraternal twins.

In the evaluation of variations in drug metabolism, twin studies enjoy several distinct advantages over family studies but they have only rarely been utilized as subjects for investigations of the genetic component of individual variations in drug metabolism (Bönicke and Lisboa, 1957; Kappas and Gallagher, 1960). Twins are by definition age corrected, and dizygotic twins can easily be selected from the same sex. As studies in rodents have shown (Vesell, 1968) and as studies in man may eventually establish, rates of drug metabolism change markedly with age and sex. Thus, the genetic analysis of data on drug metabolism from family studies

is complicated by incorporation of variations in rates of drug metabolism from differences in age and sex, two variables eliminated in twin studies. Although systematic effects of age and sex on drug metabolism have thus far not been detected in man (Vesell and Page, 1968*a*, *b*; Whittaker and Price Evans, 1970; Åsberg *et al.*, 1971), a non-random effect exerted by these factors could still have existed and not been identified. Furthermore, differences in the environment of children, parents and grandparents with respect to exposure to certain compounds capable of inducing or inhibiting the hepatic microsomal drug-metabolizing enzymes must be considered as a source of possible variation in family studies. Such environmental influences on drug metabolism arising from common exposure in the same household to inducers or inhibitors of drug-metabolizing enzymes could explain why, in the family study of Whittaker and Price Evans, there was a correlation in phenylbutazone metabolism between husbands and wives before phenobarbital administration.

We have attempted to investigate within a given individual possible correlations in rates of metabolism of different drugs. If such correlations existed, then drugs could be grouped into categories. Once the individual's rate of metabolism of one drug in the category was ascertained, his rate of handling all the others in that category could be calculated. We attempted to correlate rates of metabolism of phenylbutazone, antipyrine, bishydroxycoumarin and ethanol. The only correlation between the plasma half-lives of these drugs after a single oral dose of each was between rates of metabolism of phenylbutazone and bishydroxycoumarin (Fig. 8), using the method of Bartlett (1949). The tendency toward correlation between rates

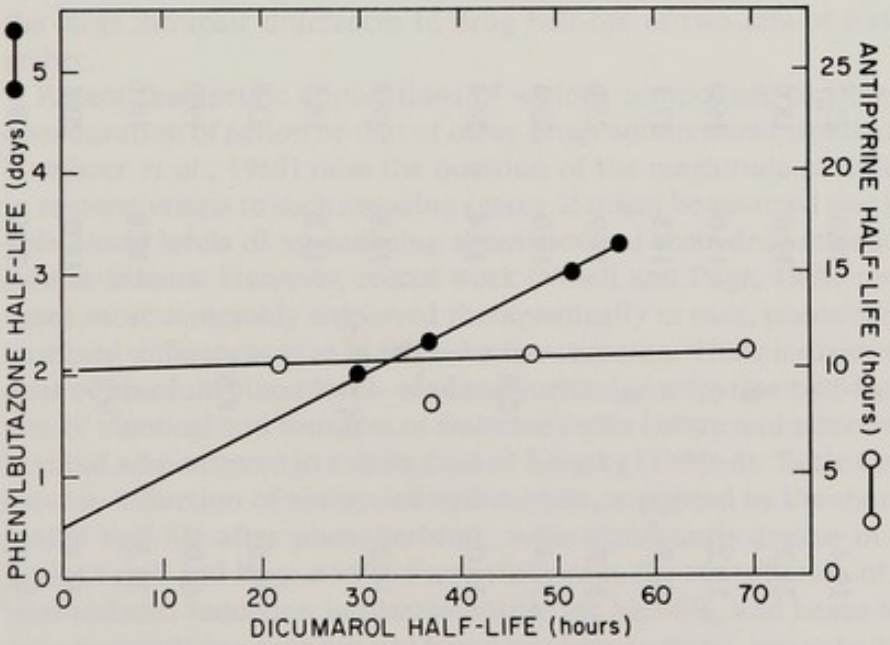


Fig. 8. Relationship between half-lives of bishydroxycoumarin and phenylbutazone and between half-lives of bishydroxycoumarin and antipyrine in various individuals according to the method of Bartlett (1949). (Reproduced from Vesell and Page, 1968*c*.)

of phenylbutazone and bishydroxycoumarin metabolism in an individual may be related to the fact that both drugs are avidly and almost entirely bound to plasma proteins. Alternatively, both drugs may be degraded by similar enzymatic steps or share a common rate-limiting step. Whatever the explanation, the existence of such a correlation raises the possibility that within an individual the rates of metabolism of other drugs may also be correlated. Recent work by Hammer *et al.* (1969) demonstrates correlation within a given individual of the rates of metabolism of desmethylimipramine, nortriptyline, and oxyphenylbutazone. Furthermore, after chronic dosage, antipyrine metabolism in an individual was shown to correlate with phenylbutazone metabolism (Davies and Thorgeirsson, 1971).

TABLE 6 *Response of plasma antipyrine half-life to phenobarbital administration with smoking, coffee, tea, and alcohol history in 16 twins*

Twins	Age, sex	Plasma antipyrine half-life		Decrease in half-life produced by phenobarbital (%)	Percentage difference between sibs in response to phenobarbital	Plasma phenobarbital levels at		Smoking (packs/day)	Coffee (cups/day)	Tea (cups/day)	Alcohol		
		Before phenobarbital (hours)	After phenobarbital (hours)			156 hours	212 hours				Beer (bottles/day)	Wine (glasses/day)	Hard liquor (oz/day)
Identical													
Dan. E.	22, M	13.6	9.6	29.4	0	20.0	23.0	0	2	0	¼	0	¼
Dav. E.	22, M	13.6	9.6	29.4		20.0	23.2	¼	0	2	¼	0	¼
A. M.	35, F	8.0	6.3	21.2	0	17.0	18.0	½	0	3	0	0	0
B. Z.	35, F	8.0	6.3	21.2		15.0	16.0	2	6–7	0	0	0	1
Bar. J.	23, F	18.2	8.4	53.8	0	16.8	17.1	0	0	0	0	0	0
Bev. J.	23, F	18.2	8.4	53.8		17.9	25.0	0	0	0	0	0	0
B. F.	26, F	10.8	7.3	32.4	2.6	16.0	23.4	0	0	0	0	0	0
B. J.	26, F	11.4	7.4	35.0		17.4	24.4	0	0	1	0	0	¼
Fraternal													
F. D.	49, M	12.0	10.3	14.2	14.2	14.8	19.4	0	1	2	0	0	0
P. D.	49, M	9.3	9.3	0		15.2	18.3	1½	5	0	0	0	1
C. K.	49, M	17.5	5.5	68.6	8.6	–	23.0	1½	2	0	1	0	18
N. R.	49, F	14.5	5.8	60.0		–	19.0	1½	4	0	0	0	0
H. H.	47, F	12.3	9.2	25.2	9.8	17.8	16.6	2	2	0	½	0	½
P. M.	47, F	6.5	5.5	15.4		16.0	20.8	1½	2	0	½	0	0
E. W.	54, F	15.0	6.9	54.0	30.7	20.0	29.0	0	4	0	0	0	½
E. E.	54, F	9.0	6.9	23.3		20.0	30.0	1	2	2	0	0	¼

Several environmental factors can alter rates of bishydroxycoumarin, diphenylhydantoin or phenylbutazone metabolism, including the size of the dose and the extent and rapidity of gastrointestinal absorption. Of course, prior ingestion of substances capable of inducing drug-metabolizing enzymes located in liver microsomes will affect the biotransformation of these, as well as most other, drugs. Various compounds induce hepatic microsomal drug-metabolizing enzymes, thereby shortening the plasma half-life of many therapeutic agents (Conney, 1967). In addition to phenobarbital and diphenylhydantoin, several substances such as 3,4-benzpyrene, 3-methylcholanthrene, nicotine (Wenzel and Broadie, 1966), and caffeine (Mitoma *et al.*, 1968), to which many individuals expose themselves, can also accelerate drug metabolism. Benzpyrene hydroxylase was enhanced in the placenta of smokers (Welch *et al.*, 1969). Therefore, a history of cigarette smoking and coffee ingestion was taken in our 28 twins (Table 2). No correlation of these habits with the rate of bishydroxycoumarin metabolism was detected; in a set of identical twins with closely similar bishydroxycoumarin half-lives, one individual did not smoke, whereas the other consumed two packs a day. Additional discordant examples occurred for both smoking and coffee consumption. To determine whether a relationship exists between these agents and activities of drug-metabolizing enzymes in human beings will require much further work. Only two of the seven sets of identical twins lived in the same household; therefore, the close resemblance between identical twins in phenylbutazone half-lives, in antipyrine half-lives, and in bishydroxycoumarin half-lives cannot be attributed to those environmental factors, such as exposure to the same inducing agents, operating on individuals sharing the same home and meals. Further support for this conclusion derives from the large intrapair differences in drug half-life of two sets of fraternal twins who lived together.

Recent therapeutic applications of various compounds capable of shortening either their own duration of action or that of other drugs administered simultaneously (Yaffe *et al.*, 1966; Ramboer *et al.*, 1969) raise the question of the magnitude of differences among individuals in responsiveness to such inducing agents. It might be assumed that subjects achieving comparable blood levels of an inducing agent elevated their drug-metabolizing enzyme activity to similar extents. However, recent work (Vesell and Page, 1969) reveals that for the inducing agent most commonly employed therapeutically in man, phenobarbital, large, genetically determined differences exist in the inductive response. These individual variations were independent of absolute blood levels of phenobarbital. Antipyrine half-lives were determined in four sets of identical and four sets of fraternal twins before and after two weeks of sodium phenobarbital administered in a daily dose of 2 mg/kg (Table 6). Table 6 reveals that intrapair differences in induction of antipyrine hydroxylase, suggested by the shortening of the plasma antipyrine half-life after phenobarbital, were significantly greater in fraternal than in identical twins (Vesell and Page, 1969). From these data, the contribution of heredity to the phenobarbital-induced reduction in plasma antipyrine half-life, and hence to the implied induction of drug-metabolizing enzymes produced by phenobarbital, was calculated to be 99%.

Phenobarbital administration in these 16 twins decreased variations in antipyrine half-life from 2.8-fold before phenobarbital to 1.8-fold after two weeks on the drug (Vesell and Page, 1969). After phenobarbital the standard deviation of the mean antipyrine half-life decreased by more than 2-fold. Appreciably diminished individual variations in drug metabolism after phenobarbital suggest that possibly when extensive individual differences in the metabolism of a drug pose therapeutic problems, relatively innocuous inducing agents could be administered to minimize such variability.

Measurement of phenobarbital in plasma revealed negligible intratwin differences in phenobarbital levels, so that large differences between fraternal twins in their inductive response to phenobarbital appear unrelated to differences in phenobarbital blood levels (Vesell and Page, 1969). Phenobarbital blood levels correlated neither with the final values for plasma antipyrine half-lives nor with the per cent reduction in plasma antipyrine half-life produced by phenobarbital treatment (Table 6). A direct relationship exists between initial antipyrine

half-lives and the per cent shortening of antipyrine half-life produced by phenobarbital administration: the longer the initial antipyrine half-life, the greater the reduction caused by phenobarbital treatment (Fig. 9). This relationship, which permits prediction of the extent to which phenobarbital will depress the initial antipyrine half-life, has potential clinical application. Since phenobarbital shortens the antipyrine half-life of slow metabolizers

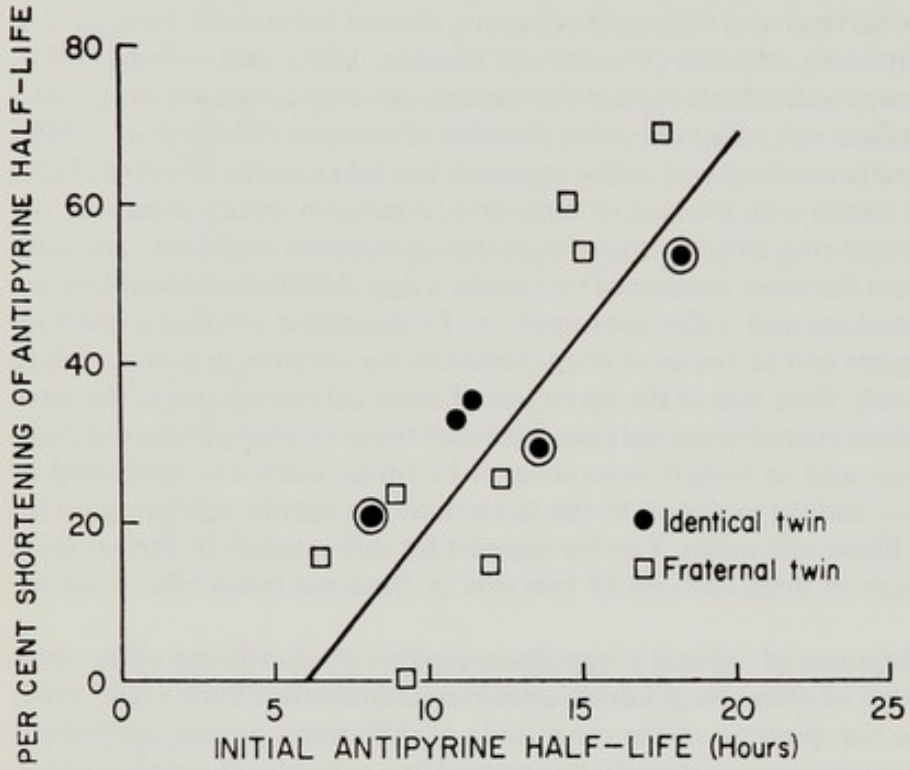


Fig. 9. Positive correlation (0.84) between the initial antipyrine half-life in plasma and the phenobarbital-induced shortening of antipyrine half-life. (Reproduced from Vesell and Page, 1969.)

more than it shortens the antipyrine half-life of fast metabolizers, use of phenobarbital to reduce toxic blood levels of various drugs would appear to aid preferentially those individuals most in need of such therapy, namely, the slow metabolizers. On the other hand, if the high blood concentrations of a certain drug are therapeutically desirable but difficult to attain, maximum effectiveness for such an agent will be easier to achieve in a slow than in a rapid drug metabolizer. Because the genes controlling variations among individuals in inducibility after phenobarbital may be different from the genes controlling variations among relatively uninduced individuals in the metabolism of such drugs as phenylbutazone or antipyrine, it is very difficult to interpret the genetic significance of a study based on the extent and nature of variations among individuals in the metabolism of a drug such as phenylbutazone after all the subjects have been induced by phenobarbital (Whittaker and Price Evans, 1970).

Although many compounds enhance the activity of hepatic microsomal drug-metabolizing enzymes, few have previously been observed to inhibit drug metabolism in man. These inhibiting drugs include methylphenidate (Garrettson *et al.*, 1969), oxyphenylbutazone (Weiner *et al.*, 1965), methandrostenolone (Weiner *et al.*, 1965), and phenyramidol (Solomon and Schrogie, 1966). We were surprised to discover that several commonly employed drugs, the tricyclic antidepressant nortriptyline, the xanthine oxidase inhibitor allopurinol, used in the treatment of gout, and disulfiram, used to inhibit alcohol dehydrogenase in alcoholics, impair drug metabolism (Vesell *et al.*, 1970, 1971*c*).

The subjects for these investigations were healthy, male, Caucasian medical students between the ages of 21 and 24; none took medications at the time of, or for one month preced-

ing, the study. Each volunteer provided his own control, which consisted of his plasma antipyrine or bishydroxycoumarin half-life, determined after a single oral dose at 9:00 A.M. of antipyrine (18 mg/kg) or bishydroxycoumarin (4 mg/kg). Afterward, each volunteer received either nortriptyline (0.2 mg/kg t.i.d., p.o.) for eight days or allopurinol (2.5 mg/kg b.i.d., p.o.) for two weeks of disulfiram (7 mg/kg daily for 4 days). 12 or 24 hours after the last dose of nortriptyline, allopurinol or disulfiram, plasma antipyrine or bishydroxycoumarin half-lives were redetermined following another oral dose of either antipyrine or bishydroxycoumarin, as described above. Figures 10, 11 and 12 demonstrate the marked effect of nortriptyline, allopurinol and disulfiram in prolonging the plasma half-lives of antipyrine and bishydroxycoumarin.

For each drug, marked differences occurred among individuals in the extent of this prolongation. For nortriptyline and allopurinol 5-fold variations (Vesell *et al.*, 1970) and for disulfiram almost 3-fold variations (Vesell *et al.*, 1971*c*) in the extent of retardation of antipyrine or bishydroxycoumarin half-lives were observed. Although no family or twin studies were

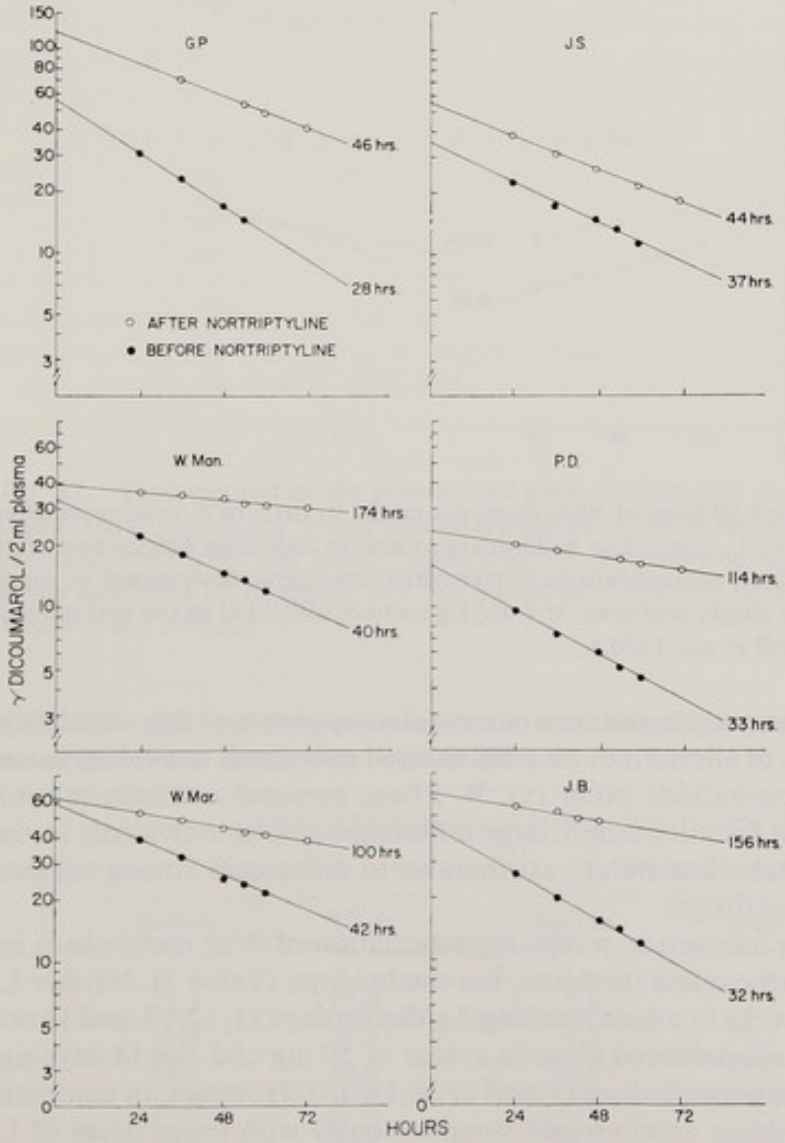


Fig. 10. Prolongation of the plasma half-lives of bishydroxycoumarin in each of 6 volunteers after 8 days on nortriptyline (0.2 mg/kg t.i.d., p.o.). The bishydroxycoumarin half-lives before and after nortriptyline are calculated from the blood concentrations of bishydroxycoumarin determined at regular intervals after administration of a single oral dose of 4 mg/kg and are indicated at the end of each straight line. (Reproduced from Vesell *et al.*, 1970.)

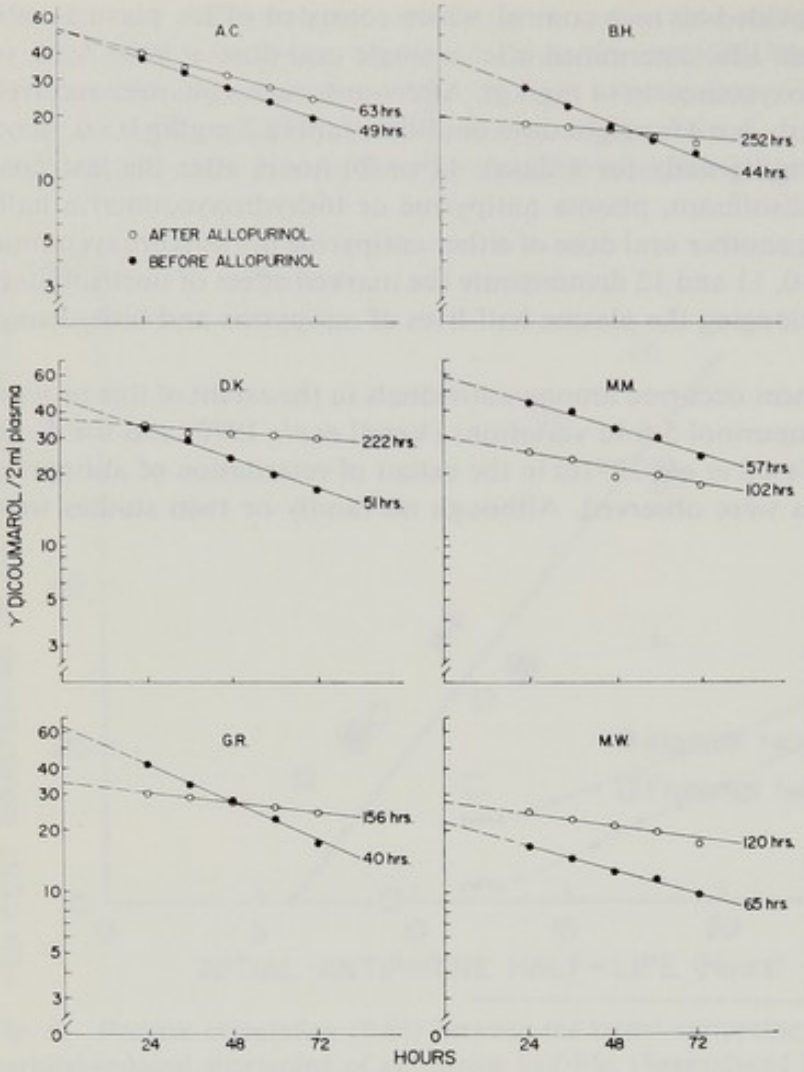


Fig. 11. Prolongation of the plasma half-lives of bishydroxycoumarin in each of 6 volunteers after 14 days on allopurinol (2.5 mg/kg b.i.d., p.o.). The bishydroxycoumarin half-lives before and after allopurinol are calculated from the blood concentrations of bishydroxycoumarin determined at regular intervals after administration of a single oral dose of 4 mg/kg and are indicated at the end of each straight line. (Reproduced from Vesell *et al.*, 1970.)

performed to determine the relative genetic and environmental components of this variability, it is known that on the daily doses of allopurinol we administered only small individual variations in allopurinol plasma concentrations occur (G. B. Elion, personal communication). Therefore, it appears that, at least for allopurinol, large differences among individuals in the degree of retardation of drug metabolism are not attributable to differences among subjects in the blood concentrations of these drugs.

An interesting example of drug interaction producing retardation of drug metabolism involves L-dopa and the dopa decarboxylase inhibitor, L-α-methyldopa (Table 7). Neither L-dopa administered alone for two weeks in a dose reaching 3 g/day on days 11, 12, 13, and 14 nor the dopa decarboxylase inhibitor administered alone in a dose of 50 mg t.i.d. for 14 days significantly altered rates of antipyrine metabolism (Vesell *et al.*,1971*a*). However, in combination, the dopa decarboxylase inhibitor administered simultaneously with lower doses of L-dopa significantly prolonged antipyrine metabolism. On this combination five of the six subjects showed retardation of plasma antipyrine half-lives. More than a 2-fold range occurred in the extent of this inhibition. It is interesting from the viewpoint of individual variations that 2 of the 6 subjects receiving the dopa decarboxylase inhibitor alone prolonged their antipyrine metabolism to an appreciable extent. Although no blood levels of L-dopa were measured in

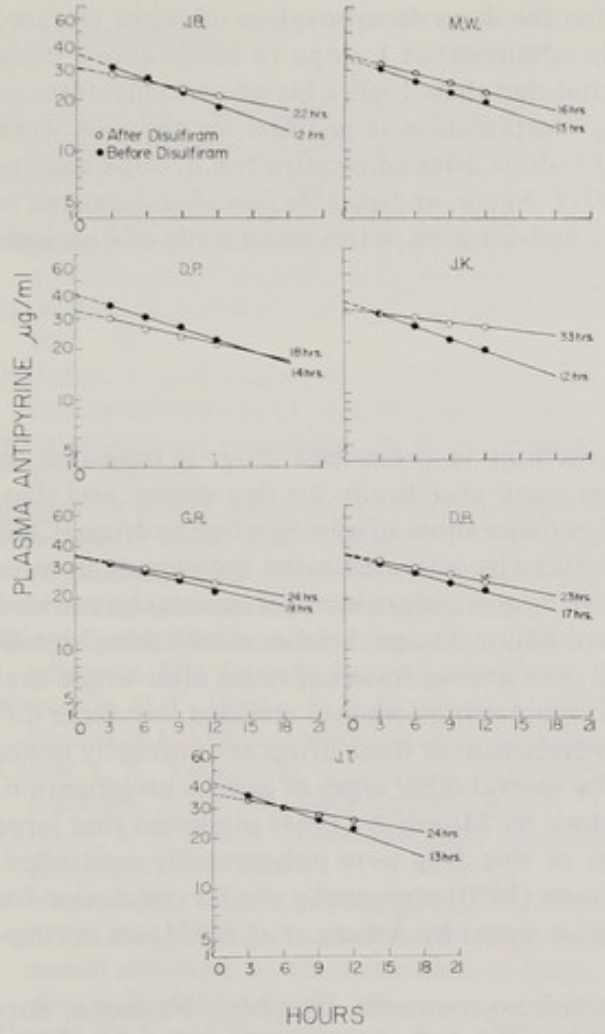


Fig. 12. Prolongation of the plasma half-lives of antipyrine in each of 7 volunteers after 4 days on disulfiram (7 mg/kg q.d., p.o.). The antipyrine half-lives 48 hours before and 24 hours after disulfiram are calculated from the blood concentrations of antipyrine determined after administration of a single oral dose of 18 mg/kg and are indicated at the end of each straight line. (Reproduced from Vesell *et al.*, 1971*c*.)

TABLE 7 *Effect of L-dopa (Group 1), L-α-methyldopa hydrazine (Group 2), and a combination of the two drugs (Group 3) on antipyrine half-life*

	Antipyrine half-life in hours after an oral dose of 18 mg/kg					
	Group 1		Group 2		Group 3*	
	Before	After	Before	After	Before	After
	12.3	12.0	7.8	7.5	8.8	13.8
	11.3	11.5	9.5	9.5	7.5	16.0
	12.3	14.0	15.0	13.0	13.8	15.0
	14.0	12.0	7.5	7.5	7.5	9.0
	13.0	13.0	11.5	16.3	12.5	16.5
			8.5	11.0	11.0	11.0
Mean	12.6	12.5	10.0	10.8	10.2	13.6

* $P<0.05$ by paired Student's t-test.

this study, we interpret the results to indicate that the dopa decarboxylase inhibitor elevated the blood concentrations of the simultaneously administered L-dopa to levels above those attained when L-dopa was administered alone and that these higher blood concentrations of L-dopa inhibited antipyrine metabolism. This interpretation is justified by previous work demonstrating elevated blood concentrations of L-dopa after administration of dopa decarboxylase inhibitors (Pletscher and Bartholini, 1971). Again, appreciable variation occurred in the extent to which L-dopa prolonged antipyrine half-life even in this small study of 6 patients receiving both L-dopa and L-α-methyldopa.

CONCLUSIONS

The magnitude of the problem of adverse reactions to prescribed drugs is indicated by several estimates that 5% of all hospitalizations occur specifically for this reason and that, during hospitalization, about 15% of patients experience some adverse reaction to drugs (Cluff *et al.*, 1965). Large variations among normal, otherwise non-medicated individuals in rates of drug clearance suggest that the extremes of toxicity and undertreatment can best be avoided if drug concentrations in blood serve as guides to adjust dosage. Studies establishing significantly less intratwin differences among identical than among fraternal twins after single oral doses of phenylbutazone, antipyrine, bishydroxycoumarin or ethanol revealed that large differences among normal individuals in rates of metabolism of these drugs are primarily under genetic control. This conclusion was supported by several other types of genetic investigation. A family study of bishydroxycoumarin metabolism by Motulsky (1964) suggested that large variations among individuals in the metabolism of this drug were polygenically controlled. A recent family study by Whittaker and Price Evans (1970) presented a similar conclusion for variations in phenylbutazone metabolism, as did a report by Åsberg *et al.* (1971) on nortriptyline metabolism in two large pedigrees.

Rates of metabolism of phenylbutazone, bishydroxycoumarin, oxyphenylbutazone, nortriptyline, desmethylimipramine and antipyrine have been shown to be correlated within a single subject under some conditions (Vesell and Page, 1968*c*; Hammer *et al.*, 1969; Davies and Thorgeirsson, 1971). This correlation encourages the development of drug categories, within which, once the rate of metabolism of one drug is determined, the rates of metabolism of the others can be readily calculated. Thus, the typing of individuals for their relative rates of metabolism of many drugs appears possible even with the use of only a few key test drugs. The biochemical basis of this correlation remains to be determined; possible explanations include a common biochemical pathway for the metabolism of several drugs or a rate-limiting compound in the pathway of drug oxidation within liver microsomes. Complications in therapy are introduced by the widespread exposure to many environmental agents capable of altering the underlying genetically controlled rate of drug metabolism (Conney *et al.*, 1971). Twin studies revealed that normal individuals vary significantly with respect to their capacity to respond to an inducing substance such as phenobarbital and, furthermore, that in otherwise non-medicated individuals this variability is under rigid genetic control (Vesell and Page, 1969). Large differences among individuals also exist with respect to the extent of retardation in the metabolism of other drugs produced by nortriptyline, allopurinol, disulfiram or L-dopa. The relative contribution of genetic and environmental factors to variations among individuals in the extent of inhibition of drug metabolism by these agents remains to be determined.

REFERENCES

ALEXANDERSON, B., PRICE EVANS, D. A. and SJÖQVIST, F. (1969): Steady-state plasma levels of nortriptyline in twins: influence of genetic factors and drug therapy. *Brit. med. J., 4*, 764.

ARNOLD, K. and GERBER, N. (1970): The rate of decline of diphenylhydantoin in human plasma. *Clin. Pharmacol. Ther., 11*, 121.

ÅSBERG, M., PRICE EVANS, D. A. and SJÖQVIST, F. (1971): Genetic control of nortriptyline kinetics in man: a study of relatives of propositi with high plasma concentrations. *J. med. Genet., 8*, 129.

BARTLETT, M. S. (1949): Fitting a straight line when both variables are subject to error. *Biometrics, 5*, 207.

BÖNICKE, R. and LISBOA, B. P. (1957): Über die Erbbedingtheit der intraindividuellen Konstanz der Isoniazidausscheidung beim Menschen. *Naturwissenschaften, 44*, 314.

BRECKENRIDGE, A. and ORME, M. (1971): Clinical implications of enzyme induction. *Ann. N. Y. Acad. Sci., 179*, 421.

BRODIE, B. B. and AXELROD, J. (1950): The fate of antipyrine in man. *J. Pharmacol. exp. Ther., 98*, 97.

BURNS, J. J., ROSE, R. K., CHENKIN, T., GOLDMAN, A., SCHULERT, A. and BRODIE, B. B. (1953): The physiological disposition of phenylbutazone (Butazolidin) in man and a method for its estimation in biological material. *J. Pharmacol. exp. Ther., 109*, 346.

BURT, C. and HOWARD, M. (1956): The multifactorial theory of inheritance and its application to intelligence. *Brit. J. statist. Psychol., 8/2*, 95.

CASCORBI, H. F., VESELL, E. S., BLAKE, D. A. and HELRICH, M. (1971): Genetic and environmental influence on halothane metabolism in twins. *Clin. Pharmacol. Ther., 12*, 50.

CLUFF, L. E., THORNTON, G., SEIDL, L. and SMITH, J. (1965): Epidemiological study of adverse drug reactions. *Trans. Ass. Amer. Phycns, 78*, 255.

CONNEY, A. H. (1967): Pharmacological implications of microsomal enzyme induction. *Pharmacol. Rev., 19*, 317.

CONNEY, A. H., WELCH, R., KUNZMAN, R., CHANG, R., JACOBSON, M., MUNRO-FAURE, A. D., PECK, A. W., BYE, A., POLAND, A., POPPERS, P. J., FINSTER, M. and WOLFF, J. A. (1971): Effects of environmental chemicals on the metabolism of drugs, carcinogens and normal body constituents in man. *Ann. N. Y. Acad. Sci., 179*, 155.

DAVIES, D. S. and THORGEIRSSON, S. S. (1971): Mechanism of hepatic drug oxidation and its relationship to individual differences in rates of oxidation in man. *Ann. N. Y. Acad. Sci., 179*, 411.

FALCONER, D. S. (1960): *Introduction to Quantitative Genetics*. Ronald Press, New York, N. Y.

GALTON, F. (1875): The history of twins as a criterion of the relative powers of nature and nurture. *J. Brit. anthrop. Inst., 5*, 391.

GARRETTSON, L. K., PEREL, J. M. and DAYTON, P. G. (1969): Methylphenidate interaction with both anticonvulsants and ethyl biscoumacetate: a new action of methylphenidate. *J. Amer. med. Ass., 207*, 2053.

GLAZKO, A. J., CHANG, T., BAUKEMA, J., DILL, W. S., GOULET, J. R. and BUCHANAN, R. A. (1969): Metabolic disposition of diphenylhydantoin in normal human subjects following intravenous administration. *Clin. Pharmacol. Ther., 10*, 498.

GOLDBAUM, L. R., DOMANSKI, T. J. and SCHLOEGEL, E. L. (1964): Analysis of biological specimens for volatile compounds by gas chromatography. *J. forens. Sci., 9*, 63.

HAMMER, W., MARTENS, S. and SJÖQVIST, F. (1969): A comparative study of the metabolism of desmethylimipramine, nortriptyline, and oxyphenylbutazone in man. *Clin. Pharmacol. Ther., 10*, 44.

HOLZINGER, H. J. (1929): The relative effect of nature and nurture influences on twin differences. *J. educ. Psychol., 20*, 243.

KALOW, W. (1962): *Pharmacogenetics: Heredity and the Response to Drugs*. W. B. Saunders, Philadelphia, Pa.

KAPPAS, A. and GALLAGHER, T. F. (1960): Study of the genetic and extragenetic determinants of alpha-ketosteroid production in man. *J. clin. Invest., 39*, 620.

KUTT, H., WINTERS, W., KOKENGE, R. and MCDOWELL, F. (1964): Diphenylhydantoin metabolism, blood levels and toxicity. *Arch. Neurol. (Chic.), 11*, 642.

LA DU, B. N. (Ed) (1965): Experimental and clinical aspects of pharmacogenetics. *Fed. Proc., 24*, 1259.

LA DU, B. N. and KALOW, W. (Eds) (1968): Pharmacogenetics. *Ann. N. Y. Acad. Sci., 151*, 691.

MITOMA, C., SORICH, T. J. and NEUBAUER, S. E. (1968): The effect of caffeine on drug metabolism. *Life Sci., 7*, 145.

MOTULSKY, A. (1957): Drug reactions, enzymes, and biochemical genetics. *J. Amer. med. Ass., 165*, 835.

MOTULSKY, A. (1964): Pharmacogenetics. *Progr. med. Genet., 3*, 49.

NEEL, J. V. and SCHULL, W. J. (1954): *Human Heredity*, p. 280. University of Chicago Press, Chicago, Ill.

O'REILLY, R. A. and AGGELER, P. M. (1970): Determinants of the response to oral anticoagulant drugs in man. *Pharmacol. Rev., 22*, 35.

OSBORNE, R. H. and DEGEORGE, F. V. (1959): *Genetic Basis of Morphological Variation. An Evaluation and Application of the Twin Study Method.* Harvard University Press, Cambridge, Mass.

PLETSCHER, A. and BARTHOLINI, G. (1971): Selective rise in brain dopamine by inhibition of extracerebral levodopa decarboxylation. *Clin. Pharmacol. Ther., 12*, 344.

RAMBOER, C., THOMPSON, R. P. H. and WILLIAMS, R. (1969): Controlled trials of phenobarbitone therapy in neonatal jaundice. *Lancet, 1*, 966.

SOBERMAN, R., BRODIE, B. B., LEVY, B. B., AXELROD, J., HOLLANDER, V. and STEELE, J. M. (1949): The use of antipyrine in the measurement of total body water in man. *J. biol. Chem., 179*, 31.

SOLOMON, H. M. and SCHROGIE, J. J. (1966): The effect of phenyramidol on the metabolism of bishydroxycoumarin. *J. Pharmacol. exp. Ther., 154*, 660.

STORRS, E. E. and WILLIAMS, R. J. (1968): A study of monozygous quadruplet armadillos in relation to mammalian inheritance. *Proc. nat. Acad. Sci. USA, 60*, 910.

VESELL, E. S. (1968): Factors altering the response of mice to hexobarbital. *Pharmacology, 1*, 7.

VESELL, E. S. (1969): Recent progress in pharmacogenetics. *Advanc. Pharmacol. Chemother., 7*, 1.

VESELL, E. S. (Ed) (1971): Drug metabolism in man. *Ann. N. Y. Acad. Sci., 179*, 1.

VESELL, E. S., NG, L., PASSANANTI, G. T. and CHASE, T. N. (1971*a*): Inhibition of drug metabolism by L-dopa in combination with a dopa decarboxylase inhibitor. *Lancet, II*, 370.

VESELL, E. S. and PAGE, J. G. (1968*a*): Genetic control of drug levels in man: phenylbutazone. *Science, 159*, 1479.

VESELL, E. S. and PAGE, J. G. (1968*b*): Genetic control of drug levels in man: antipyrine. *Science, 161*, 72.

VESELL, E. S. and PAGE, J. G. (1968*c*): Genetic control of Dicumarol levels in man. *J. clin. Invest., 47*, 2657.

VESELL, E. S. and PAGE, J. G. (1969): Genetic control of phenobarbital-induced shortening of plasma antipyrine half-lives in man. *J. clin. Invest., 48*, 2202.

VESELL, E. S., PAGE, J. G. and PASSANANTI, G. T. (1971*b*): Genetic and environmental factors affecting ethanol metabolism in man. *Clin. Pharmacol. Ther., 12*, 192.

VESELL, E. S. and PASSANANTI, G. T. (1971): Clinical chemical determination of drug concentrations in biological fluids. *Clin. Chem., 17*, 851.

VESELL, E. S., PASSANANTI, G. T. and GREENE, F. E. (1970): Impairment of drug metabolism in man by allopurinol and nortriptyline. *New Engl. J. Med., 283*, 1484.

VESELL, E. S., PASSANANTI, G. T. and LEE, C. H. (1971*c*): Impairment of drug metabolism in man by disulfiram. *Clin. Pharmacol. Ther., 12*, 785.

VOGEL, F. (1959): Moderne Probleme der Humangenetik. *Ergebnisse der Inneren Medizin und Kinderheilkunde, 12*, 52.

WEINER, M., SHAPIRO, S., AXELROD, J., COOPER, J. R. and BRODIE, B. B. (1950): The physiological disposition of dicumarol in man. *J. Pharmacol. exp. Ther., 99*, 409.

WEINER, M., SIDDIQUI, A. A., BOSTANCI, N. and DAYTON, P. G. (1965): Drug interactions: the effect of combined administration on the half-life of coumarin and pyrazolone drugs in man. *Fed. Proc., 24*, 153.

WELCH, R. M., HARRISON, V. E., GOMMI, B. W., POPPERS, P. J., FINSTER, M. and CONNEY, A. H. (1969): Stimulatory effect of cigarette smoking on the hydroxylation of 3,4-benzpyrene and the N-demethylation of 3-methyl-4-monomethylaminoazobenzene by enzymes in human placenta. *Clin. Pharmacol. Ther., 10*, 100.

WENZEL, D. G. and BROADIE, L. L. (1966): Stimulatory effect of nicotine on the metabolism of meprobamate. *Toxicol. appl. Pharmacol., 8*, 455.

WHITTAKER, J. A. and PRICE EVANS, D. A. (1970): Genetic control of phenylbutazone metabolism in man. *Brit. med. J., 4*, 323.

YAFFE, S. J., LEVY, G., MATSUZAWA, T. and BALIAH, T. (1966): Enhancement of glucuronide-conjugating capacity in a hyperbilirubinemic infant due to apparent enzyme induction by phenobarbital. *New Engl. J. Med., 275*, 1461.

Werner Kalow

Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada

Pharmacogenetics of drugs used in anaesthesia

Many drugs used for therapy of disease have slow and protracted actions and it requires the special art of the clinical pharmacologist to measure their effects. By contrast, drugs to be useful in anaesthesia have to act rapidly, their effects are striking and their actions should not last any longer than the surgery which is aided by their use. When drug responses are sharply defined, individual differences in response also show up clearly. It is therefore no chance that genetic factors have been recognized which affect the response to drugs used in anaesthesia. This applies particularly to the anaesthetic agents themselves, and to some muscle relaxants which are used as auxiliary agents.

Let me first consider the general anaesthetics, of which the most important group are the volatile anaesthetics. At the present time the most widely used agents of this class are halothane and methoxyflurane. Of special concern are unusual or toxic effects produced by these agents.

A rare toxic effect which occurs on the basis of hereditary predisposition is malignant hyperthermia (Britt and Kalow, 1970*a*,*b*; Ryan and Papper, 1970). In typical cases body temperature may rise to extreme degrees and in about three-quarters of all cases there is evidence of rigidity. Whether the rigidity is present or not, the hyperthermic reaction has been fatal to two-thirds of the affected patients. In the operating theatre the first observation is frequently an increased heart rate, and it may then be noted that the patient feels hot. In other cases the first indication is a failure of the muscles to relax after an injection of succinylcholine. The injection is therefore repeated whereupon some or all muscles become rigid. In some cases all muscles become completely stiff and this rigidity persists into death as rigor mortis. In spite of administration of oxygen, venous oxygen tension tends to be low and carbon dioxide high. There is severe acidosis. Plasma potassium is high and calcium low. In the rigid cases there is evidence of muscle damage in the form of release of muscle enzymes such as creatine phosphokinase (CPK) and myoglobin into plasma. Acute death is often due to cardiac failure, delayed death due to renal damage produced by the released myoglobin. There are some reasons to believe that the hyperthermic reaction with rigidity is a somewhat different disease than the more rare hyperthermia without rigidity (Britt and Kalow, 1970*a*; Kalow *et al.*, 1970).

A number of studies have shown that this condition occurs on the basis of a hereditary predisposition. Almost 10 years ago, Denborough *et al.* (1962) in Australia reported a sibship of which 10 members had died after anaesthesia, while they could keep the eleventh member alive. In retrospect it seems that Denborough was dealing with what we now would call malignant hyperthermia, but it is not entirely clear whether or not rigidity occurred in these cases. The pedigree strongly suggested inheritance of the predisposition as autosomal dominant. Two years ago, Britt *et al.* (1969) reported a pedigree with 18 members affected by hyperthermia. None of these had been rigid. Again the pedigree indicated inheritance of the predis-

position as an autosomal dominant. Recently, we encountered a patient who had survived a severe episode of malignant hyperthermia with rigidity (Kalow and Britt, 1971) (Fig. 1). Two paternal uncles had died from the same condition. Hence our patient, a young man, must have inherited the defect from his father, so that this small pedigree represents specific evidence that the predisposition to hyperthermia with rigidity is also inherited by autosomal dominance.

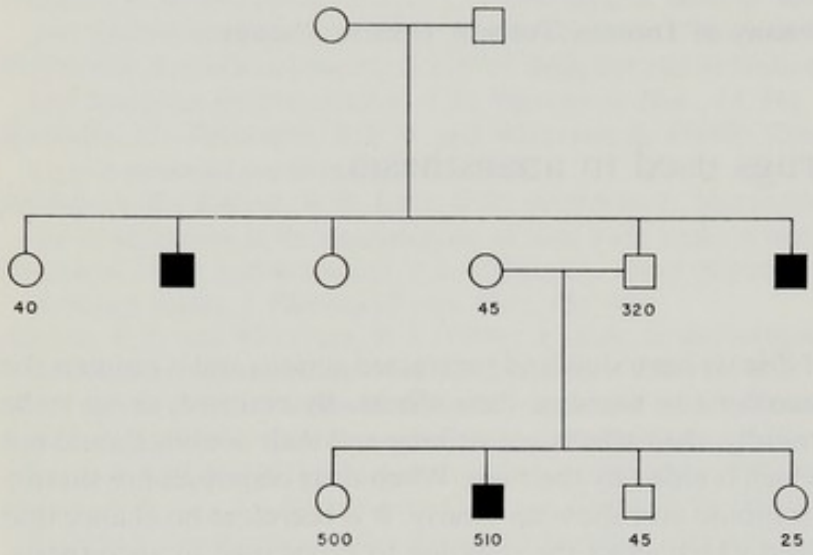


Fig. 1. Pedigree of a family predisposed to malignant hyperthermia with rigidity. Males are indicated by squares, females by circles. The black squares indicate males who exhibited hyperthermia with rigidity. The episode was fatal in two brothers while their nephew survived his severe attack. A number under a symbol indicates the level of creatine phosphokinase (CPK) in plasma. Values above 70 must be regarded as abnormal. (After Kalow and Britt, 1971).

In this last pedigree, as well as in several published pedigrees (Denborough *et al.*, 1970; Isaacs and Barlow, 1970; Zsigmond *et al.*, 1971), affected members and some relatives show in daily life an elevated plasma level of CPK. Much attention is being paid to this fact, since it is hoped that preoperative CPK determinations may help one to recognize patients disposed to malignant hyperthermia. Within each affected sibship transfer of elevated CPK levels is again by autosomal dominance.

While most cases of malignant hyperthermia have been observed in Caucasians, some cases have also been described among Negroes and Orientals (Britt and Kalow, 1970*a*).

The disease incidence is uncertain. In the Hospital for Sick Children in Toronto, the incidence as estimated by temperature measurements during all operative procedures over several years was 1:15,000. However, a statement from Berne, Switzerland, indicated that there were no cases among 90,000 patients (Tschirren, 1970). There were 5 cases among 300,000 in Johannesburg, South Africa, but only one among a similar number in Capetown (Harrison, 1971). The disease incidence permits no conclusion as to the gene frequency, since the predisposition without appropriate anaesthesia would not permit recognition of the gene. While there is no question that the condition is rare, we are aware of over 200 cases, mostly from Canada and the United States.

Most often affected are children and young adults. Both sexes are equally affected in the younger age group but males predominate after the teens.

The investigation of malignant hyperthermia has been aided by the observation of a similar occurrence in pigs (Berman *et al.*, 1970; Woolf *et al.*, 1970), particularly in Landrace and Poland China pigs. In these animals, as in man, the predisposition is inherited as an autosomal dominant.

The agent most often responsible for malignant hyperthermia has been halothane, but

this is probably due to its widespread use, since it has also occurred after other general anaesthetics. Succinylcholine seems to be a triggering agent, which speeds development of rigidity and perhaps intensifies it. However, it is clear that the hyperthermia with rigidity can occur without succinylcholine. The greatest puzzle is an apparent role of atropine. To our surprise a statistical analysis of our data (Britt and Kalow, 1970*a*) showed that most cases who develop rigidity had received atropine or similar anti-cholinergic agent, while non-rigid cases had not. While the difference was significant on the one in thousand level, there is no known pharmacological basis for this effect. Another unresolved puzzle is the fact that some patients tolerated a first anaesthetic well, but developed hyperthermia during a second anaesthesia a few years later.

There are several pieces of evidence to indicate that malignant hyperthermia with rigidity occurs on the basis of a defect in skeletal muscle. The first evidence came from the clinical observation that rigidity did not develop in a limb when a tourniquet prevented its being reached by halothane (Satnick, 1969). A second piece of evidence is the increase of CPK levels in predisposed persons (see above). Increased CPK levels in plasma usually indicate muscle damage, and they occur, for instance, in Duchenne muscular dystrophy. Third, several authors have observed in affected families cases of mild myopathy which is characterized by wasting of some muscles and hypertrophy (or pseudohypertrophy) of others (Denborough *et al.*, 1970; Steers *et al.*, 1970). Fourth, we had in our own data (Britt and Kalow, 1970*a*) statistical indications of an association between malignant hyperthermia and the presence of various musculoskeletal disorders. Fifth, *in vitro* testing of muscle of affected persons showed an unusual susceptibility to caffeine-induced contracture and unusual potentiation of this effect by halothane (Fig. 2) (Kalow *et al.*, 1970).

Production of a caffeine contracture requires higher doses than could occur in a living subject and it is therefore strictly an *in vitro* experimental tool. However, caffeine is known to produce the contracture by an effect on calcium uptake by sarcoplasmic reticulum within the

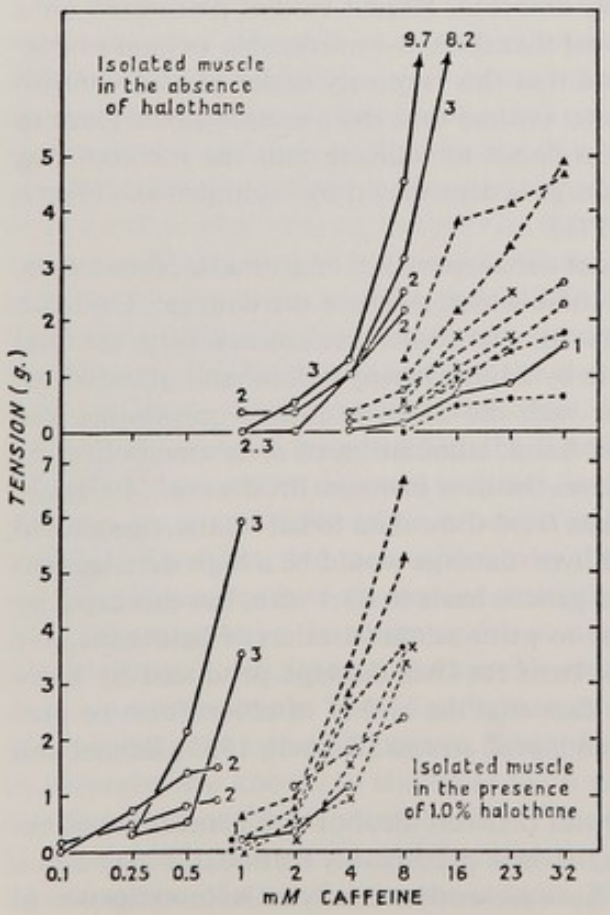


Fig. 2. Caffeine contracture of isolated muscle from volunteers disposed to malignant hyperthermia and from controls.

The abscissa (log scale) indicates the concentration of caffeine in the tissue bath; the ordinate gives the maximal increase of isometric contracture produced by caffeine. The lower plot shows the measurements obtained when 1% halothane was added to the oxygen/carbon dioxide mixture bubbling through the bath.

----- = control muscle
_____ = patient's muscle
1 = patient A (no rigidity) quadriceps
2 = patient B (rigidity) quadriceps
3 = patient C (rigidity) quadriceps
○ = quadriceps
× = soleus
▲ = gastrocnemius
● = rectus abdominis

(After Kalow *et al.*, 1970)

muscle fibre (Weber, 1968; Sandow, 1965, 1970). We therefore sought and found the opportunity to isolate the sarcoplasmic reticulum of two patients a year after they had survived a severe episode of malignant hyperthermia with rigidity (Kalow *et al.*, 1970). It turned out that the uptake of radiocalcium by these isolated preparations was inhibited by halothane, while there was no such inhibition in the controls. Since intracellular calcium transport regulates not only the contraction-relaxation cycle but also the metabolic activity of muscle, we seem to have pinpointed the key defect responsible for the unusual reaction to halothane. This does not necessarily mean that we have pinpointed the primary genetic lesion; this could reside, for instance, in the nerve which releases trophic factors which – independent from acetylcholine – affect muscle structure and functional ability. However, our observation has potential therapeutic consequences.

The caffeine contracture is known to be counteracted by the local anaesthetic agent procaine, but not by the local anaesthetic lidocaine (Bianchi and Bolton, 1967). It is therefore logical to attempt therapy of an attack of malignant hyperthermia with rigidity by an intravenous injection of procaine or procainamide. Berman (1971) has recently shown that this is effective treatment in hyperthermic pigs and indeed I am now aware of 5 recent and as yet unpublished human cases where this treatment has been highly successful and led to the survival of the patient.

Halothane is thought to have occasionally produced liver damage (Gall, 1968; Wilkinson, 1970). The incidence must be extremely rare. One suspects that this liver damage occurs mostly when halothane is repeatedly administered within days or a few weeks. We have no direct evidence that there is a hereditary basis for halothane-induced liver damage, but there is information which suggests this as a working hypothesis until the contrary is proven. In former years it has been taken for granted that volatile anaesthetics like halothane would be eliminated from the body by exhalation of the unchanged drug. In the meantime it has been shown that many anaesthetics are metabolized to a noticeable degree in the human body (Brown and Vandam, 1971). The prominent metabolic step in the degradation of halothane is the removal of a bromine molecule and probably chlorine. Recent studies performed on a series of twins (Cascorbi *et al.*, 1971) have indicated that there is considerable person-to-person variation in this dehalogenation reaction, and that this is mostly under genetic control. The heritability value was 0.63. There is reason to assume that this reaction takes place in liver microsomes, and there is now additional evidence to indicate that the metabolizing capacity of human liver microsomes for several drugs is determined by multifactorial inheritance (Whittaker and Evans, 1970; Vesell *et al.*, 1971).

There is additional evidence that in the process of dehalogenation of aromatic compounds, unstable chemical radicals are formed, and it is these which produce the damage. Evidence has been presented recently by Brodie *et al.* (1971*a*, *b*), who could save animals from the fatal effects of carbon tetrachloride and bromobenzene by administering beforehand glutathione. The glutathione would instantaneously combine with the unstable radical metabolite and thereby reduce its biological effects. On the other hand, stimulation of drug metabolism by pretreatment of animals with phenobarbital increases the liver damage (Brodie *et al.*, 1971*a*, *b*; Nayak *et al.*, 1970). If it is permissible to extrapolate from these data to halothane, one should expect that a prerequisite for halothane-induced liver damage would be a high dehalogenation capacity of a person. This could be high on a genetic basis to start with, but this capacity could also be enhanced by enzyme induction due to prior administration of halothane. We may therefore well have at least a partial genetic basis for liver damage produced by halothane. This interpretation is strengthened by the fact that the ability of chloroform to produce tissue damage in mice varies strongly between inbred strains (Russell, 1955; Bennet and Whigham, 1964).

Since there are many pharmacological similarities between alcohol and general anaesthetics, and since their mode of action at the cellular level is presumably identical, I may add a few remarks about a peculiar effect of alcohol. We have presently a family under investigation in

which the propositus died some years ago at age 17 from a cardiac attack after he had shared a bottle of wine with some friends. The diagnosis was verified by autopsy. A year prior to his death he had taken some champagne at a wedding party. The prominent syndrome at that time was muscle pain in arms and legs, which was sufficiently discomforting for him to seek help in the emergency ward of the Montreal General Hospital. Recently, his sister, now aged 18, complained about muscle pain after small doses of alcohol. Under experimental conditions at the Toronto General Hospital she experienced mild pain in her limbs after half an ounce of gin. Successive test doses of one, and then one and a half ounces, had fundamentally similar but increasing effects. There may have been a slight rise of creatine phosphokinase (CPK) and LDH1 in plasma, suggesting some enzyme release from muscle. However, to make the effects more distinct, a test dose of two ounces of gin was given. After that the girl developed both severe muscle pain and chest pain with ECG changes typical of cardiac hypoxia. Fortunately, the girl recovered quickly from this cardiac attack. A few weeks later she had another non-fatal cardiac attack following some exertion during a ball game. We then found a literature report (Sussman *et al.*, 1970) of a similar sensitivity to alcohol in chronic lactic acidosis, and we still have to investigate our patient to find out whether this is also her basic defect.

Though incomplete, these observations raise a number of questions. First, chronic alcohol intake causes skeletal muscle damage in some subjects which may lead to a profound wasting of muscle (Editorial, 1966; Perkoff *et al.*, 1967; Wolf, 1969). In others alcoholism causes a cardiac myopathy. Since neither condition occurs in all alcoholics, tests for hereditary predispositions are warranted. Second, since alcohol is an anaesthetic and since – as our observations suggest – muscle damage can follow an acute exposure, it needs to be investigated whether skeletal muscle damage can be produced by general anaesthetics on a similar basis as by alcohol. If the damage is not accompanied by the high fever typical of malignant hyperthermia, it may be overlooked, and the release of muscle enzymes may be ascribed to the effects of surgery rather than to those of the anaesthetic.

It is old knowledge that barbiturates including thiopental given intravenously to produce anaesthesia, can initiate an attack of acute intermittent porphyria (AIP) (Ward, 1965). The whole sequence of events between barbiturate intake and the paralysis or other neurological defect typical of porphyria is not clear, but some recent observations have opened new avenues of investigation (Kappas *et al.*, 1971). A porphyric attack is accompanied by an increase of delta amino levulinic acid synthetase (ALA synthetase) (Sweeney *et al.*, 1970). This is a key enzyme in the biosynthetic pathway leading to the porphyrins and finally to haem. It has been established in experimental porphyrias that formation of this enzyme is stimulated by barbiturates and other drugs (Hutton and Gross, 1970). In order to facilitate the study of this enzyme induction, Sassa and Granick (1970) developed a cell culture system using liver cells from chick embryos. This simple test system permitted the screening of many compounds. Kappas *et al.* (1971) then found that the most potent inducers were not drugs but certain steroids, and particularly steroid metabolites. Particularly potent were 5β metabolites. Testosterone, for instance, is reduced at the 5 position by either an α reductase or a β reductase (Fig. 3). These are two different enzymes, in different locations in the liver cell. The difference between the α and β reduction products appears to be minor if one looks at conventional structural formulae, but stereochemically the 5α reduction leads to a planar molecule, while the 5β reduction leads to an angular molecule. In normal subjects the extent of α and β reductions are about alike. In patients with acute intermittent porphyria the 5α reduction is diminished due to a deficiency of the specific reductase. As a consequence, the 5β reduction product is overwhelming. The 5β reductase as the remaining enzyme may assume a key role which it does not normally possess. Since the 5β reductase is a microsomal enzyme and since barbiturates are known to stimulate the formation of numerous liver microsomal enzymes, it is possible that the barbiturate effect is due to induced formation of the steroid 5β reductase. In other words, there is a possibility that barbiturates cause a porphyric attack by stimulating the formation of a natural enzyme inducer. However, this is not the whole story.

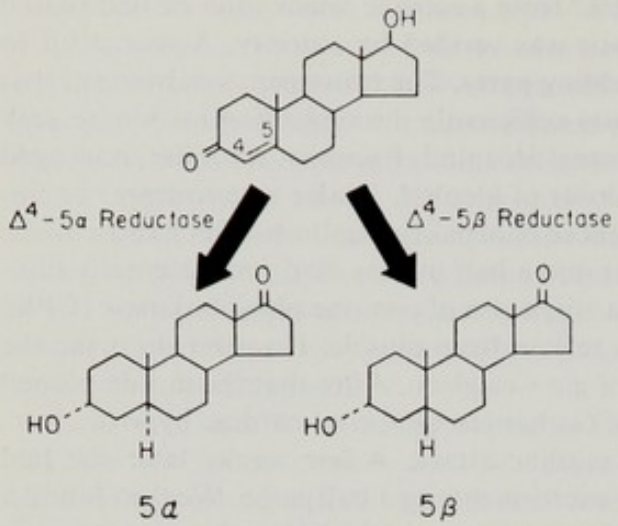


Fig. 3. Graphic representation of the normal pattern of testosterone metabolism in man. The reductive transformation of the hormone, shown at the top, proceeds, on average, equally along the 5α and the 5β pathways. (After Kappas *et al.*, 1971.)

Following the investigations on porphyric subjects, Kappas *et al.* (1971) noticed that the overwhelming 5β reduction is even more pronounced in myxoedema than in porphyria. It was then found that the thyroid hormone, triiodothyronine (T_3), is an inducer of the 5α steroid reductase. It is therefore possible that the deficiency of the 5α reductase in porphyric patients does not represent a primary defect, but that it is a consequence of either a disordered thyroid metabolism in porphyria, or a specific failure of the 5α reductase system to respond to thyroid hormone. Thus this research is still open-ended. However, these fundamental studies starting with chick liver embryos direct attention to the endocrine glands in porphyria. On purely clinical grounds, Stein and Tschudy (1970) investigated endocrine functions in porphyric patients and suspected that one of the bases of the porphyric attack may be a disturbance in the hormonal regulation of water and salt metabolism. In short, the puzzle of barbiturates and porphyria has gained completely new dimensions, although the final explanations are still elusive.

One of the most important auxiliary drugs used in anaesthesia is the short-acting muscle relaxant succinylcholine. It has been established many years ago that prolonged effects of this drug can be accounted for by the presence of cholinesterase variants, which do not metabolize the drug at the rapid rate which is seen in most persons (Litwiller, 1969; Lehmann and Liddell, 1969; Whittaker, 1970*a*; Goedde and Altland, 1971; La Du, 1971). Of these esterase variants the first discovered and most frequently occurring is the so-called atypical cholinesterase, which is characterized by resistance to dibucaine inhibition and by a low affinity for succinylcholine. A considerably more rare variant is the so-called fluoride type which can be detected by its resistance to fluoride inhibition. The reasons for prolonged succinylcholine effects in the presence of this variant are presumably the same as for atypical esterase, although this has not been as extensively investigated. Allelic to the genes for the dibucaine and fluoride resistants is the so-called silent gene which is very rare and produces a lack or severe deficiency of cholinesterase activity. The gene locus has been called E_1 and the genes producing the usual, atypical, fluoride-resistance variants and the esterase deficiency have been designated E_1^u, E_1^a, E_1^f and E_1^s, respectively. This E_1 locus is linked with the transferrin locus T_f (Robson *et al.*, 1966). This is the state of affairs which has prevailed for many years.

Recent or current investigations have covered or are covering mainly 3 areas, namely the isoenzyme composition of cholinesterase, methods for the determination of cholinesterase variants and population studies.

A comprehensive review of the isoenzyme pattern of cholinesterase was published recently by LaMotta and Woronick (1971). In most human sera several investigators have distinguished 4 isoenzymes. In purified preparations or with special methods of investigation 5, 7 or up to 12 isoenzymes have been noted. Roughly 90% of the activity resides ordinarily in the band originally referred to as the fourth band. The differences between the isoenzymes

are primarily in molecular weight, and there is a pattern of interconvertibility of isoenzymes so that the cholinesterase must be thought to consist of aggregates of subunits. If the plasma contains atypical esterase, each of the isoenzymes displays the characteristic of this variant. An additional major component is observed in some sera, and was referred to by Harris *et al.* (1963) as C5+ component. This may contain 30% of the total esterase activity. It behaves like an additional isoenzyme, the presence or absence of which is determined by a locus which has been referred to as E_2 locus and which is non-allelic to the E_1 locus.

In a rare variant with about three times normal cholinesterase activity, and referred to as E Cynthiana variant by Yoshida and Motulsky (1969), additional isoenzymes similar to, but distinguishable from, the C5+ band have been found. The locus which determines this variant has not yet been established. For the pharmacologist and clinician this variant is of interest since its presence conveys some resistance to succinylcholine.

In the context of isoenzymes the work of Altland and Goedde (1970) on the products of the silent gene should be mentioned. Some residual activity of the plasma cholinesterase in these cases has been described by several investigators. Since the residual esterase differs in enzymatic behaviour from the usual cholinesterase, it could be a trace enzyme occurring in all subjects (Rubinstein *et al.*, 1970; Scott, 1970). However, this is unlikely (Gaffney and Lehmann, 1969), particularly since at least in some cases the trace enzyme reacts immunologically like usual cholinesterase (Altland and Goedde, 1970). This observation, and the fact that the silent gene is allelic with the gene for atypical esterase, is compatible with the assumption that cholinesterase deficiency is due to an amino acid substitution which grossly reduces esterase activity. Altland and Goedde demonstrated the residual activity after gel electrophoresis and found different isoenzyme patterns in almost every case. Hence there are apparently numerous 'silent genes'. Many subjects with cholinesterase deficiency may be heterozygotes for two different silent genes. Since there are several alleles which all produce a marginally active enzyme, the term 'silent gene' is inappropriate and further investigations should lead to its replacement.

Numerous papers have been published over the years dealing with methods of determination of cholinesterase variants. In view of their numbers, these papers cannot be disregarded in a review, but it is difficult to provide a good survey of this work in brief terms. The best way seems to provide separate looks at the substrates which were used, at the inhibitors and activators, and at the methods of measuring hydrolysis rates. The data are presented in Tables 1, 2, 3. It should be obvious that elements from each table could be combined in various ways to yield an almost endless variety of methods. Therefore, a few remarks must suffice. Morrow and Motulsky (1968) have worked out a cheap, simple, and rapid assay. Their method has the rather common fault, that it does not show up the fluoride-resistant variant. Those who are interested in mass surveys should note that there are 3 methods which have been adapted for use with the Technicon Autoanalyzer (Ashby *et al.*, 1970; Boutin and Brodeur, 1970; Garry, 1971). Whittaker (1970*a*) has tested enzyme activation by various alcohols and enzyme inhibition by very high concentrations of sodium chloride. The strongly contrasting behaviour

TABLE 1 *Methods used for cholinesterase typing (selected recent publications)*

Spectrophotometry (Whittaker 1968*a*, *b*, 1969, 1970*b*; King and Dixon, 1970; Smith and Foldes, 1969; Whittaker and Hardisty, 1969; Garry, 1971*a*; Hanel and Mogensen, 1971)
Spectrophotometry – automated (Garry, 1971*b*)
pH change – pH stat (Ashby *et al.*, 1970)
pH change – automated (Boutin and Brodeur, 1970)
Diazo dye formation (Morrow and Motulsky, 1968)
Radio labelling of succinylcholine (Goedde *et al.*, 1968)
Immunoassay (Altland and Goedde, 1970)
Succinylcholine *in vivo* (Cohen *et al.*, 1970)

TABLE 2 *Substrates used for cholinesterase typing (selected recent publications)*

Acetylcholine (Ashby *et al.*, 1970; Boutin and Brodeur, 1970)
Butyrylcholine (Ashby *et al.*, 1970)
Benzoylcholine (Whittaker, 1968*a*, *b*; King and Dixon, 1970; Hanel and Mogensen, 1971)
Succinylcholine (Goedde *et al.*, 1968)
Succinylmonocholine (Goedde *et al.*, 1968)
Acetylthiocholine (Garry, 1971*a*, *b*)
Propionylthiocholine (Garry, 1971*a*, *b*)
Butyrylthiocholine (Garry, 1971*a*, *b*)
Procaine (Smith and Foldes, 1969)
Tetracaine (Smith and Foldes, 1969)
Aspirin (La Du, 1971)
α-Naphthyl acetate (Morrow and Motulsky, 1968)
o-Nitrophenyl butyrate (Whittaker and Hardisty, 1969)

TABLE 3 *Inhibitors and activators used for cholinesterase typing (selected recent publications)*

Dibucaine (Ashby *et al.*, 1970; Boutin and Brodeur, 1970; King and Dixon, 1970)
Fluoride (Boutin and Brodeur, 1970; King and Dixon, 1970; Garry, 1971*a*)
Chloride (Whittaker and Hardisty, 1969; King and Dixon, 1970; Garry, 1971*a*)
Phosphate (Garry, 1971*a*, *b*)
Tris(hydroxymethyl)aminomethane (Tris) (Garry, 1971*a*, *b*)
Na^+, K^+, Mg^{++}, Ca^{++}, Ba^{++} (Garry, 1971*a*)
R02-0683* (Morrow and Motulsky, 1968)
Alcohols (Whittaker, 1968*a*)
n-Butyl alcohol (Whittaker, 1968*b*)
Formaldehyde (Whittaker, 1969; Whittaker and Hardisty, 1969)
Thyroxin, thiouracil (Whittaker, 1970*b*)
Urea (Hanel and Mogensen, 1971)
Succinylcholine (Garry, 1971*a*; LaMotta and Woronick, 1971)
Decamethonium, tetramethylammonium, physostigmine, neostigmine (Kalow, 1962)
Chlorpromazine

* Dimethylcarbamate of 2-hydroxy-5-phenyl benzyl-trimethylammonium bromide (Roche Laboratories, Nutley, N.J.)

of the enzyme variants in these systems has led to a tentative identification of new cholinesterase variants.

A large number of different populations have been tested for cholinesterase over the years. The most recent compilation of the results is by Lubin *et al.* (1971). The data reveal that the gene for atypical esterase (E_1^a) varies substantially in different populations. The highest frequency ever reported was in Ashkenazi Jews from Iraq and Iran where the gene frequency was 0.051 (Szeinberg *et al.*, 1966). In populations of European origin the gene frequency is usually found to be between 0.014 to 0.019, while in most Oriental populations and in Negroes the frequency is very much lower than that. On the other hand, the frequency of the silent gene (E_1^s) is very rare in the Caucasian population and probably does not exceed 0.003 (Simpson and Kalow, 1964). By contrast, Scott *et al.* (1970) found among a group of 1500 Eskimoes in Alaska a gene frequency of 0.116, that is an at least 40 times greater frequency than found among Caucasians. Hence the risk of prolonged apnoea after succinylcholine due to the presence of genetic variants of cholinesterase can be expected to differ substantially in different populations.

How great actually is this risk? This question can only be answered empirically. Over the past few years about 500 blood specimens were submitted for cholinesterase typing by numer-

TABLE 4 *Cholinesterase variants in plasma samples submitted for testing because of prolonged apnoea following succinylcholine*

Genotype	Observed no. of patients with prolonged apnoea	Expected no. in random population of equal size*	Observed/ expected
$E_1^uE_1^u$) $E_1^uE_1^s$)	199**	467	0.4
$E_1^aE_1^a$) $E_1^aE_1^s$)	168	0.2	840
$E_1^fE_1^f$) $E_1^fE_1^s$)	2	0.03	66
$E_1^sE_1^s$	7	0.005	1400
$E_1^uE_1^a$	81	20	4
$E_1^uE_1^f$	10	5	2
$E_1^aE_1^f$	20	0.1	200
Unclassified variant	5	–	–
	492	492 (approx.)	

Combined observations from the laboratories of N. E. Simpson, Kingston, Ontario and of W. Kalow, Toronto, Ontario.

* The expected numbers are calculated on the assumption of the following gene frequencies:

$E_1^u = 0.972$; $E_1^a = 0.020$; $E_1^f = 0.005$; $E_1^s = 0.003$

The gene products are usual, atypical, fluoride-resistant, and silent cholinesterase, respectively.

** About 35 patients had an esterase activity substantially below normal average.

ous anaesthetics to the laboratory of Dr. Nancy Simpson in Kingston, and to my own laboratory in Toronto. The combined data are shown in Table 4. Since most determinations were made on a routine basis and did not permit family investigations we were not able to distinguish between all genotypes. If we accept at face value the ratios between observed and expected numbers, they indicate that phenotype S would convey the greatest sensitivity to succinylcholine, followed by phenotype A and finally phenotype F. Otherwise, the ratios in this table would mean that the assumption of gene frequencies in the random population was grossly erroneous, which is unlikely. The most striking observation is, however, that in confirmation of previous observations about a third of the patients have normal cholinesterase activity by our criteria of evaluation. These patients offer the greatest challenge.

It is likely that not all anaesthetists who submitted samples used the same criteria for prolonged apnoea. It is also possible that among the 164 persons with prolonged apnoea whose esterase was assessed as normal, were some with a temporary or even permanent esterase deficiency, which has not been or could not be recognized with our test system. However, I feel confident that these arguments would not apply to all these patients and that the prolonged effect in some patients occurred in spite of normal cholinesterase activity. There must be other factors for which we have to search at the site of action of succinylcholine in skeletal muscle. I would like to recall what I said in the beginning about malignant hyperthermia. In this condition, succinylcholine is presumably only a trigger which produces rigidity, but once produced the rigidity persists in spite of normal cholinesterase activity. That the action of succinylcholine depends on the state of the muscle is known to every anaesthetist since pretreatment with curare diminishes the succinylcholine effect. Payne and Holmdahl (1959) have shown that succinylcholine becomes less and less effective after successive injections.

In the two dominant diseases, myotonia congenita and dystrophia myotonica, succinylcholine produces muscular contractions instead of relaxation (Örndahl, 1962; Thiel, 1967; Cody, 1968). However, this contraction is usually of short duration and does not last any longer than the normally occurring relaxation. A very unusual effect of succinylcholine in a patient with progressive muscular dystrophy has been described by Pinelli (1970). The dose necessary to produce a paralysis was 3 times higher than in control subjects, but instead of minutes it took 4 days for the effect to wear off. While this does not seem to be always the case in patients with muscular dystrophy, this observation should stimulate further investigations.

There are other effects of succinylcholine in normal subjects with individual differences in response, but genetic investigations have not been performed. Thus, in some subjects, the use of succinylcholine is followed by muscle pain (Bush and Roth, 1961; Cooke *et al.*, 1963). This might be related to the initial muscle stimulation, that is, the fasciculations which tend to precede the muscle paralysis, but it is more likely related to direct damage of the cell membrane. This is reflected by the observation that succinylcholine is sometimes causing a release of myoglobin (Jensen *et al.*, 1968; Ryan *et al.*, 1970) and CPK (Tammisto and Airaksinen, 1966; Perkoff *et al.*, 1969). In short, the actions of succinylcholine on muscle still present us with a number of puzzles even though this drug is one of the most widely used and best investigated agents.

I am sure that the pharmacogenetic investigations of drugs used in anaesthesia have not yet come to an end.

REFERENCES

ALTLAND, K. and GOEDDE, H. W. (1970): Heterogeneity in the silent gene phenotype of pseudocholinesterase of human serum. *Biochem. Genet.*, *4*, 321.

ASHBY, T. M., SUGGS, J. E. and JUE, D. L. (1970): Detection of atypical cholinesterase by an automated pH stat method. Report of a family with both atypical and silent gene alleles. *Clin. Chem.*, *16*, 503.

BENNET, R. A. and WHIGHAM, A. (1964): Chloroform sensitivity of mice. *Nature* (*Lond.*), *204*, 1328.

BERMAN, M. C. (1971): *Presentation of the International Symposium on Malignant Hyperthermia, Toronto, May 15/16, 1971.* To be published.

BERMAN, M. C., HARRISON, G. G., BULL, A. B. and KENCH, J. E. (1970): Changes underlying halothane-induced malignant hyperpyrexia in Landrace pigs. *Nature* (*Lond.*), *225*, 653.

BIANCHI, C. P. and BOLTON, T. C. (1967): Action of local anesthetics on coupling systems in muscle. *J. Pharmacol. exp. Ther.*, *157*, 388.

BOUTIN, D. and BRODEUR, J. (1970): An automated method for simultaneous determination of serum pseudocholinesterase activity, dibucaine number and fluoride number. *Clin. Biochem.*, *3*, 245.

BRITT, B. A. and KALOW, W. (1970*a*): Malignant hyperthermia: a statistical review. *Canad. Anaesth. Soc. J.*, *17*, 293.

BRITT, B. A. and KALOW, W. (1970*b*): Malignant hyperthermia: aetiology unknown! *Canad. Anaesth. Soc. J.*, *17*, 316.

BRITT, B. A., LOCHER, W. G. and KALOW, W. (1969): Hereditary aspects of malignant hyperthermia. *Canad. Anaesth. Soc. J.*, *16*, 89.

BRODIE, B. B., CHO, A. K., KRISHNA, G. and REID, W. D. (1971*a*): Drug metabolism in man: past, present and future. *Ann. N. Y. Acad. Sci.*, *179*, 11.

BRODIE, B. B., REID, W. D., CHO, A. K., SIPES, G., KRISHNA, G. and GILLETTE, J. R. (1971*b*): Possible mechanism of liver necrosis caused by aromatic organic compounds. *Proc. nat. Acad. Sci. USA*, *68*, 160.

BROWN, B. R., Jr and VANDAM, L. D. (1971): A review of current advances in metabolism of inhalation anesthetics. *Ann. N. Y. Acad. Sci.*, *179*, 235.

BUSH, G. H. and ROTH, F. (1961): Muscle pains after suxamethonium chloride in children. *Brit. J. Anaesth.*, *33*, 151.

CASCORBI, H. F., VESELL, E. S., BLAKE, D. A. and HELRICH, M. (1971): Halothane biotransformation in man. *Ann. N. Y. Acad. Sci.*, *179*, 244.

CODY, J. R. (1968): Muscle rigidity following administration of succinylcholine. *Anesthesiology*, *29*, 159.
COHEN, P. J., REYNOLDS, R. C. and NAIDL, J. (1970): A simple test for abnormal pseudocholinesterase. *Anesthesiology*, *32*, 281.
COOKE, M., MCCLELLAND, R. M. A., SINCLAIR, J. H. and HAYTON-WILLIAMS, D. S. (1963): Muscle pains after intramuscular suxamethonium chloride. *Brit. J. Anaesth.*, *35*, 121.
DENBOROUGH, M. A., FORSTER, J. F. A., LOVELL, R. R. H., MAPLESTONE, P. A. and VILLIERS, J. D. (1962): Anaesthetic deaths in a family. *Brit. J. Anaesth.*, *34*, 395.
DENBOROUGH, M. A., KING, J. O., EBELING, P. and ZAPF, P. (1970): Myopathy and malignant hyperpyrexia. *Lancet*, *I*, 1138.
EDITORIAL (1966): Alcohol and disease of muscle. *Lancet*, *II*, 1153.
GAFFNEY, P. J. and LEHMANN, H. (1969): Residual enzyme activity in the serum of a homozygote for the silent pseudocholinesterase gene. *Hum. Hered.*, *19*, 234.
GALL, E. A. (1968): Report of the Pathology Panel. The National Halothane Study. *Anesthesiology*, *29*, 233.
GARRY, P. J. (1971*a*): Serum cholinesterase variants: examination of several differential inhibitors, salts, and buffers used to measure enzyme activity. *Clin. Chem.*, *17*, 183.
GARRY, P. J. (1971*b*): A manual and automated procedure for measuring serum cholinesterase activity and identifying enzyme variants. *Clin. Chem.*, *17*, 192.
GOEDDE, H. W. and ALTLAND, K. (1971): Suxamethonium sensitivity. *Ann. N.Y. Acad. Sci.*, *179*, 695.
GOEDDE, H. W., HELD, K. R. and ALTLAND, K. (1968): Hydrolysis of succinyldicholine and succinylmonocholine in human serum. *Molec. Pharmacol. (N.Y.)*, *4*, 274.
HANEL, H. K. and MOGENSEN, J. V. (1971): Urea inhibition of human pseudocholinesterase. A new method of detecting atypical pseudocholinesterase in homozygotes. *Brit. J. Anaesth.*, *43*, 51.
HARRIS, H., HOPKINSON, D. A., ROBSON, E. B. and WHITTAKER, M. (1963): Genetical studies on a new variant of serum cholinesterase detected by electrophoresis. *Ann. hum. Genet.*, *26*, 359.
HARRISON, G. G. (1971): *Discussion Remark at International Symposium on Malignant Hyperthermia, Toronto, May 15/16, 1971.* To be published.
HUTTON, J. J. and GROSS, S. R. (1970): Chemical induction of hepatic porphyria in inbred strains of mice. *Arch. Biochem.*, *141*, 284.
ISAACS, H. and BARLOW, M. B. (1970): The genetic background to malignant hyperpyrexia revealed by serum creatine phosphokinase estimations in asymptomatic relatives. *Brit. J. Anaesth.*, *42*, 1077.
JENSEN, K., BENNIKE, K.-A., HANEL, H. K. and OLESEN, H. (1968): Myoglobinuria following anaesthesia including suxamethonium. *Brit. J. Anaesth.*, *40*, 329.
KALOW, W. (1962): *Pharmacogenetics; Heredity and the Response to Drugs*, Chapter 6, p. 75. W. B. Saunders, Philadelphia-London.
KALOW, W. and BRITT, B. A. (1971): Inheritance of malignant hyperthermia. In: *Proceedings, International Symposium on Malignant Hyperthermia, Toronto, May 15/16, 1971.* To be published.
KALOW, W., BRITT, B. A., TERREAU, M. E. and HAIST, C. (1970): Metabolic error of muscle metabolism after recovery from malignant hyperthermia. *Lancet*, *II*, 895.
KAPPAS, A., BRADLOW, H. L., GILLETTE, P. N. and GALLAGHER, T. F. (1971): Abnormal steroid hormone metabolism in the genetic liver disease acute intermittent porphyria. *Ann. N.Y. Acad. Sci.*, *179*, 611.
KING, J. and DIXON, R. I. (1970): A source of error in the determination of inhibitor constants of serum cholinesterase. *Brit. J. Anaesth.*, *42*, 698.
LA DU, B. (1971): Plasma esterase activity and the metabolism of drugs with ester groups. *Ann. N.Y. Acad. Sci.*, *179*, 684.
LAMOTTA, R. V. and WORONICK, C. L. (1971): Molecular heterogeneity of human serum cholinesterase. *Clin. Chem.*, *17*, 135.
LEHMANN, H. and LIDDELL, J. (1969): Human cholinesterase (pseudocholinesterase): genetic variants and their recognition. *Brit. J. Anaesth.*, *41*, 235.
LITWILLER, R. W. (1969): Succinylcholine hydrolysis: a review. *Anesthesiology*, *31*, 356.
LUBIN, A. H., GARRY, P. J. and OWEN, G. M. (1971): Sex and population differences in the incidence of a plasma cholinesterase variant. *Science*, *173*, 161.
MORROW, A. C. and MOTULSKY, A. G. (1968): Rapid screening method for the common atypical pseudocholinesterase variant. *J. Lab. clin. Med.*, *71*, 350.
NAYAK, N. C., CHOPRA, P. and RAMALINGASWAMI, V. (1970): The role of liver cell endoplasmic reticulum and microsomal enzymes in carbon tetrachloride toxicity: an *in vivo* study. *Life Sci.*, *9, Part 1*, 1431.

Örndahl, G. (1962): Myotonic human musculature: stimulation with depolarizing agents. *Acta med. scand., 172*, 739 and 753.

Payne, J. P. and Holmdahl, M. H. (1959): The effect of repeated doses of suxamethonium in man. *Brit. J. Anaesth., 31*, 341.

Perkoff, G. T., Abernathy, R. and Ruiz, M. (1969): Effect of succinylcholine on creatine phosphokinase (CPK) in anesthetized dogs. *J. Lab. clin. Med., 74*, 153.

Perkoff, G. T., Marconi, M. D., Bleisch, V. and Klinkerfuss, G. (1967): Spectrum of myopathy associated with alcoholism. *Ann. intern. Med., 67*, 481.

Pinelli, P. (1970): The effects of edrophonium, suxamethonium and ACTH in some neuromuscular diseases. In: *Muscle Diseases*, pp. 138–149. Editors: J. N. Walton, N. Canal and G. Scarlato. ICS 199, Excerpta Medica, Amsterdam.

Robson, E. B., Sutherland, I. and Harris, H. (1966): Evidence for linkage between the transferrin locus (Tf) and the serum cholinesterase locus (E_1) in man. *Ann. hum. Genet., 29*, 325.

Rubinstein, H. M., Dietz, A. A., Hodges, L. K., Lubrano, T. and Czebotar, V. (1970): Silent cholinesterase gene: variations in the properties of serum enzyme in apparent homozygotes. *J. clin. Invest., 49*, 479.

Russell, E. S. (1955): Significance of physiological patterns of animal strains in biological research. *Brit. med. J., I*, 826.

Ryan, J. F., Kagan, L. J. and Hyman, A. I. (1970): The relationship of succinylcholine to myoglobinemia. *Fed. Proc., 29*, 548 (Abstract).

Ryan, J. F. and Papper, E. M. (1970): Malignant fever during and following anesthesia. *Anesthesiology, 32*, 196.

Sandow, A. (1965): Excitation-contraction coupling in skeletal muscle. *Pharmacol. Rev., 17*, 265.

Sandow, A. (1970): Skeletal muscle. *Ann. Rev. Physiol., 32*, 87.

Sassa, S. and Granick, S. (1970): Induction of α-aminolevulinic acid synthetase in chick embryo liver cells in culture. *Proc. nat. Acad. Sci. USA, 67*, 517.

Satnick, J. H. (1969): Hyperthermia under anesthesia with regional muscle flaccidity. *Anesthesiology, 30*, 472.

Scott, E. M. (1970): Properties of the trace enzyme in human serum cholinesterase deficiency. *Biochem. biophys. Res. Commun., 38*, 902.

Scott, E. M., Weaver, D. D. and Wright, R. C. (1970): Discrimination of phenotypes in human serum cholinesterase deficiency. *Amer. J. hum. Genet., 22*, 363.

Simpson, N. E. and Kalow, W. (1964): The 'silent' gene for serum cholinesterase. *Amer. J. hum. Genet., 16*, 180.

Smith, J. C. and Foldes, F. F. (1969): An improved method for the recognition of atypical plasma cholinesterase. Personal communication.

Steers, A. J. W., Tallack, J. A. and Thompson, D. E. A. (1970): Fulminating hyperpyrexia during anaesthesia in a member of a myopathic family. *Brit. med. J., 2*, 341.

Stein, J. A. and Tschudy, D. P. (1970): Acute intermittent porphyria. A clinical and biochemical study of 46 patients. *Medicine (Baltimore), 49*, 1.

Sussman, K. E., Alfrey, A., Kirsch, W. M., Zweig, P., Felig, P. and Messner, F. (1970): Chronic lactic acidosis in an adult. A new syndrome associated with an altered redox state of certain NAD/NADH coupled reactions. *Amer. J. Med., 48*, 104.

Sweeney, V. P., Pathak, M. A. and Asbury, A. K. (1970): Acute intermittent porphyria. Increased ALA-synthetase activity during an acute attack. *Brain, 93*, 369.

Szeinberg, A., Pipano, S. and Ostfeld, E. (1966): Frequency of typical pseudocholinesterase in different population groups in Israel. *Acta anaesth. scand., Suppl. 24*, 199.

Tammisto, T. and Airaksinen, M. (1966): Increase of creatine kinase activity in serum as sign of muscular injury caused by intermittently administered suxamethonium during halothane anaesthesia. *Brit. J. Anaesth., 38*, 510.

Thiel, R. E. (1967): The myotonic response to suxamethonium. *Brit. J. Anaesth., 39*, 815.

Tschirren, B. (1970): Narkoseführung bei Süchtigkeit. *Anaesthesist (Berl.), 19*, 191.

Vesell, E. S., Passananti, G. T., Greene, F. E. and Page, J. G. (1971): Genetic control of drug levels and of the induction of drug metabolizing enzymes in man. Individual variability in the extent of allopurinol and nortriptyline inhibition of drug metabolism. *Ann. N.Y. Acad. Sci., 179*, 752.

Ward, R. J. (1965): Porphyria and its relation to anesthesia. *Anesthesiology, 26*, 212.

Weber, A. (1968): The mechanism of the action of caffeine on sarcoplasmic reticulum. *J. gen. Physiol., 52*, 760

WHITTAKER, J. A. and EVANS, D. A. P. (1970): Genetic control of phenylbutazone metabolism in man. *Brit. med. J.*, *4*, 323.

WHITTAKER, M. (1968*a*): The pseudocholinesterase variants. Differentiation by means of alkyl alcohols. *Acta genet. (Basel)*, *18*, 325.

WHITTAKER, M. (1968*b*): Differential inhibition of human serum cholinesterase with n-butyl alcohol: recognition of new phenotypes. *Acta genet. (Basel)*, *18*, 335.

WHITTAKER, M. (1969): The serum cholinesterase variants. Differentiation by means of formaldehyde. *Clin. chim. Acta*, *26*, 141.

WHITTAKER, M. (1970*a*): Genetic aspects of succinylcholine sensitivity. *Anesthesiology*, *32*,143.

WHITTAKER, M. (1970*b*): The effect *in vitro* of thyroxine, glucagon, thyrotrophin and antithyroid drugs on 'usual' and 'atypical' human plasma cholinesterase. *J. Endocr.*, *47*, 177.

WHITTAKER, M. and HARDISTY, C. A. (1969): Properties of 'usual' and 'atypical' serum cholinesterases using o-nitrophenyl butyrate as substrate. *Clin. Chem. 15*, 445.

WILKINSON, C. J. (1970): Postanesthetic hepatic dysfunction. *Arch. Surg.*, *101*, 359.

WOLF, P. (1969): Alcoholic skeletal muscular necrosis syndrome. In: *Biochemical and Clinical Aspects of Alcohol Metabolism*, pp. 283–287. Editor: V. M. Sardesai. Charles C. Thomas, Springfield, Ill.

WOOLF, N., HALL, L., THORNE, C., DOWN, M. and WALKER, R. (1970): Serum creatine phosphokinase levels in pigs reacting abnormally to halogenated anaesthetics. *Brit. med. J.*, *3*, 386.

YOSHIDA, A. and MOTULSKY, A. G. (1969): A pseudocholinesterase variant (E Cynthiana) associated with elevated plasma enzyme activity. *Amer. J. hum. Genet.*, *21*, 486.

ZSIGMOND, E. K., STARKWEATHER, W. H. and DUBOFF, G. S. (1971): Genetic abnormality of muscle creatine phosphokinase in a family with malignant hyperpyrexia. *Clin. Pharmacol. Ther.*, *12, Part 1*, 305.

Robert A. O'Reilly

Departments of Medicine, Santa Clara Valley Medical Center, San Jose, Stanford University School of Medicine, Palo Alto, the University of California School of Medicine, San Francisco, and the Institute for Medical Research of Santa Clara County, San Jose, Calif., U. S. A.

Genetic factors in the response to oral anticoagulant drugs*

Variation in the response of the blood coagulation system to oral anticoagulant drugs in man is usually continuous and forms a unimodal frequency distribution. This response is probably under polygenic control. Mutation has resulted in an allele associated with marked resistance or tolerance to large doses of oral anticoagulants in two human kindreds and in four reported geographic loci of the Norwegian brown rat. The pattern of simple mendelian inheritance as an autosomal dominant characteristic and the bimodal distribution of the hypoprothrombinemic responses in the resistant kindreds and rats indicate the monogenic nature of the trait. The genetic control of the response to the oral anticoagulant drugs is reviewed herein – the polygenic factors for normal man and animal and the monogenic trait in the resistant kindreds and rats.

POLYGENIC CONTROL IN NORMAL MAN AND ANIMAL

Variations in the blood level of drug and the response of the one-stage prothrombin time after administration of a single oral dose of sodium warfarin, 0.75 mg/kg body weight, in 15 normal human subjects are shown in Tables 1, 2 and Figure 1 (O'Reilly *et al.*, 1963). The biologic half-life of the drug varied from 32 to 61 hours, The area under the curve for the hypoprothrombinemic response showed a 10-fold variation. Both of these variations formed a continuous distribution, which is indicative of polygenic control. Similar findings have been reported for bishydroxycoumarin (Dicumarol) (O'Reilly *et al.*, 1964*b*; Weiner *et al.*, 1950), ethyl biscoumacetate (Tromexan) (Brodie *et al.*, 1952), and phenindione (Schulert and Weiner, 1954). The genetic control for the metabolism of bishydroxycoumarin in man was established by finding very similar drug half-lives in the plasma of identical twins, but very different half-lives in the plasma of fraternal twins (Vesell and Page, 1968).

During the studies that led to the discovery of bishydroxycoumarin in 1939, marked variation was found in the hypoprothrombinemic response of rabbits to a standard dose of the anticoagulant (Smith, 1938; Campbell *et al.*, 1941). The nonresponsive or resistant rabbits were believed to have inherited a recessive monogenic trait (Overman *et al.*, 1942), but the genetic data were subsequently considered inadequate and never published (Link, 1943-44). In another study of 700 rabbits (Fig. 2) the sensitive and resistant responses to bishydroxycoumarin represented distinct populations at the extremes of a unimodal distribution for the hypoprothrombinemia, indicating polygenic control (Millar *et al.*, 1964). As shown in Figure 2 the

* Supported in part by U.S.P.H.S. Grant HE8058-09.

TABLE 1 *Plasma warfarin concentrations in 15 normal subjects after a single loading dose**

Subject	Age (yr)	Sex	Total dose (mg)	Plasma warfarin concentration in mg/l of plasma after					
				24 hr	36 hr	48 hr	72 hr	96 hr	120 hr
N-1	50	M	50	1.2	0.8	0.5	0.3	0.0	0.0
N-2	31	M	50	3.9	3.2	2.3	2.0	1.1	0.8
N-3	40	M	60	4.9	4.5	4.2	3.1	3.0	2.0
N-4	46	F	40	5.9	4.6	3.6	2.5	1.9	0.8
N-18	31	M	55	3.9	2.6	2.1	1.5	1.0	0.5
N-19	22	M	50	4.6	4.0	3.4	2.2	1.9	1.2
N-20	22	M	45	4.3	3.6	3.2	2.2	2.0	1.5
N-22	22	M	43	5.1	4.1	3.4	2.9	2.0	1.6
N-25	23	M	55	4.7	3.5	3.2	2.1	1.3	0.9
N-28	22	M	62.5	7.8	5.2	3.9	2.6	1.9	1.0
N-29	22	M	77.0	4.6	3.8	3.1	2.1	1.5	1.1
N-30	23	M	52.5	5.6	4.8	4.2	3.0	2.0	1.5
N-31	24	M	62.5	5.4	4.2	3.3	2.5	1.7	0.9
N-39	26	M	62.5	4.3	3.2	2.5	1.6	0.8	0.3
N-40	22	M	64.0	3.7	3.0	2.5	1.7	0.7	0.4
Mean				4.7	3.7	3.0	2.2	1.5	1.0
SD				±1.5	±1.1	±0.9	±0.8	±0.7	±0.4

* 0.75 mg/kg of body weight.

TABLE 2 *One-stage prothrombin response in 15 normal subjects after a single loading dose of warfarin**

Subject	One-stage prothrombin activity in % of normal after						
	0 hr	24 hr	36 hr	48 hr	72 hr	96 hr	120 hr
N-1	100	59	54	50	60	72	84
N-2	100	37	25	21	38	47	59
N-3	100	38	26	17	16	16	19
N-7	100	63	48	34	27	34	40
N-19	100	36	30	26	22	31	35
N-20	100	41	37	36	47	47	60
N-22	100	45	40	37	50	58	62
N-25	95	43	31	30	49	49	53
N-28	100	52	42	36	45	54	80
N-29	100	34	27	25	42	55	74
N-30	100	48	42	38	48	64	74
N-31	100	52	42	35	44	60	55
N-39	100	30	31	36	57	70	79
N-40	100	52	36	28	30	60	80
Mean	100	45	36	32	41	52	62
SD	±<1	±9	±8	±8	±13	±15	±19

* 0.75 mg/kg of body weight.

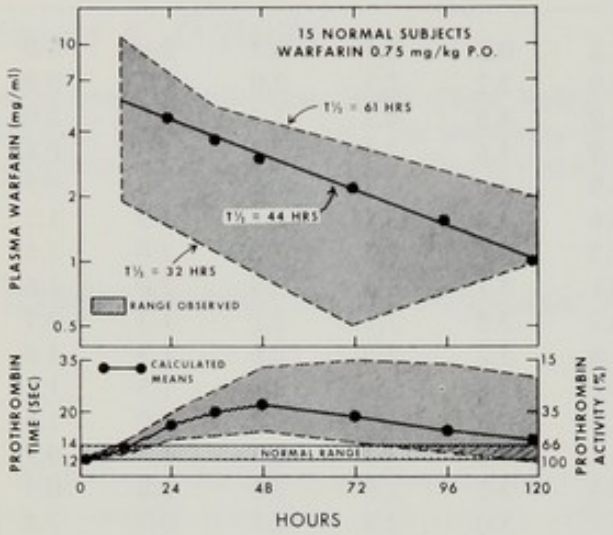


Fig. 1

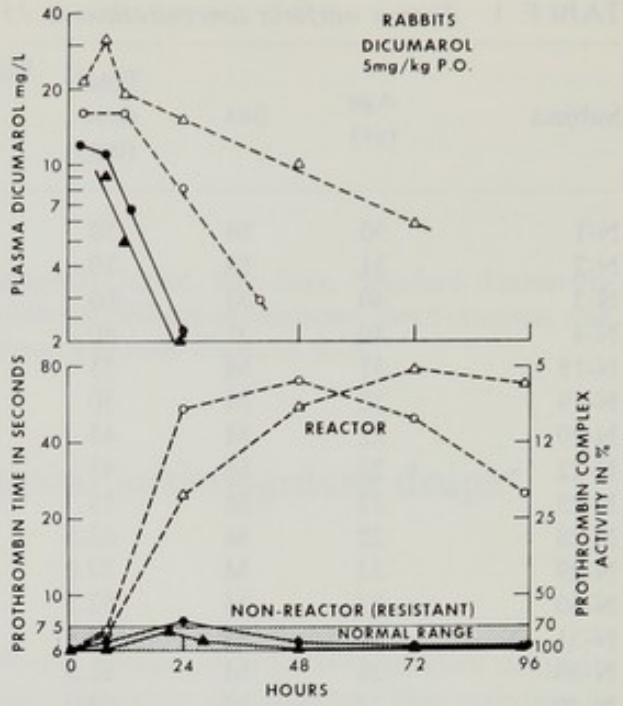


Fig. 2

Fig. 1. Means and ranges of plasma warfarin concentrations and one-stage prothrombin times after a single oral dose of sodium warfarin, 0.75 mg/kg body weight, in 15 normal subjects.

Fig. 2. Plasma drug concentrations and one-stage prothrombin times after a single oral dose of bishydroxycoumarin (Dicumarol), 5 mg/kg body weight, in 4 normal rabbits. (From O'Reilly *et al.*, 1968.)

resistant rabbits after administration of bishydroxycoumarin orally had far lower plasma levels of drug than responsive rabbits because of faster biotransformation of the drug (Jaques *et al.*, 1957).

In normal rats the existence of genetic control for the disposition of warfarin was demonstrated by selectively inbreeding two lines of animals from a random bred colony of Sprague-Dawley rats in which the response of clotting factor VII (proconvertin) was used for selection (Pyörälä and Nevanlinna, 1968). One line, characterized by a rapid metabolism of warfarin (5 hours), showed little hypoproconvertinemic response, while the other line, characterized by a slow metabolism of drug (30 hours), showed a marked response.

MONOGENIC TRAIT FOR HEREDITARY RESISTANCE IN RATS

Hereditary resistance to the oral anticoagulant drugs occurs in man and rat. In 1960 wild rats (*Rattus norvegicus*) trapped in western Scotland survived the rodenticidal effect of warfarin (Boyle, 1960). Forced ingestion of warfarin in the laboratory chow of these rats verified the tolerance (Cuthbert, 1963). Additional populations of resistant rats were found subsequently in Denmark (Lund, 1964), Wales (Drummond, 1966), and The Netherlands (Ophof and Laneveld, 1968). In 1966 a monogenic basis for the resistance as an autosomal dominant trait was found for the rats from Scotland (Evans and Sheppard, 1966). The resistant rats in Denmark were found to be less viable; on removal of the selective pressure imposed by the anticoagulant rodenticide, the proportion of resistant mutants decreased substantially in 4 years (Lund, 1967). Two research groups successfully bred the genetic factor for the resistance of the rats from Wales into laboratory strains of rats by 6 generations of

back-crossing (Greaves and Ayres, 1967; Pool *et al.*, 1968). One group reported linkage of the murine gene for anticoagulant resistance and coat color (Greaves and Ayres, 1969), but this finding could not be confirmed by others (Pool, 1969). There are no reports on any hereditary condition associated with an *increased* responsiveness to anticoagulant drugs.

MONOGENIC TRAIT FOR HEREDITARY RESISTANCE IN MAN

INCREASED RATE OF ELIMINATION OF THE ANTICOAGULANT

The mechanisms for pharmacogenetic abnormalities can be divided into two categories. One is a genetic polymorphism for drug metabolism (pharmacokinetic), as occurs with succinylcholine chloride and isoniazid (Evans, 1963; Kalow, 1962). The other is a genetic polymorphism for the response to the drug (pharmacodynamic), as occurs with primaquine sensitivity and perhaps the heritable resistance to oral anticoagulants. A possible example of the first mechanism has been reported for oral anticoagulants in 2 patients with the nephrotic syndrome (Lewis *et al.*, 1967; Rapaport, 1969). Marked hypoalbuminemia was present and the plasma half-life for warfarin was very rapid without any urinary excretion of unchanged drug (Lewis *et al.*, 1967). In these patients, in whom no family studies could be performed, the rapid half-life of the anticoagulant could result from a genetic polymorphism for drug metabolism or could be associated with the nephrotic syndrome.

CHANGES IN SENSITIVITY OF THE ANTICOAGULANT RECEPTOR

Propositus of kindred M. In 1961 a 73-year-old man of English ancestry was referred to the late Dr. Paul Aggeler and me because of a lack of response to repeated large doses of sodium warfarin (O'Reilly *et al.*, 1964*a*). His clinical history is shown in Figure 3. He had recently sustained a heart attack for which the anticoagulant drug had been prescribed. We found that a daily dose of 145 mg of warfarin was required to maintain the hypoprothrombinemic response at therapeutic levels. In a series of 105 patients receiving long-term anticoagulant therapy, the mean dose of warfarin was 6.8 ± 2.8 mg/day. The propositus therefore required a daily dose that was an incredible 49 standard deviations above the mean for other patients.

Propositus of kindred McC. In 1967 a 41-year-old man of Scottish ancestry was referred to me because of the lack of hypoprothrombinemic response to 50 mg/day of sodium warfarin (O'Reilly, 1970). The anticoagulant had been prescribed for a recurrent attack of pulmonary embolism (Fig. 4). This patient required a daily dose of 75–80 mg of warfarin, about 25 standard deviations above the mean dose for the average patient. The data for the 1.5 mg/kg of body weight dose orally and intravenously of sodium warfarin are seen in Figure 5. The warfarin levels and prothrombin responses for both routes were nearly identical. The blood level of warfarin for the propositus was within the range for the 45 normal subjects given the same dose, but the hypoprothrombinemic response was almost nil. A single dose of 600 mg warfarin resulted in a 'normal' hypoprothrombinemic response only for the first 36 hours.

The dose-response relation for warfarin in the propositi of kindreds M and McC was compared to that in normal subjects (Fig. 6). The maximum reduction of the activity of the prothrombin complex achieved with varying amounts of sodium warfarin was plotted linearly as a function of the dose plotted logarithmically. The slopes of the 3 regression lines for the data showed no significant difference. The rightward position of the lines for the propositi meant that a much larger dose of anticoagulant was required to achieve a given prothrombin complex response. The propositi were equally resistant to another coumarin anticoagulant,

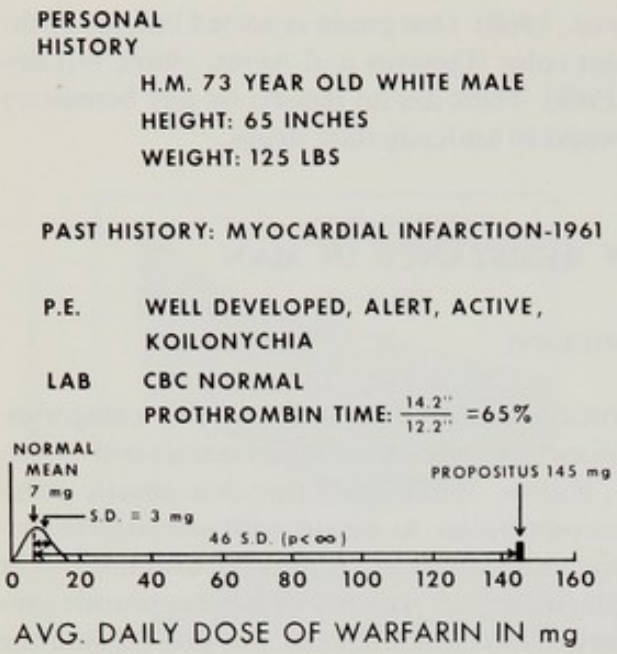


Fig. 3. Clinical summary and graph of chronic daily dose of warfarin for propositus of kindred M and for 105 patients on long-term therapy.

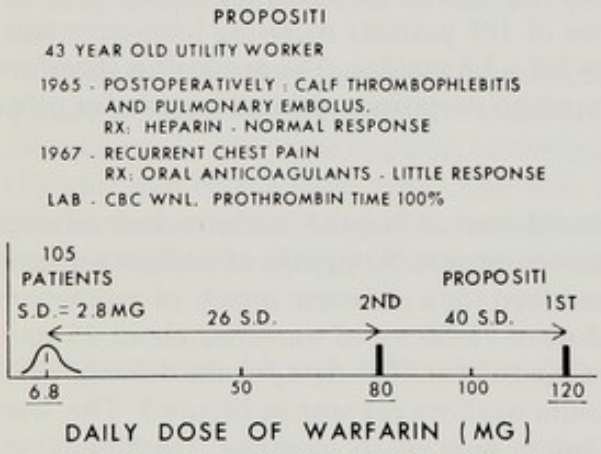


Fig. 4

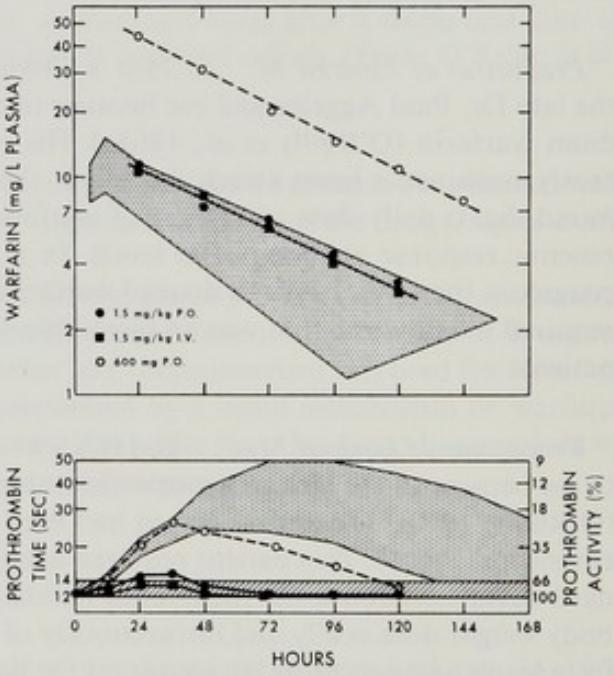


Fig. 5

Fig. 4. Clinical summary and graph of chronic daily dose of warfarin for propositus of kindred McC and for 105 patients on long-term therapy.

Fig. 5. Plasma warfarin levels and one-stage prothrombin time after single doses of sodium warfarin, 1.5 mg/kg body weight orally and intravenously and 600 mg orally, in the propositus of kindred McC. The shaded area represents the data obtained in 45 normal subjects after a single oral dose of sodium warfarin, 1.5 mg/kg body weight, and the hatched area represents the normal range of the prothrombin time. (From O'Reilly, 1970.)

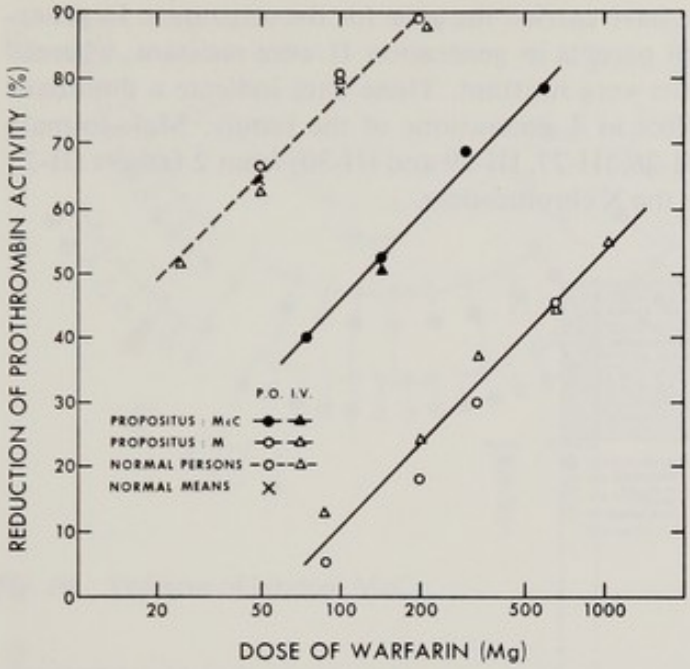


Fig. 6. Comparison of the dose-response relationship in normal subjects and the propositi of kindreds M and McC. The response is expressed on a linear scale as the maximum reduction of one-stage prothrombin activity and is plotted as a function of a single oral dose of sodium warfarin on a logarithmic scale. Regression lines were fitted to the data by the method of least squares.

bishydroxycoumarin (Dicumarol), as well as to the indanedione type of oral anticoagulant, phenindione.

Members of kindreds M and McC. No member of either kindred at the time of study had been treated with any medication containing a hypnotic, a sedative, or vitamin K for the preceding 2.5 months. A comparison of the concentration of warfarin and the one-stage prothrombin activity (method of Quick) of plasma samples determined 48 hours after a single dose of 1.5 mg/kg body weight sodium warfarin, in members of the McC family and in the 45 normal subjects (Fig. 7), is shown here. On the abscissa are the blood levels of warfarin at 48 hours with the 99% confidence limits for normal subjects shown by the dotted lines, and on the ordinate the prothrombin activity at 0 hours (the circles) and 48 hours (the dots) after drug administration. The stippled area represents the 95% confidence limits for the response in the 45 normal subjects. Nineteen of the kindred members showed little or no prothrombin response, but their blood levels of warfarin were within the range of the normal subjects. Figure 8 shows a frequency distribution for the reduction of prothrombin activity from 0 to 48 hours for *both* kindreds after a single oral dose of 1.5 mg/kg body weight of sodium warfarin. The dotted lines represent the 99% confidence limits for 45 normal subjects given the same dose; the mean was 82% and the standard deviation was 4%. The 34 members of both kindreds with a normal response are indicated by the cross-hatched bars and the 25 members with a resistant response are indicated by the dark bars. The means of the two groups are 18 standard deviations apart. The results indicate a clear-cut bimodality for the response, which allowed an unambiguous assessment of these findings as genetic data.

Figure 9 shows the pedigree of kindred M. Resistant members occurred in all 3 generations, the majority of the members tested were found to be resistant, and the resistance occurred in and was transmitted by both males and females. The pedigree of kindred McC is shown in Figure 10. Since the mother (I-3) of the propositus (II-19) was normal, the deceased father (I-2) and his living blood relatives could have possessed the gene for resistance. Two of 3 children tested and 4 of 7 grandchildren of member I-5 showed a resistant response. This

finding indicates that both I-2 and I-5 must have carried the gene for the resistance. In generation III, 10 of the 19 children of resistant parents in generation II were resistant, whereas none of the 11 children of 3 normal parents were resistant. These data indicate a dominant character transmitted as a single gene effect in 4 generations of the family. Male-to-male transmission of the resistance to 4 sons (III-26, III-27, III-29 and III-30) from 2 fathers (II-22 and II-23) ruled out a locus for the gene on the X chromosome.

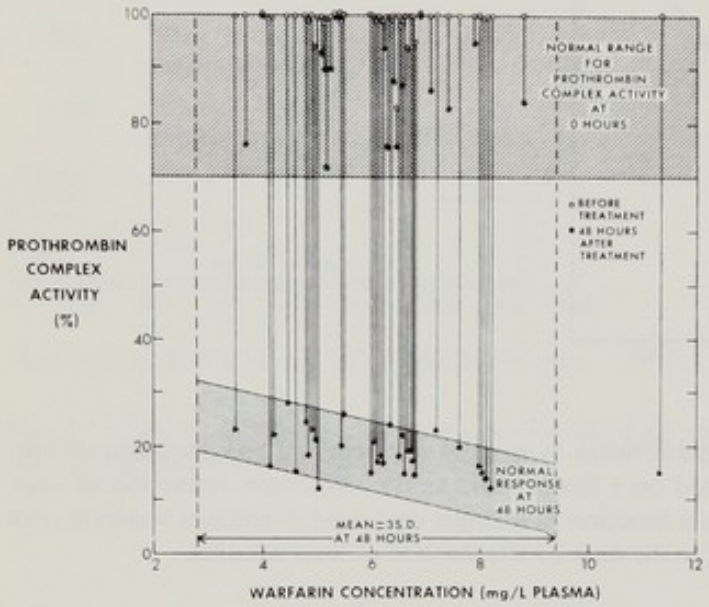


Fig. 7. Plasma warfarin concentrations 48 hours after, and one-stage prothrombin activity before and 48 hours after, a single dose of sodium warfarin in 51 members of kindred McC. Not shown is member IV-4 whose plasma warfarin level was 4.4 mg/l and one-stage prothrombin activity declined from 100 to 95% in 48 hours. The hatched area represents the normal range for the one-stage prothrombin activity and the shaded area represents the 95% confidence limits for the normal response at 48 hours. The broken vertical lines indicate the 99% confidence limits for the warfarin levels at 48 hours in the 45 normal subjects.

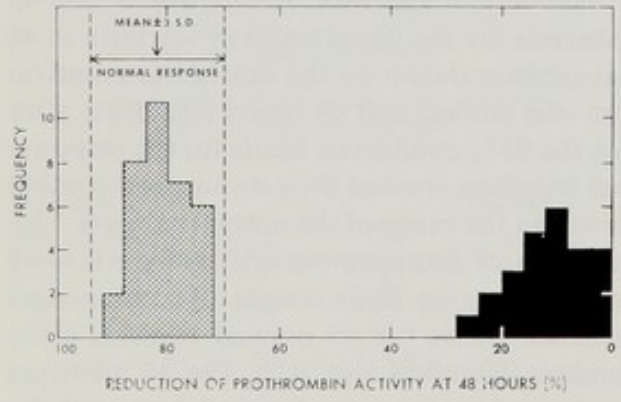


Fig. 8

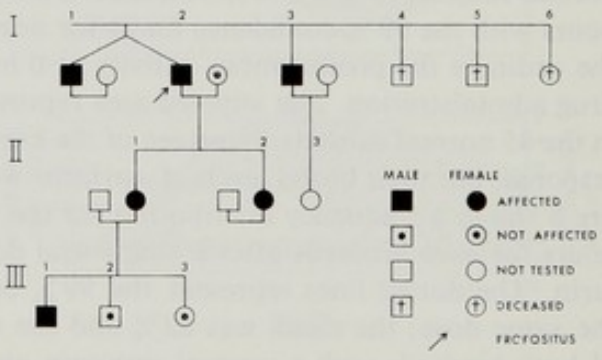


Fig. 9

Fig. 8. Reduction of one-stage prothrombin activity from 0 to 48 hours after a single oral dose of sodium warfarin, 1.5 mg/kg body weight, for the 59 members of kindred M and McC. The dotted lines represent the 99% confidence limits (mean ± one standard deviation equals 82 ± 4% for the 0 to 48 hours' response in 45 normal subjects given in the same single dose. The 34 members of both kindreds with a normal response are indicated by the cross-hatched bars and the 25 members with a resistant response are indicated by the dark bars. The means of these two groups (82 and 10%, respectively) are 18 standard deviations apart.

Fig. 9. Pedigree of kindred M.

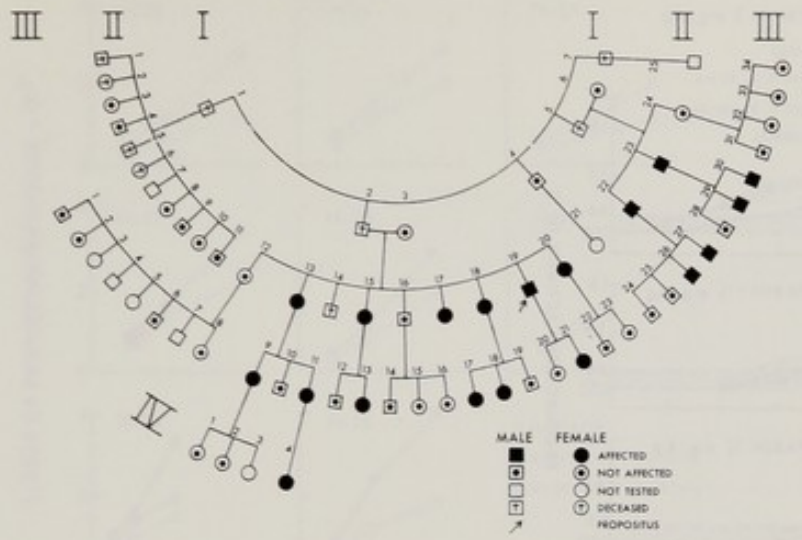


Fig. 10. Pedigree of kindred McC.

MECHANISM FOR THE RESISTANCE

As many pharmacologic mechanisms can be responsible for resistance to a drug, a patient and his family must be studied in detail (O'Reilly, 1966). Some of the possible mechanisms of resistance to oral anticoagulant drugs are the following:

1. decreased gastrointestinal absorption of the drug;
2. increased metabolic transformation, excretion, or volume of distribution of the drug;
3. increased production of clotting factors dependent on vitamin K;
4. prolonged biologic half-life of clotting factors dependent on vitamin K;
5. presence of an alternate pathway for production of clotting factors, bypassing vitamin K;
6. increased activity or altered metabolism of vitamin K; and
7. presence of an enzyme or receptor site with altered affinity or permeability for vitamin K or anticoagulant drug.

Most of these possibilities have been ruled out in the M and McC kindreds (O'Reilly *et al.*, 1968). The first two mechanisms of altered metabolism of the anticoagulant drug were ruled out by data just presented. The third and fourth mechanisms were ruled out by finding normal or decreased activities and normal biologic half-lives of the vitamin K-dependent clotting factors (O'Reilly and Aggeler, 1965; O'Reilly, 1970). The fifth mechanism was ruled out by finding a relative rather than an absolute resistance to oral anticoagulant drugs (O'Reilly *et al.*, 1968). Hypervitaminosis K or altered metabolism of vitamin K was ruled out by the marked responsiveness to very small doses of the vitamin during long-term anticoagulant therapy of both propositi (O'Reilly, 1970) and by the normal whole body and subcellular distribution of vitamin K in the warfarin-resistant rat (Thierry *et al.*, 1970). If warfarin truly regulates vitamin K metabolism (Bell and Matschiner, 1970) an abnormal regulatory mechanism could result in resistance to oral anticoagulants. The final possibility of a change in the sensitivity or permeability of the receptor site is consistent with all the data. Comparison of the dose-response relation in the propositi and the normal subjects (Fig. 6) showed a parallelism that, in the absence of an excess of vitamin K, is indicative of an altered affinity or permeability of the receptor site for the anticoagulant and the vitamin (O'Reilly and Aggeler, 1965).

VITAMIN K AND ORAL ANTICOAGULANT DRUGS AS COMPETITIVE ANTAGONISTS

The theory of a genetic alteration of the receptor site for vitamin K and anticoagulant as the mechanism of hereditary resistance to oral anticoagulants requires that in the normal

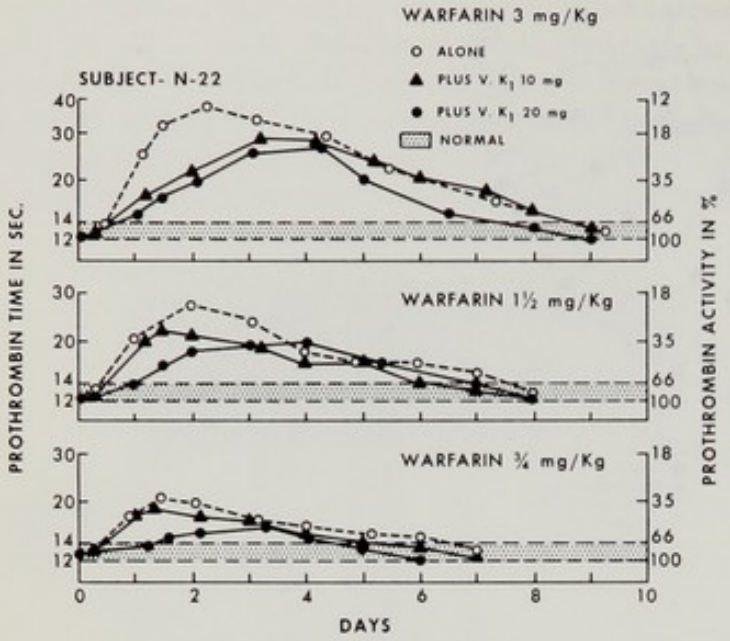


Fig. 11. One-stage prothrombin response after single oral doses of sodium warfarin and vitamin K_1 (phytonadione) in 1 normal subject.

state the two substances be competitive antagonists. Previous studies in dogs had suggested a competitive mechanism in which vitamin K participated as the prosthetic group of an enzyme system responsible for the synthesis of factors II, VII, IX, and X and the oral anticoagulant participated as a competitive inhibitor for the enzyme sites in a manner consistent with the law of mass action (Quick and Collentine, 1951; Almquist, 1952). Subsequent work in rats by two different groups did not support this theory as competitive inhibition could not be demonstrated for a range of anticoagulant doses beyond the average therapeutic levels (Babson *et al.*, 1956; Lowenthal and MacFarlane, 1964).

To determine whether or not vitamin K and oral anticoagulants act as competitive antagonists in man, 8 normal subjects were studied with varied single doses of oral sodium warfarin and vitamin K_1 administered simultaneously (Fig. 11). The response was determined by measuring the area under the prothrombin time curve plotted semilogarithmically, as described previously (Aggeler and O'Reilly, 1969). Note that the hypoprothrombinemic response is directly proportional to the warfarin dose and inversely proportional to the vitamin K_1 dose in all instances. When the data were plotted by the graphical method of Lineweaver and Burk, as described previously (O'Reilly and Aggeler, 1965), and corrected for the threshold concentration of warfarin (Kirschner and Stone, 1951), the results in Figure 12 were obtained. The findings in all 8 subjects of common intercepts and different slopes are indicative of a competitive mechanism (Chen and Russell, 1950).

VITAMIN K STATUS DURING VITAMIN K DEPLETION AND REPLETION

To assess the minimum daily need for vitamin K, 4 normal subjects and the propositus of kindred McC with hereditary resistance to oral anticoagulants were restricted for 28 days to a diet deficient in vitamin K (about 25 μg/day) and supplemented with antibiotics to suppress production of vitamin K by gastrointestinal bacteria (Fig. 13). The diet, tolerated with some difficulty, consisted of unlimited quantities only of boiled rice, egg whites, gelatin, salt, pepper, granulated sugar, coffee, tea, water, and a vitamin and mineral supplement containing no vitamin K (O'Reilly, 1971). In none of the normal subjects did the one-stage prothrombin time become abnormal, but by 13 days the propositus showed only 31% of normal prothrombin activity. These data indicate that the propositus had a greater requirement for vita-

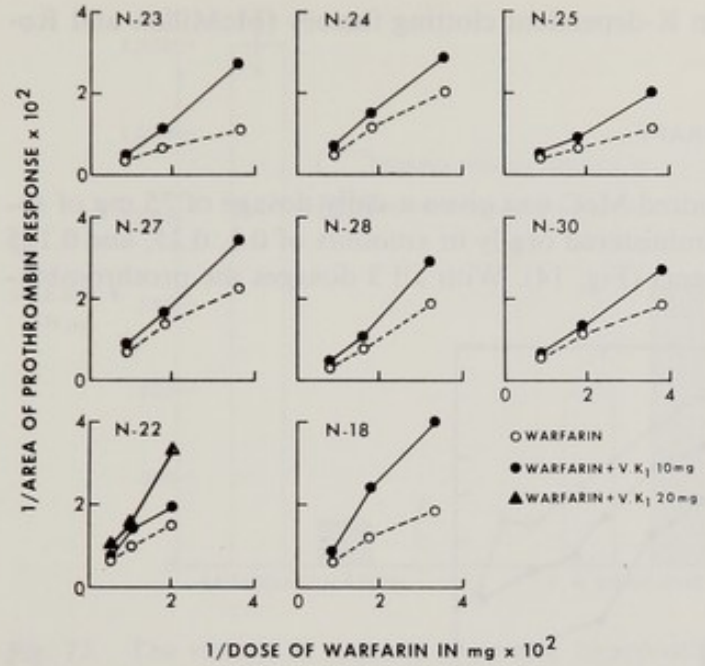


Fig. 12. Double reciprocal plot of the single oral dose of warfarin on the *abscissa* and of the area under the prothrombin response curve on the *ordinate* for 8 normal subjects.

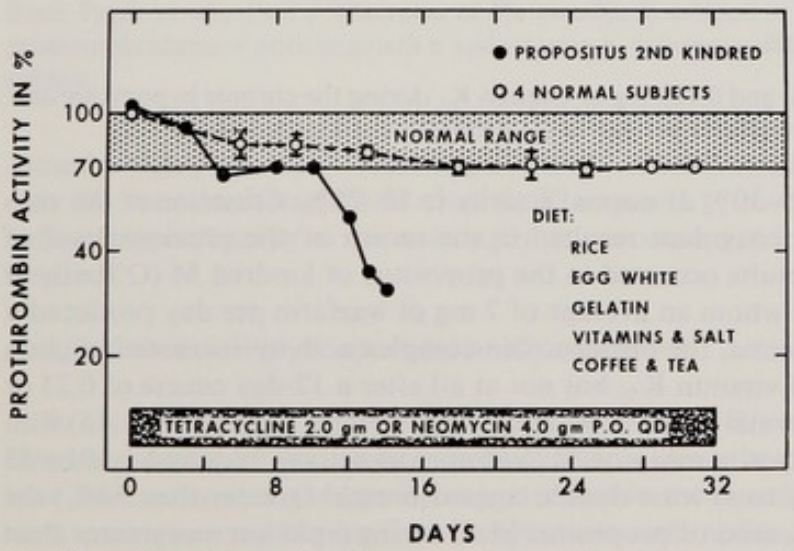


Fig. 13. Mean (± standard error of the mean) one-stage prothrombin times in per cent of normal activity for 4 normal subjects and the propositus of kindred McC after 13–30 days of a vitamin K-deficient diet and oral antibiotics. The propositus became hypoprothrombinemic on the regimen within 13 days while the normal subjects did not in 30 days.

min K than normal subjects during a period of vitamin K depletion. Similar findings have been reported previously for hereditary resistance to oral anticoagulants in rats (Hermodson *et al.*, 1969).

Graduated amounts of vitamin K_1 were administered to propositus McC with his prothrombin activity at 31% and during maintenance of the vitamin K-deficient diet and the oral antibiotic regimen. Eighty micrograms of vitamin K_1, administered intravenously, were required daily to restore the prothrombin activity of the propositus to the normal range. The only study on vitamin K deficiency in man was carried out in 7 sick patients in whom about 50 μg vitamin K_1 resulted in a similar ratio of restoration of the one-stage prothrombin activity (Frick *et al.*, 1967). The greater need for vitamin K_1 in propositus McC during repletion of the vitamin was also observed in the resistant rats (Hermodson *et al.*, 1969) and in a child

with congenital deficiency of the vitamin K-dependent clotting factors (McMillan and Roberts, 1966).

VITAMIN K DURING ANTICOAGULANT THERAPY

In another study the propositus of kindred McC was given a daily dosage of 75 mg of sodium warfarin. Vitamin K_1 was then administered orally in amounts of 0.5, 0.25, and 0.125 mg/day for 12 days in separate experiments (Fig. 14). With all 3 dosages the prothrombin-

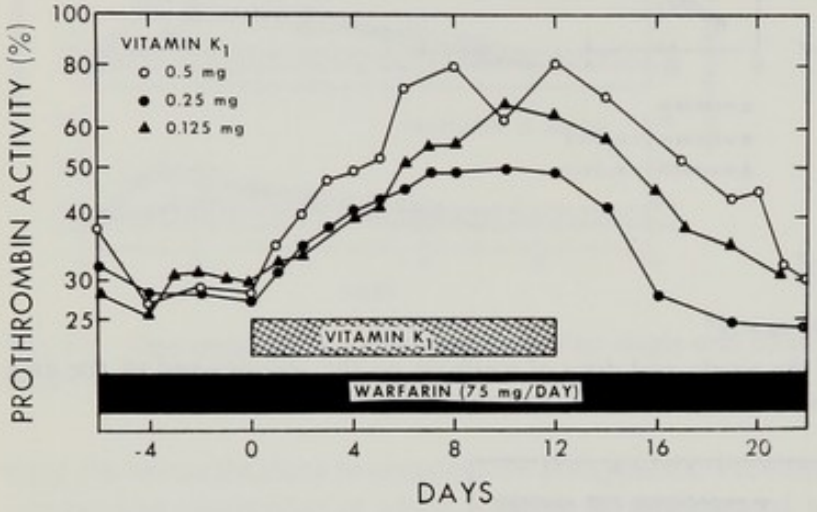


Fig. 14. Antidotal effect of 0.5, 0.25, and 0.125 mg of vitamin K_1 during the chronic hypoprothrombinemia induced by 75 mg of sodium warfarin per day in the propositus of kindred McC.

complex activity increased from 25–30% of normal activity to 50–75%. Cessation of the vitamin and continuation of the anticoagulant resulted in the return of the previous level of hypoprothrombinemia. Similar results occurred in the propositus of kindred M (O'Reilly *et al.*, 1968). In 4 normal subjects in whom an average of 7 mg of warfarin per day produced a similar level of hypoprothrombinemia, the prothrombin-complex activity increased slightly after a 12-day course of 0.5 mg of vitamin K_1, but not at all after a 12-day course of 0.25 or 0.125 mg (O'Reilly, 1970). Six normal subjects on long-term warfarin therapy (Fig. 15) with the one-stage prothrombin activity at a mean of 32% of normal activity required $1,501 \pm 48$ μg (mean $\pm$ SEM) of vitamin K_1 to at least double toward normal (greater than 64%) the activity. Thus, while the vitamin K need of propositus McC during repletion was greater than normal patients during vitamin K deficiency, it was 12 times less than normal subjects during anticoagulant therapy. Normal subjects require 30 times (1500/50) more vitamin K_1 to reverse the hypoprothrombinemia of anticoagulant therapy than of vitamin K deficiency, whereas the propositus required only 1.5 times (120/80) as much, which is said to be consistent with an allosteric mechanism for the warfarin-vitamin K interaction at the receptor site (Olson, 1970). Hereditary resistance to oral anticoagulants is possibly an inherited vitamin-dependency state, as reported for vitamins B_6 and B_{12} (Rosenberg, 1969). These genetic disturbances affect a specific reaction of the vitamin and cause an increased requirement for the vitamin.

The amount of vitamin K required to reverse the hypoprothrombinemia of normal and resistant rats during anticoagulant therapy was determined by measuring the one-stage prothrombin time with the P and P method (Pool *et al.*, 1968) 48 hours after drinking *ad libitum* various concentrations of sodium warfarin and vitamin K_1 (Fig. 16). Warfarin at a dose level of 10 parts per million (ppm) for normal rats and 250 ppm for resistant rats caused a similar decline in the P and P activity (O'Reilly, 1971). The addition of 10 ppm vitamin K_1 completely prevented any fall in the P and P activity for the resistant rats, but had no protective effect at all in the normal rats. Even 3 ppm and 1 ppm of vitamin K_1 in the resistant rats

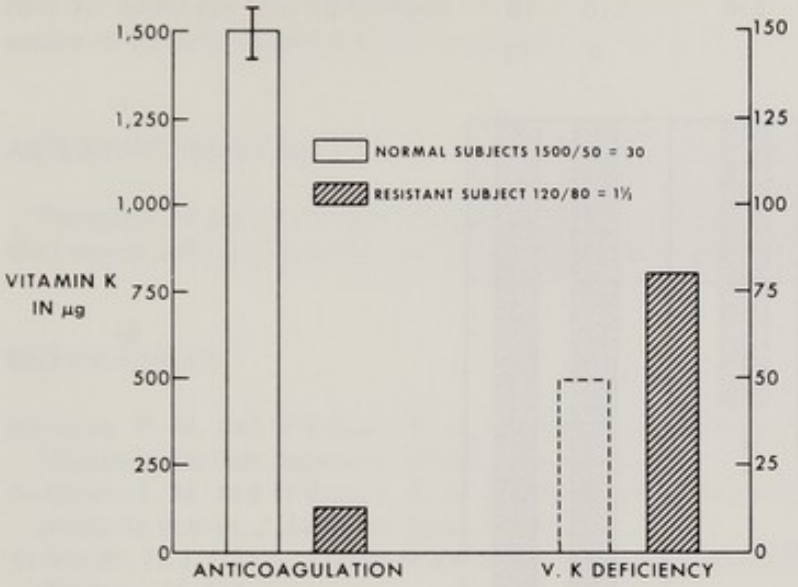


Fig. 15. The vitamin K required to reverse hypoprothrombinemia expected by long-term therapy with oral anticoagulants and by vitamin K deficiency for normal subjects and for the propositus of kindred McC. The propositus required 12.5 less vitamin K than normal subjects during anticoagulant therapy, yet required more vitamin during vitamin K deficiency. (The data for the dotted bar are from Frick *et al.*, 1967.) The ratio of the vitamin K needed in normal subjects to reverse the hypoprothrombinemia of anticoagulation and vitamin K deficiency was 30 compared with 1.5 in the resistant subject.

caused a highly significant lessening of the fall of the P and P activity. These data clearly indicate the decreased amount of vitamin K required to reverse the hypoprothrombinemia of anticoagulant therapy in the resistant rats, as occurred in the propositi of the resistant human kindreds.

POSSIBLE MECHANISMS OF AN ALTERED RECEPTOR SITE

To account for the paradox of an increased vitamin K requirement during its deficiency yet a decreased amount required to reverse anticoagulant therapy (O' Reilly, 1971), two theories have been advanced. One group suggests that in the hereditary resistance to oral anticoagulants a mutation has resulted in a receptor site with moderately decreased affinity for vitamin K and markedly decreased affinity for anticoagulant drug (Thierry *et al.*, 1970). The other group explains the data by a cooperative interaction between two sites in the altered receptor protein (Olson, 1970). In the absence of anticoagulant the vitamin K binding site shows a reduced affinity and hence an increased requirement for vitamin K, whereas in the presence of warfarin, a cooperative interaction between the binding sites for anticoagulant and vitamin could result in an increased affinity and hence a decreased requirement for vitamin K. These theories can be studied by isolating the hepatic receptor sites for the vitamin and anticoagulant and performing binding studies in normal and resistant rats. The continued study of these pharmacogenetic variants in man and rat may clarify the normal mechanisms of action of vitamin K and the oral anticoagulant drugs (Editorial, 1969).

IMPLICATIONS FOR EVOLUTION

This hereditary resistance of man and rat has heuristic value for evolution and natural selection. The resistant rats have become the predominant strain in several areas where warfarin has been used as a rodenticide (Drummond, 1966). The warfarin in the environment acted as

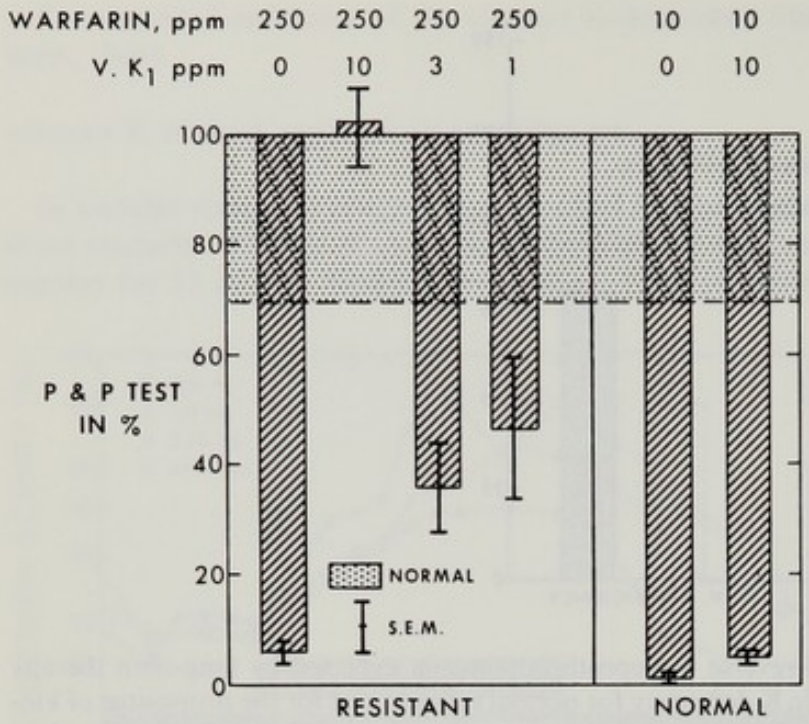


Fig. 16. Mean (± standard error of the mean) hypoprothrombinemic responses from 0–48 hours, measured by the P and P test, in normal and resistant rats after continuous oral ingestion of various concentrations of sodium warfarin and vitamin K. The stippled area indicates the normal range of the P and P test in per cent of normal activity.

an agent of natural selection that favored the survival of resistant rats. It has not been ascertained whether this genetic adaptation was present in some of the original wild rats before contact with warfarin or the hereditary alteration was a consequence of this contact (Waddington, 1957). In the resistant human kindreds all of the members except the propositi had no previous contact with oral anticoagulant drugs. Therefore, resistance in man to warfarin, like that in bacteria to streptomycin (Cavalli-Sforza and Lederberg, 1956), is a preadaptation founded on the selection of pre-existing mutants rather than on a mutation induced by the adaptive conditions (Moody, 1970). The selection of the warfarin-resistant phenotypes in rats led to a marked increase in the gene frequency for the resistance because this rare gene was at a marked advantage in this particular environment. The genetic variability of the organisms allowed this positive selection of a random mutation for resistance to warfarin and this adaptation to the new environment. Some view this relationship between the organism and its environment as a cybernetic loop in which the self-regulating system is carried beyond the individual organism to encompass the complex of environment, phenotype, and genetic pool (Piaget, 1970). Genotypic variations as a response to problems set by the environment give evolution a dialectical thrust.

SUMMARY

The response to oral anticoagulant drugs in man and animal is under genetic control. This normal control of the blood coagulation system is polygenic. Mutation has resulted in an allele associated with marked resistance or tolerance to large doses of oral anticoagulants in man and rat. Because the resistant kindreds and rats show a pattern of simple mendelian inheritance for the autosomal dominant characteristic and a bimodal distribution for the hypoprothrombinemic responses, the trait for the resistance is monogenic. This pharmacogenetic trait can be characterized as a mutation of the receptor site for anticoagulant drugs and vita-

min K. Through this experiment of nature wrought by God in man and animal the mode of action of vitamin K and the oral anticoagulant drugs may become rational to mankind.

ACKNOWLEDGEMENTS

The author is grateful to Mrs. Catherine Motley, Miss Susan Nutter, and Mrs. Marjorie Kline for their expert technical, editorial, and typographical assistance, respectively.

REFERENCES

AGGELER, P. M. and O'REILLY, R. A. (1966): Pharmacological basis of oral anticoagulant therapy. *Thrombos. Diathes. haemorrh. (Stuttg.), Suppl. 21*, 227.

AGGELER, P. M. and O'REILLY, R. A. (1969): Effect of heptabarbital on the response to bishydroxycoumarin in man. *J. Lab. clin. Med., 74*, 229.

ALMQUIST, H. J. (1952): Letters to the editor: relation of prothrombin level to vitamin K intake. *Arch. Biochem., 35*, 463.

BABSON, A. L., MALAMENT, S., MANGUN, G. H. and PHILLIPS, G. E. (1956): Effect of simultaneous administration of vitamin K_1 and dicumarol on the prothrombin in rat plasma. *Clin. Chem., 2*, 243.

BELL, R. G. and MATSCHINER, J.T. (1970): Vitamin K activity of phylloquinone oxide. *Arch. Biochem., 141*, 473.

BOYLE, C. M. (1960): Case of apparent resistance of *Rattus norvegicus* Berkenhout to anticoagulant poisons. *Nature (Lond.), 188*, 517.

BRODIE, B. B., WEINER, M., BURNS, J. J., SIMSON, G. and YALE, E. K. (1952): Physiological disposition of ethylbiscoumacetate (Tromexan) in man and a method for its estimation in biological material. *J. Pharmacol. exp. Ther., 106*, 453.

CAMPBELL, H. A., SMITH, W. K., ROBERTS, W. L. and LINK, K. P. (1941): Studies on the hemorrhagic sweet clover disease. II. Bioassay of hemorrhagic concentrates by following the prothrombin level in the plasma of rabbit blood. *J. biol. Chem., 138*, 1.

CAVALLI-SFORZA, L. L. and LEDERBERG, J. (1956): Isolation of pre-adaptive mutants in bacteria by sib selection. *Genetics, 41*, 367.

CHEN, G. and RUSSELL, D. (1950): Quantitative study of blood pressure response to cardiovascular drugs and their antagonists. *J. Pharmacol. exp. Ther., 99*, 401.

CUTHBERT, J. H. (1963): Further evidence of resistance to warfarin in the rat. *Nature (Lond.), 198*, 807.

DRUMMOND, D. (1966): Rats resistant to warfarin. *New Sci., 30*, 771.

EDITORIAL (1969): Pharmacogenetics. *Calif. Med., 111*, 312.

EVANS, D. A. P. (1963): Pharmacogenetics. *Amer. J. Med., 34*, 639.

EVANS, D. A. P. and SHEPPARD, P. M. (1966): Some preliminary data on the genetics of resistance to anticoagulants in the Norway rat. *W. H. O. Seminar on Rodents and Rodent Ectoparasites. Vector Control, 66*, 144.

FRICK, P. G., RIEDLER, G. and BROGLI, H. (1967): Dose response and minimal daily requirement for vitamin K in man. *J. appl. Physiol., 23*, 387.

GREAVES, J. H. and AYRES, P. (1967): Heritable resistance to warfarin in rats. *Nature (Lond.), 215*, 877.

GREAVES, J. H. and AYRES, P. (1969): Linkages between the genes for coat colour and resistance to warfarin in *Rattus norvegicus*. *Nature (Lond.), 224*, 284.

HERMODSON, M. A., SUTTIE, J. W. and LINK, K. P. (1969): Warfarin metabolism and vitamin K requirement in the warfarin-resistant rat. *Amer. J. Physiol., 217*, 1316.

JAQUES, L. B., FROESE, E. P., O'TOOLE, R. and SPINKS, J. W. T. (1957): Relation between duration of hypoprothrombinemia with dicumarol and the level of the drug in the liver. *Arch. int. Pharmacodyn., 111*, 478.

KALOW, W. (1962): *Pharmacogenetics: Heredity and the Response to Drugs*, p. 231. W. B. Saunders Co., Philadelphia, Pa.

KIRSCHNER, L. B. and STONE, W. E. (1951): Action of inhibitors at the myoneural junction. *J. gen. Physiol., 34*, 821.

LEWIS, R. J., SPIVACK, M. and SPAET, T. H. (1967): Warfarin resistance. *Amer. J. Med., 42*, 620.

LINK, K. P. (1943-44): Anticoagulant from the spoiled sweet clover hay. *Harvey Lect., 34*, 162.

LOWENTHAL, J. and MACFARLANE, J. A. (1964): Nature of the antagonism between vitamin K and indirect anticoagulants. *J. Pharmacol. exp. Ther.*, *143*, 273.

LUND, M. (1964): Resistance to warfarin in the common rat. *Nature (Lond.)*, *203*, 778.

LUND, M. (1967): Resistance of rodents to rodenticides. *Wld Rev. Pest Control*, *6*, 131.

MCMILLAN, C. W. and ROBERTS, H. R. (1966): Congenital combined deficiency of coagulation factors II, VII, IX, and X. Report of a case. *New Engl. J. Med.*, *274*, 1313.

MILLAR, G. J., JAQUES, L. B. and HENRIET, M. (1964): Prothrombin time response of rabbits to dicumarol. *Arch. int. Pharmacodyn.*, *150*, 197.

MOODY, P. A. (1970): *Introduction to Evolution, 3rd ed.*, p. 416. Harper and Row, New York, N.Y.

OLSON, R. E. (1970): Mode of action of vitamin K. *Nutr. Rev.*, *28*, 171.

OPHOF, A. J. and LANEVELD, D.W. (1968): Warfarin resistance in The Netherlands. *WHO Vector Biol. Control*, *68/109*, 1 (unpublished document).

O'REILLY, R. A. (1966): Discussion: resistance to coumarin anticoagulant drugs in man. *Thrombos. Diathes. haemorrh. (Stuttg.)*, *Suppl. 21*, 280.

O'REILLY, R. A. (1970): Second reported kindred with hereditary resistance to oral anticoagulant drugs. *New Engl. J. Med.*, *282*, 1448.

O'REILLY, R. A. (1971): Vitamin K in hereditary resistance to oral anticoagulant drugs. *Amer. J. Physiol.*, *221*, 1327.

O'REILLY, R. A. and AGGELER, P. M. (1965): Coumarin anticoagulant drugs: hereditary resistance in man. *Fed. Proc.*, *24*, 1266.

O'REILLY, R. A., AGGELER, P. M., HOAG, M. S., LEONG, L. S. and KROPATKIN, M. (1964*a*): Hereditary transmission of exceptional resistance to coumarin anticoagulant drugs: first reported kindred. *New Engl. J. Med.*, *271*, 809.

O'REILLY, R. A., AGGELER, P. M. and LEONG, L. S. (1963): Studies on the coumarin anticoagulant drugs: pharmacodynamics of warfarin in man. *J. clin. Invest.*, *42*, 1542.

O'REILLY, R. A., AGGELER, P. M. and LEONG, L. S. (1964*b*): Studies on the coumarin anticoagulant drugs: comparison of the pharmacodynamics of Dicumarol and warfarin in man. *Thrombos. Diathes. haemorrh. (Stuttg.)*, *11*, 1.

O'REILLY, R.A., POOL, J.G. and AGGELER, P.M. (1968): Hereditary resistance to coumarin anticoagulant drugs, in man and rat. *Ann. N. Y. Acad. Sci.*, *151*, 913.

OVERMAN, R. S., STAHMANN, M. A., SULLIVAN, W. R., HEUBNER, C. F., CAMPBELL, H. A. and LINK, K. P. (1942): Studies on the hemorrhagic sweet clover disease. VII. The effect of 3,3′-methylenebis-(4-hydroxycoumarin) on the prothrombin time of the plasma of various animals. *J. biol. Chem.*, *142*, 941.

PIAGET, J. (1970): *Structuralism*, p. 50. Translated and edited by C. Maschler. Basic Books, Inc., New York, N.Y.

POOL, J. G. (1969): Personal communication.

POOL, J. G., O'REILLY, R. A., SCHNEIDERMAN, L. J. and ALEXANDER, M. (1968): Warfarin resistance in the rat. *Amer. J. Physiol.*, *215*, 627.

PYÖRÄLÄ, K. and NEVANLINNA, H. R. (1968): Effect of selective and non-selective inbreeding on the rate of warfarin metabolism in the rat. *Ann. Med. exp. Biol. Fenn.*, *46*, 35.

QUICK, A. J. and COLLENTINE, G. E. (1951): Role of vitamin K in the synthesis of prothrombin. *Amer. J. Physiol.*, *164*, 716.

RAPAPORT, S.I. (1969): Unpublished observations.

ROSENBERG, L. E. (1969): Inherited aminoacidopathies demonstrating vitamin dependency. *New Engl. J. Med.*, *281*, 145.

SCHULERT, A. R. and WEINER, M. (1954): Physiological disposition of phenylindanedione in man. *J. Pharmacol. exp. Ther.*, *110*, 451.

SMITH, W. K. (1938): Failure of alfalfa to prevent the hemorrhaghic sweet clover disease. *Science*, *87*, 419.

THIERRY, M. J., HERMODSON, M. A. and SUTTIE, J. W. (1970): Vitamin K and warfarin distribution and metabolism in the warfarin-resistant rat. *Amer. J. Physiol.*, *219*, 854.

VESELL, E. S. and PAGE, J. G. (1968): Genetic control of Dicumarol levels in man. *J. clin. Invest.*, *47*, 2657.

WADDINGTON, C. H. (1957): *The Strategy of the Genes*, p. 186. The Macmillan Co., New York, N.Y.

WEINER, M., SHAPIRO, S., AXELROD, J., COOPER, J. R. and BRODIE, B. B. (1950): Physiological disposition of Dicumarol in man. *J. Pharmacol. exp. Ther.*, *99*, 409.

Chapter X Chromosome mapping

J. H. Renwick

London School of Hygiene and Tropical Medicine, London, United Kingdom

Man's map maps man

Recently, Dr. E. A. Murphy in Baltimore drew my attention to the need for different words to emphasise the distinction between the task of studying the physical arrangement of the gene loci or other features on a chromosome and the task of creating a conceptualised *map* of this. After some casting around I found just the word I think we were wanting. The location of hidden treasure – in this case the precious gems or genes of our priceless inheritance of great antiquity – we might call *trovating*, by extension from the *treasure-trove*, and particularly appropriate in France from whence came the old French word, trove. The physical position of a locus we might call a *trove-point* and a set of such points would constitute the *trove* for a particular chromosome. The distance of a *trove-point* from a fixed reference point, say the centromere, would be its *trovation*, measured in physical units of length or as a proportion of arm-length.

On the other hand, the conceptualised *map* – the orthodox genetic map of formal genetics – is measured in terms of crossover units – morgans or centimorgans, where a morgan is the length of map that corresponds to a physical chromatid segment that sports a batting average of 1 crossover event per meiosis. There are good reasons for assessing this average in the past – that is, retrospectively – but it would not be suitable to go into that here.

The relationship between the *map* and the *trove* is like that between the two sides of a concertina – where corresponding points retain the same sequence but distances between them may be expanded or contracted in different regions on the two sides. The relationship between *map-positions* and *trovations* would just involve a scaling factor if, and only if, the distribution of crossover events experienced by strands that have emerged from meiosis were uniform. In those circumstances, which are most unlikely, the crossover scale would be strictly proportional to the physical scale.

Here then is a simplified scheme of the various steps and their products. The aim of *trovative genetics*, which is well advanced in Drosophila but in few other genera, is first to *assign* gene loci to particular chromosomes at a categorising stage and *trovate* them in a later metrical stage at their respective *trove-points*. The set of such *trove-points* on a chromosome is a *trove*. The *trovation* of a locus might be expressed as the proportional distance down the appropriate arm of the chromosome or in some physical units of length.

Some of the cell-hybrid methods may soon be adaptable to give this *troval* information directly, but most of the present experimental and observational data even from hybrid cells do not allow direct trovation. For recombinational data, geneticists conceive of a *set of genetic maps* – a consistent, conceptual scheme in which distances are additive and measured in terms of crossover incidences. Information from various sources may be condensed into such a set of maps. This could become an intermediate stage en route to *trovating* and, of course, the ordering of the *map-points* on a *chromosome-map* should be exactly the same

as the ordering of the corresponding *trove-points* on the *trove* of the chromosome itself.

As an aid to thinking, it is useful to recognise also a conceptual equivalent to assignment or, more precisely, *co-assignment*. This is the disclosure of *synteny* – the presence together on the same chromosome-map of two specified map-points — and we might call the process *syntenising*. Just as synchrony is the state of being together in time; so synteny is the state of being together on the *taenia* or ribbon, the chromosome or chromosome-map. *Asynteny* is the contrary state. In the standard Bayesian analysis, posterior probability statements are derived for the categorisation statements of synteny or asynteny; and probability distributions are attached to the map-distance and, in favourable circumstances, to the map-position.

Even these abstract maps cannot be derived *directly* from the data from pedigrees since these data reflect not the proportion of crossover events but the proportion of *odd numbers* of crossover events, that is the proportion of recombinants. The mathematical function that describes this translation between recombination fraction or proportion and crossover proportion is the mapping function. (We need another translation from the *map* to the *trove* and we may refer to this as the *troving* function). We do not yet know what particular form of these functions we should be using and it may not be the same in males and females. As far as the mapping function is concerned, there are times when it does not matter except in the middle of the range, because all the plausible functions agree in giving a simple equivalence at the lower end of the range and many or most of them agree in an upper limit of 0.5 for the recombination fraction. Non-human genetics and its devotees grew up in a non-Bayesian environment of statistical inference so we have inherited from them words such as *linked*, which imply that the linkage estimate can be used by itself for categorisation of pairs of loci into unlinked and linked, depending upon whether the true recombination fraction or proportion is 0.5 or less than that. Such inferences can be made validly for pairs of loci that are close or for which there are extensive data, but this is merely because, under those circumstances, the contributions of prior considerations would be overridden whatever form they took. This is not true in general and it would seem clear that a Bayesian system of inference that can summarise the situation at any point of time, whatever the extent of the data, is preferable. In such a system we make no inferences or estimates directly from the likelihoods or their standardised logarithmic equivalents – the lods. Likelihoods are used only as factors for calculating the odds on *synteny* and for estimating *map-distances* conditional on synteny. If, as in counselling, we should require an estimate of the recombination fraction or proportion, we translate back from this estimate of map-distance.

The data for *formal genetics* come from numerous sources — the joint segregation of phenotypes, genotypes and karyotypes in pedigrees or in hybrid cells, the measurement of chromosome arms and particular features of them, chiasma counts on these arms in the two sexes and at different ages, etc. The integrated analysis procedure that uses and harmonises the findings from all these sources clearly will not be all that simple, but the Bayesian type of analysis that is now evolving seems to have the capacity for indefinite adaptability to absorb new sources and types of data and has built-in safeguards against inconsistency and absurdity.

In case any of you have forgotten Bayes Theorem – it *is* 200 years old – it can be stated simply as follows: The probability of a hypothesis in the light of a set of data is proportional to the prior probability of the hypothesis multiplied by the probability of the set of data given that hypothesis. It comes from a conditional probability statement and we owe a debt to Professor Cedric Smith for showing its great suitability in this field, over 12 years ago.

Lowell R. Weitkamp

Division of Genetics, Departments of Anatomy and Pediatrics, University of Rochester School of Medicine and Dentistry, Rochester, N.Y., U.S.A.

Human autosomal linkage groups*

Genetic linkage between two loci exists when the loci are situated on the same homologous pair of chromosomes.** Linkage may be determined without reference to the chromosome pair involved, if the loci are closely linked, or it may be inferred from chromosomal location. The assignment of loci to particular autosomes will be discussed in a subsequent paper. I wish to mention, however, the interrelation between gene assignment and gene linkage. With the advent of new staining techniques it is now possible to distinguish between all 22 pairs of autosomes (O'Riordan *et al.*, 1971; Gagné *et al.*, 1971; Sumner *et al.*, 1971) and in some instances to distinguish differences between homologues (Schnedl, 1971; Wahlström, 1971; Uchida and Lin, 1971).

The building up of linkage groups and the analysis of distant linkage relations may in future more often depend on known chromosomal location, much as linkage analysis of sex-linked characters does at present. On the other hand, many loci will undoubtedly be assigned to specific autosomes by virtue of being in known linkage groups.

PEDIGREE ANALYSIS AND SOMATIC CELL HYBRIDIZATION: COMPLEMENTARY METHODS FOR DETECTING AND ESTIMATING LINKAGE

To a first approximation, linkage may be recognized by the non-random assortment of two inherited characters, expressed as a recombination fraction less than 0.5. Classically, non-independence of segregation at cell division in man has been recognized through pedigree analysis, the first autosomal linkage being described by Mohr, in 1951. Within little more than a year ago, gene linkage relationships have been recognized following the assortment of inherited characters upon cloning interspecific somatic cell hybrids (Santachiara *et al.*, 1970; Ruddle *et al.*, 1970; Shows, 1970, 1971; Westerveld and Meera Khan, 1971). In the latter case periodic loss of human chromosomes with the concomitant loss of linked genetic loci bears a formal relationship to genetic assortment.

* Supported in part by U.S. Public Health Service Career Development Award 1-K04-HD50248 and by U.S. Public Health Service Contract NIH-71-2404.

** Although 'linkage' is commonly used in this sense (*e.g.* Neel and Schull, 1954; Harris, 1959; Giblett, 1969; Fraser-Roberts, 1970; Levitan and Montagu, 1971), it is also defined as the occurrence of two loci sufficiently close together on one chromosome pair such that assortment is recognized as being non-independent (*e.g.* Stern, 1960; McKusick, 1964; Race and Sanger, 1968; Harris, 1970). The latter is the original operational usage (Morgan, 1964). Respecting this definition, Renwick (1969) has used 'syntenic' to indicate two loci are on the same chromosome.

The two methods, having different limitations, complement each other and together extend considerably current capability for the detection of genetic linkage in man. Pedigree analysis is possible only when at least one parent is heterozygous at both loci, and in order to demonstrate linkage there must be a sufficient number of such matings. With this approach, however, any of a large number of different characters which demonstrate simple Mendelian inheritance may be analyzed for linkage with the available marker systems. The somatic cell method is limited to characters expressed in hybrid cell culture, primarily certain enzymes, but it has the advantage that the recognition of non-random assortment in the loss of human phenotypes upon cloning interspecific hybrids, such as man-mouse (Boone and Ruddle, 1969; Nabholz *et al.*, 1969) or man-Chinese hamster (Káo and Puck, 1970) does not require heterozygosity at the human loci. The technique may well be refined to the point where it is possible to predetermine the loss of a specific human autosome from a variety of interspecific hybrid cells (Pontecorvo, 1971).

TABLE 1 *Human autosomal linkage groups (listed vertically and laterally in order of discovery)*

Loci	Original reference
1. Lutheran/Secretor/myotonic dystrophy	Mohr, 1951; Mohr, 1954; Renwick *et al.*, 1971*b*
2. Rhesus/elliptocytosis/6-PGD/PGM_1	Chalmers and Lawler, 1953; Weitkamp *et al.*, 1970*a*; Westerveld and Meera Khan, 1971; Renwick, 1971
3. ABO/nail-patella syndrome/AK	Renwick and Lawler, 1955; Rapley *et al.*, 1967; Schleutermann *et al.*, 1969
4. Hemoglobin β/hemoglobin δ	Ceppellini, 1959
5. Duffy/congenital zonular cataract/Amy_2	Renwick and Lawler, 1963; Hill *et al.*, 1972
6. Transferrin/cholinesterase E_1	Robson *et al.*, 1966
7. Albumin/group-specific component	Weitkamp *et al.*, 1966
8. γG1/γG2/γG3/γA2	Natvig *et al.*, 1967; Kunkel *et al.*, 1969*b*
9. MNSs/sclerotylosis	Deminatti *et al.*, 1968
10. $HL\text{-}A_{LA}$/$HL\text{-}A_4$/PGM_3/P	Kissmeyer-Nielson *et al.*, 1969; Lamm *et al.*, 1971; Fellous *et al.*, 1971
11. LDH B/peptidase B	Santachiara *et al.*, 1970; Ruddle *et al.*, 1970
12. LDH A/esterase A_4	Shows, 1970

In Table 1, 12 autosomal linkage groups are listed in the order of their discovery. All linked pairs of loci in the first and third through ninth groups were discovered by pedigree analysis, and, as it turns out, no more than one phenotype in each of the 9 groups is known to be expressed in hybrid cell culture. Thus, none of these linkage relationships have been confirmed by the cell hybridization technique. On the other hand, the last two pairs of linked loci, producing enzymes infrequently variable in man, were discovered using man-mouse hybrids. The possibility of confirming the latter linkage relations by family studies is remote. For example, a number of populations have been surveyed for variants of LDH B (Vesell, 1965; Das *et al.*, 1970) and peptidase B (Lewis and Harris. 1967; Blake *et al.*, 1970); however, among Caucasians and Negroes, the two populations examined for both enzymes, the probability of finding a double heterozygote appears to be less than 1 in 10^6.

The second and tenth linkage groups represent examples of the combined use of cell hybri-

dization and pedigree analysis. The loci for Rh and elliptocytosis (Chalmers and Lawler, 1953) and for Rh and 6-PGD (Weitkamp *et al.*, 1971*b*) had been previously shown to be linked by pedigree analysis, but such methods had not yielded evidence suggestive of linkage between the loci for Rh and PGM_1 (Gedde-Dahl and Monn, 1967; Lamm *et al.*, 1970) or between 6-PGD and PGM_1 (Weitkamp *et al.*, 1970*b*). Following the demonstration of a correlation between loss or retention of PGM_1 and 6-PGD in clones of man-hamster hybrids (Westerveld and Meera Khan, 1971), however, analysis of further pedigrees was undertaken and has revealed loose but definite linkage between the Rh and PGM_1 loci (Renwick, 1971; Robson, 1971; see Table 2). Possible linkage of this group with the locus for peptidase C, suggested by cell hybridization (Nguyen *et al.*, 1971), remains to be explored.

TABLE 2 *Lod scores for the PGM_1/Rh linkage*

Source	Parents scored	Recombination fraction				
		0.05	0.10	0.20	0.30	0.40
Lamm *et al.*, 1970	52	−22.07	−10.81	− 2.43	−0.02	–
Renwick, 1971	51	–	− 5.37	2.29	2.95	1.41
Robson, 1971	132 ♂	−38.50	−16.08	− 0.04	3.58	2.41
	180 ♀	−72.96	−37.45	− 9.89	−1.04	0.67
This paper	34 ♂	− 8.33	− 2.56	1.26	1.70	0.83
	37 ♀	−20.87	−12.10	− 4.79	−1.84	−0.58
Total	486	–	−84.37	−13.60	5.33	4.74

In the tenth group the HL-A loci had been shown to be linked to the PGM_3 locus by pedigree methods (Lamm *et al.*, 1971). Although small positive lod scores were previously reported for the PGM_3/P relationship (Lamm *et al.*, 1970), linkage of these two loci was not suspected until the demonstration of linkage between the HL-A and P loci by cell hybridization (Fellous *et al.*, 1971). Possible linkage of the adenosine deaminase (ADA) locus with P (*cf.* Weitkamp, 1971) can now be explored by noting the assortment of ADA in relation to PGM_3 and HL-A as well as P upon cloning interspecific cell hybrids. It is quite unlikely that the loci for all of these factors are linked closely enough to measure map distances between each of the various pairs of loci by pedigree analysis.

THE PROBABILITY OF DETECTING LINKAGE

Recent acceleration in the description of linkage is apparent in Table 1; three-quarters of the linked pairs of loci have been reported in the last 5 years. It is interesting to view this progress in the light of some calculations on the practical possibilities for detecting linkage made by Mohr in 1964. Assuming relative lengths of the autosomes based on cytological measurements (Tjio and Puck, 1958) and an even distribution of genetic loci throughout the length of the chromosome, a computer stimulation was used to predict on average the distribution among the autosomes of a given number of marker systems. For example, with 14, 17 or 30 markers the average number of autosomes with more than one marker is 3, 4 or 8, respectively. The linkage between LDH B and peptidase B was observed in the context of examining 14 autosomal markers (Santachiara *et al.*, 1970; Ruddle *et al.*, 1970), at least 10 of which demonstrated good segregation in clones of man-mouse hybrid cells (Ruddle, 1970). By the time linkage between LDH A and esterase A_4 was established (Shows, 1970, 1971), the linkage relationship of approximately 16 autosomal enzyme markers had been determined (Ruddle

et al., 1971; Shows, 1971). Cell hybridization has the advantage that under certain conditions chromosome breakage occurs very infrequently and, therefore, recombination even between loosely linked loci should be minimal (Ruddle, 1970). The detection of linkage between 6-PGD and PGM_1, two loci which from pedigree data have a map distance approximating 50 centimorgans in males (*cf.* Table 3), provides empirical confirmation of this proposition. Thus from Mohr's calculated probabilities, the number of linkage relations discovered by this method should increase substantially in the near future.

Crossing over prevents such efficient detection of linkage based on pedigree analysis. The amount of information required to establish that any given value of the recombination fraction, θ, is significantly different from $\theta=0.5$ increases rapidly as θ approaches 0.5. For example, in Morton's (1955) sequential test the average number of two child double back cross matings required to establish linkage at a specified level of confidence is 67 for $\theta=0.2$, 355 for $\theta=0.3$ and 5,700 for $\theta=0.4$. Furthermore, since double crossing over is more likely to occur the greater the distance between the loci, the relative genetic distance or map interval between loci is not directly proportional to the recombination frequency. Several approximate formulae have been used to describe the relationship between map interval and recombination fraction (*cf.* Renwick, 1969), but whichever is used, the relative map distance is generally not underestimated by more than 5% for values of θ less than 0.3.

The probability that two randomly selected loci will be situated within a map distance of 30 centimorgans of each other, *i.e.* have a recombination frequency less than 0.3, depends on the total autosomal map length. Based on chiasma counts in the human male (Ford and Hamerton, 1956; McIlree *et al.*, 1966) this has been estimated at 26.5 morgans (Mohr, 1964; Renwick, 1969). Assuming a map length of 28.5 morgans, the computer simulation of Mohr (1964) indicates that the probability of a chosen locus being within 20 centimorgans of one of 14, 17, or 30 markers is 0.17, 0.20 and 0.32, respectively. Thus, the number of marker systems currently available provide roughly a 1/5 to 1/3 chance of success for the detection of linkage with a new character through pedigree data.

RECOMBINATION FREQUENCY: EFFECT OF EXTRINSIC FACTORS

In lambda phage there is a fair correspondence between the genetic map and the physical map as measured by the position of loops formed when a DNA strand containing a deletion is hybridized with a normal strand (Szybalski, 1970). However, in eukaryotes the frequency of meiotic recombination is affected by a wide variety of environmental and genetic factors (*cf.* Simchen and Stamberg, 1969 for review). Such effects vary considerably in degree and may encompass the entire genome or be limited to one or more specific chromosomal segments. Changes in the recombination frequency in response to environmental agents may vary in different regions and further may vary in these regions according to different genetic backgrounds. In fact, genetic controls of recombination frequency may be loosely linked or unlinked to the regions being regulated.

In higher organisms the general rule has been that, where there is a difference, the autosomal recombination frequency is lower in the heterogametic sex (Haldane, 1922). Dunn and Bennett (1967) have tabulated estimates of the paternal and maternal recombination frequency for 53 linkage pairs in the house mouse. Among 23 pairs for which the estimates for the two sexes were significantly different ($P<0.05$), the lower estimate was for males in 18 cases and females in 5. Measured in terms of male recombination frequency, the 5 linkage pairs for which the female estimate of θ was lower covered a distance of approximately 43 centimorgans in linkage groups VI and VII. The map distance covered by the 18 pairs for which the male estimate was lower included a different section of linkage group VII and sections of 9 other linkage groups, for a total of about 163 centimorgans. In the house mouse there appears to be a region-specific effect of sex on recombination frequency.

Maximum likelihood estimates of the maternal and paternal recombination fraction for 7

loosely linked human autosomal pairs are listed in Table 3. It should be noted that since nail-patella syndrome and AK are closely linked (Schleutermann *et al.*, 1969), the estimates for ABO/nail-patella and ABO/AK cover the same region of the chromosome. The estimates for 6-PGD/Rh and PGM_1/Rh, on the other hand, encompass contiguous regions of the chromosome (Renwick, 1971). The susceptibility ratio, s, is defined as the ratio of the female to

TABLE 3 *Ratios of female to male recombination fractions for human autosomal linkage pairs* ($s = \hat{\theta}_f/\hat{\theta}_m$)

Linkage pair	$\hat{\theta}_f$	$\hat{\theta}_m$	s	References
1. ABO/nail-patella	0.145	0.085	1.71	Renwick and Schultze, 1965; Renwick, 1968
2. Lu/Se	0.163	0.105	1.56	Cook, 1965; Renwick, 1968
3. Tf/Chol E_1	0.19	0.12	1.58	Robson *et al.*, 1966; Renwick, 1968
4. ABO/AK	0.17	0.10	1.70	Renwick, 1968; Weitkamp *et al.*, 1971*b*
5. 6-PGD/Rh	0.29	0.20	1.45	Weitkamp *et al.*, 1971*b*
6. HL-A/PGM_3	0.36	0.15	2.40	Lamm *et al.*, 1971
7. PGM_1/Rh	>0.40	0.31	–	Robson, 1971; this paper

TABLE 4 *Number of maternal and paternal recombinations for closely linked factors**

Factors	Number of recombinants**			References
	$\hat{\theta}$ (%)	Maternal	Paternal	
1. Hb β (structural) /Hb δ	close	0	0	Mishu and Nance, 1969
2. Hb β (structural) /Hbβ (thalassemia)	close	0	0	Weatherall, 1969
3. Hb β (thalassemia)/Hb δ**	? 3–4	0	1	Pearson and Moore, 1965; Weatherall, 1964
4. Fy/congenital zonular cataract	close	0	0	Renwick and Lawler, 1963
5. Rh/elliptocytosis	3	3	1	Morton, 1956; Clarke *et al.*, 1960; Geerdink *et al.*, 1967
6. Alb/Gc	2	2	2	Weitkamp *et al.*, 1966, 1969, 1970*c*
7. MN/Ss***	0.3	2	1	Gedde-Dahl *et al.*, 1967
8. γG_2, γG_3 γG_1	? 0.01–0.1	1	1	Natvig *et al.*, 1967
9. $\gamma G_2/\gamma G_3$ γG_1	close	1	0	Natvig *et al.*, 1967
10. Sclerotylosis/Mn	4	0	1	Deminatti *et al.*, 1968
11. AK/nail-patella syndrome	close	0	0	Schleutermann *et al.*, 1969
12. $\gamma G_{1,2,3}/\gamma A_2$	close	0	0	Kunkel *et al.*, 1969*b*; Van Loghem *et al.*, 1970
13. HL-A_{LA}/HL-A_4	0.8	7	6	Bodmer *et al.*, 1970; Svejgaard *et al.*, 1971; Mayr and Mickerts, 1971

* When parental sex could not be determined, the recombinant was omitted. One possible recombinant in the Rhesus system (Steinberg, 1965) was omitted because mutation seemed an equally plausible explanation.

** The family of Weatherall (1964), which included one recombinant, was omitted because one of six offspring was genetically excluded.

*** Three recombinants which were not confirmed by re-testing have been omitted.

male recombination fraction (Renwick, 1968). From Table 3 it is apparent that the trend is for recombination to be more frequent in the female. Given the small amount of information available for the PGM_3/HL-A linkage, the evidence suggests a fairly consistent effect of sex on recombination frequency among these particular linkage pairs.

A count of the number of maternal and paternal recombinants between certain closely linked factors is shown in Table 4. If the 4 recombinants between the Rh and elliptocytosis loci are omitted because these loci are known to be included within either the Rh/PGM_1 or Rh/6-PGD linkages, there is little evidence favoring an overall difference between males and females in the recombination frequency for 5 additional small sections of chromosome. The total data are not inconsistent with the conclusion drawn for the more completely studied house mouse: namely, that there may be a region-specific effect of sex on recombination, more often resulting in a higher recombination frequency in females.

The limited data available for pairs of loci in linkage groups V and XIII in the mouse suggest that with increasing age there is decreasing recombination in females and increasing recombination in males (Reid and Parsons, 1963). Furthermore, the number of chiasmata per oocyte decreases significantly with increasing maternal age, whereas in a preliminary study of male mice there was a slight increase in chiasmata with increased age (Henderson and Edwards, 1968). In their report on recombination within the HL-A system, Svejgaard *et al.* (1971) noted that 10 of the 11 recombinants occurred in sibships of 3 or more, thus permitting the identification of the recombinant child. In 8 of the 10 families this child belonged to the last half of the sibship. No account of the position in the sibship of the recombinant child for other linkage groups appears to have been made. However, Renwick and Schultze (1965), in comparing the regression of recombination fractions on mean parental age in the Lu/Se and ABO/nail-patella linkages, noted no effect of maternal age and divergent but non-significant effects of paternal age.

Of the 29 recombinants reported for the 8 closely linked pairs of loci listed in Table 4, 24 occur in sibships of three or more. One sibship of two has also been scored for the position of the recombinant child since in this case the fresh occurrence of a very rare IgG haplotype indicated the second child was the recombinant. The order in the sibship for 12 maternal and 13 paternal recombinants is shown in Table 5. An increased occurrence of recombinants in the latter half of the sibship is significant ($P < 0.05$); and, contrary to the mouse, there is no apparent difference between males and females. Thus, 8 closely linked pairs of loci in 6 linkage groups which do not as a whole show a pronounced effect of sex on meiotic recombination frequency, do suggest increased recombination with advancing parental age.

TABLE 5 *Position in sibship of recombinants from 8 closely linked pairs of loci (cf. Table 4)*

	Maternal	Paternal
First half	3	4
Second half	9	9

More loosely linked loci may also be scored for the position of the recombinant child, providing the phase of linkage is known or the sibships are sufficiently large. Three chromosomal regions have enough data published in suitable form to obtain an estimate of the effect of age on recombination for each region separately, *viz.*: Lu/Se/myotonic dystrophy, AK/nail-patella/ABO, and Rh/6-PGD. $\hat{\theta}_m$ and $\hat{\theta}_f$ in these linkages vary from 0.085 to 0.29, the only value greater than 0.20 being $\hat{\theta}_f$ for the 6-PGD/Rh linkage (*cf.* Table 3). For the purpose of determining the position of a recombinant in a sibship, sibships of two or more tested individuals were used if the phase of linkage was known. When the phase of linkage was unknown, sibships of three or more were used if $\hat{\theta}$ is approximately ≤ 0.1 (*i.e.* $\hat{\theta}_m$ for AK/ABO, NP/ABO

and Lu/Se); for the remaining situations only families with 4 or more tested children were used. Some families were uninformative. For example, double back cross sibships of four containing 0, 2 or 4 recombinants were discarded as being uninformative for determining the position of the recombinant in the sibship. In this case, at $\theta=0.2$ the probability that a sibship containing either one or three recombinants has only one recombinant is 0.94. Even at $\theta=0.3$ the probability for one recombinant is 0.84. At $\theta=0.1$ the probability of one rather than two combinants in a sibship of three is 0.90.

TABLE 6 *Position in sibship of recombinants for the Lutheran/Secretor and Lutheran/myotonic dystrophy linkage pairs*

	Maternal	Paternal
First half	1	0
Second half	2	8

The results for 10 families informative for the Lutheran/Secretor linkage and one informative for the Lutheran/myotonic dystrophy linkage are shown in Table 6. Disregarding sex or considering males alone, the data are significant for increased recombination with advanced parental age ($P<0.01$). In addition, in only 3 of the 8 families consisting of 4 or more informative children was the recombinant other than the last child in the sibship. The results lie in the same direction as those obtained by Renwick and Schultze (1965), but apparently differ in degree primarily because of different selection criteria. Interestingly, the most recent paper in which data on the Lutheran/Secretor linkage were presented in a form suitable for this type of analysis was published 10 years ago (Greenwalt, 1961). The hypothesis of an effect of parental age, at least in males, on recombination in the section of chromosome demarcated by these two loci should be readily testable with more recently acquired data.

In contrast, the information on the AK, nail-patella/ABO and Rh/6-PGD linkages (Tables 7 and 8) does not indicate an effect of paternal age on recombination. The trend is for more recombinants with younger mothers, the results being significant for the Rh/6-PGD linkage ($p<0.05$). When combined, the results in man (Table 9) are consistent with earlier studies in the house mouse, *i.e.* that with increased parental age there is significantly increased recombination in males ($p<0.05$) and possibly decreased recombination in females. Importantly,

TABLE 7 *Position in sibship of recombinants for the AK, nail-patella/ABO linkage*

	Maternal	Paternal
First half	10	5
Second half	3	5

TABLE 8 *Position in sibship of recombinants for the Rh/6-PGD linkage*

	Maternal	Paternal
First half	14	3
Second half	4	4

TABLE 9 *Position in sibship of recombinants for the linkage pairs in Tables 5–8*

	Maternal	Paternal
First half	28	12
Second half	18	26

however, the effect of age may vary according to chromosome region as well as according to sex.

Recently, somatic cell hybrids have been used for mapping X-linked genes under conditions of chromosome instability (Miller *et al.*, 1971). It remains to be seen to what extent specific chromosome-breaking agents may effect random autosomal breakage and the manner in which map distances determined by this means will correlate with distances derived from *in vivo* recombination frequency. Whether recombination between the Lutheran and Secretor loci is more frequent in older males or less frequent in younger males is a moot point, but one which will be amenable to investigation when *in vitro* recombination between markers covering this section of chromosome can be compared to *in vitro* and age-specific meiotic recombination within other sections of chromosome.

SIGNIFICANCE OF HUMAN LINKAGE DATA

While it is difficult to predict in specific terms what insight or advantage may derive from increased knowledge of the linkage relations of human loci, one may prognosticate from the areas in which benefits have already or soon will be established. I will consider only two: the resolution of clinical and biochemical heterogeneity and genetic counseling.

Very similar or indistinguishable effects may be produced by variant alleles at quite different loci. For example, more than 15 different genes have been postulated to produce congenital deafness (Mayo, 1970). Many phenotypically homogeneous entities are known to be autosomal of some families and in others X-linked. One would suspect that there is a substantial amount of unrecognized autosomally determined genetic heterogeneity in man. Linkage studies should have great value in the detection and analysis of such heterogeneity.

All 5 clinical syndromes listed in Table 1 – myotonic dystrophy, elliptocytosis, nail-patella syndrome, congenital zonular pulverulent cataract, and sclerotylosis – appear to be suitable candidates for a test of heterogeneity in the recombination fraction. Elliptocytosis (Morton, 1956) and nail-patella syndrome (Renwick and Schultze, 1965) have already been examined; and, in fact, in some families elliptocytosis is closely linked to the Rhesus blood group while in others it is unlinked or loosely linked. The odds favoring two values of θ are about 1000:1 (Smith, 1963). Morton (1956) considered two possible explanations: polymorphism for a chromosomal rearrangement, *e.g.* an inversion, and two or more genetic loci capable of producing the elliptocytosis phenotype. Although neither hypothesis could be excluded, the latter explanation was favored. Subsequent studies of the 'linked' and 'unlinked' varieties (Bannerman and Renwick, 1962; Geerdink *et al.*, 1967) have shown phenotypic similarities within families, but the data are still insufficient to reveal a correlation with the linkage relations of the locus. Since the Rhesus blood group is now known to be linked to 6-PGD and PGM_1, data should be forthcoming on the linkage relations of the 'Rh-linked' and 'Rh-unlinked' varieties of elliptocytosis with these two enzymes. It may soon be possible to distinguish formally between chromosomal inversion polymorphism and elliptocytosis mimic loci.

Our preliminary results for the Rh/6-PGD linkage indicate odds of about 7:1 favoring two as opposed to a single value for the recombination fraction, the most likely values of $\hat{\theta}$ being at 0.05 and 0.35 (Weitkamp *et al.*, 1971*b*). I wish to make clear that some crude assumptions

were used in deriving these odds, and they must be viewed conservatively. The possibility of heterogeneity in recombination frequency, however, has special interest in view of the Rh/elliptocytosis data. Shou'd the Rh/6-PGD as well as the Rh/elliptocytosis linkage in fact have two values for the recombination fraction, chromosomal rearrangement is still not the only plausible explanation. In leukocytes, 6-PGD A homozygotes and the uncommon B homozygotes each have a single band of activity, the latter migrating cathodal to the former. Heterozygotes have three bands, two in the same position as those in homozygotes and a third more intensely staining band with intermediate mobility. The electrophoretic pattern in erythrocytes, however, does not fit the single locus, dimer protein model suggested by the leukocyte pattern. Instead, B homozygotes have isozymes in the same three positions as heterozygotes, the amount of activity for each band in the homozygotes being shifted in favor of the more cathodally migrating isozymes. One hypothesis, proposed by Tuchinda *et al.* (1968), is that two 6-PGD loci are active in the erythrocyte. It is conceivable that phenotypically indistinguishable B type variant alleles could exist at either of the postulated loci, thus providing an alternative mechanism for variability in the recombination fraction among families for the Rh/6-PGD linkage.

The addition of the PGM_1 locus to the Rh linkage group permits for the first time in human autosomal linkage observation of double recombination. Data on recombination in 14 offspring of 6 triply heterozygous phase known individuals are shown in Table 10. Seven of the recombinations occurred between the PGM_1 and Rh loci and four between the Rh and 6-PGD loci. There was only one paternal recombinant, but among females there were three instances of double recombination.

TABLE 10 *PGM_1/Rh/6-PGD linkage. Recombinants among 14 offspring of 6 triply heterozygous phase known individuals*

Parent	Recombinants			
	PGM_1/Rh	Rh/6-PGD	Both	Neither
Male (2)	1	0	0	4
Female (4)	3	1	3	2
Total	4	1	3	6

Since the odds favoring two true values for the recombination frequency between the Rh and the 6-PGD loci are not enormous as they are in the case of the Rh/elliptocytosis linkage, another possible explanation lies in the genetic controls of recombination. One large family (NP1U1) in which $\hat{\theta}$ is 0.5 for the Rh/6-PGD linkage shows close linkage for the nail-patella and ABO loci, thus indicating no generalized excess of recombination in the pedigree (Renwick, 1970). However, polygenic control of recombination frequency limited to a specific region of chromosome has been demonstrated in Drosophila (Chinnici, 1971). The recently described linkage with the PGM_1 locus provides yet another approach to evaluating heterogeneity in recombination for this section of chromosome. Is there variability in the recombination fraction between PGM_1 and Rh? More specifically, do families which have infrequent recombination between Rh and 6-PGD have a frequency of recombination between Rh and PGM_1 similar to that in families in which the Rh/6-PGD linkage is apparently loose? Since the female recombination frequency between PGM_1 and Rh approaches free recombination only male parents appear suitable for such an analysis. Among the 34 families informative for male recombination listed in Table 2, there is one triply heterozygous phase unknown father with 5 or more informative offspring. One other similar family has been found among 16 families segregating for 6-PGD variants supplied by Lovrien (1971). Relative probability expressions for the PGM_1/Rh and Rh/6-PGD linkages for the two families are shown in Table

11. There are at least two double recombinants in family 10854 and, assuming the phase of linkage most likely from the genotypes of the offspring, one additional double recombinant in family 0329. The data are obviously insufficient to warrant strong inference, but together with the information from the two triply heterozygous phase known fathers do suggest that a comparison of the relative frequency of recombination between the two pairs of loci in different families may be worthwhile. The point I wish to emphasize here is that analysis of the linkage relations of the four characters in the Rh group will probably provide answers to questions of interest to clinical, cytological and biochemical geneticists.

TABLE 11 *Recombination in the Rh/6-PGD and PGM_1/Rh linkage pairs. Triply heterozygous phase unknown males with 5 or more offspring*

Identification	Rh/6-PGD		PGM_1/Rh	
	Probability expression	θ	Probability expression	θ
10854	$\theta^5(1-\theta)^5$	0.5	$\theta^6(1-\theta)^4+\theta^4(1-\theta)^6$	0.5
0329	$\theta^9(1-\theta)^3+\theta^3(1-\theta)^9$	0.25	$\theta^6(1-\theta)+\theta(1-\theta)^6$	0.15

In addition to the clinical syndromes, allelic variation at many of the other loci listed in Table 1 is uncommon. For instance, no individual heterozygous for two serum albumin variants has yet been reported; and, therefore, the usual tests for genetic heterogeneity, based on segregation analysis, have not been possible. A variety of electrophoretic variants of albumin, however, all are produced by a locus closely linked to the locus for the polymorphic Gc protein (Weitkamp *et al.*, 1970*c*), a finding consistent with chemical evidence that albumin is a single polypeptide chain (Schultze and Heremans, 1966).

Several years ago an unusual, genetically determined plasma protein was reported which was antigenically indistinguishable from normal albumin, but which migrated much more slowly and was present in much smaller quantity than normal albumin in the same plasma sample (Fraser *et al.*, 1959). It appeared likely that the variant phenotype was due to the presence of an increased concentration of albumin dimer (Poulik *et al.*, 1961) and two alternative mechanisms were advanced to account for the phenomenon: (1) the production or increased activity of an unknown substance which enhances the dimerization of normal albumin; or (2) the production of a structurally abnormal albumin monomer which has an accentuated tendency to dimerize (Laurell and Niléhn, 1966). The latter hypothesis would obviously require the existence of the same linkage relationship between the Gc and albumin loci that was demonstrated in other families; such linkage was found (Weitkamp *et al.*, 1968). Although peptide mapping of a portion of the albumin molecule has subsequently failed to reveal any difference between normal and the dimer variant albumin (Jamieson and Ganguly, 1969), other studies do suggest the phenotype is due to an abnormal monomer which has an increased tendency to dimerize (Weitkamp *et al.*, 1972).

Accepting the definition of a locus as the unit of genetic information which codes for the amino acid sequence of a single polypeptide chain, it is clear that recombination alone does not permit distinction between one or more loci. Intragenic recombination has been demonstrated for haptoglobin (Smithies *et al.*, 1962), hemoglobin (Baglioni, 1962) and γG globulin (Kunkel *et al.*, 1969*a*). Recombination *frequency*, however, can be used to make this distinction. The human genome probably contains at least 500,000 cistrons (Vogel, 1964; Bodmer *et al.*, 1970). Based on an average map length, considering both sexes, of 33 morgans (Renwick, 1969), a 1% recombination frequency would represent a distance of about 150 cistrons.

The calculation can be checked against the observed recombination frequency between loci

thought to be proximate. The finding of a 'Lepore' type of hybrid γG3-γG1 molecule (Kunkel *et al.*, 1969*a*) suggests these two loci are very closely linked. No certain recombinants have been found in more than 1400 children from 500 families (Natvig *et al.*, 1967). In studying 18 unusual combinations between γG1 and γG3 found in 5,000 sera, Natvig *et al.* reported two crossovers. The estimated recombination rate of 2 in 5,000, indicating a map distance of 6 cistrons, is in remarkably good agreement. This type of reasoning has been used to point out that the LA and 4 series of HL-A antigenic determinants, with a recombination frequency of 0.008, must belong to separate loci (Bodmer *et al.*, 1970). A similar argument may apply to the MN and Ss antigens, although here the recombination rate is lower and the confidence limits are wider.

Mohr in 1954 first proposed linkage between the Lutheran blood group and dominantly inherited dystrophia myotonica, a diffuse systemic disease characterized by myotonia, muscular wasting, cataracts, atrophy of the endocrine glands and other features. Recently, this linkage has been confirmed with the finding of linkage between the myotonic dystrophy and Secretor loci (Renwick *et al.*, 1971*a*,*b*; Harper *et al.*, 1971). The linkage appears fairly close ($\hat{\theta}=0.04$ to 0.12) and may be particularly useful for genetic counseling because individuals with myotonic dystrophy generally do not develop symptoms until the second or third decade. The differential diagnosis of myotonic dystrophy is not difficult, but before counseling is attempted on the basis of its linkage relations it will be necessary to test for heterogeneity in the recombination fraction. One cannot assume that a single locus is responsible for this disease, nor for that matter that the probability of recombination with the marker loci is independent of the sex and age of the parent.

In considering the potential usefulness of genetic linkage for counseling I would like to turn to a more common problem: sickle cell anemia. In the United States each year perhaps 3,000–4,000 births occur in families in which the risk for sickle cell disease is 25% with each pregnancy. At present, direct determination of the genotype at the hemoglobin β locus during the first trimester is not practicable. Prenatal diagnosis of this disease could have enormous impact on the medical care of a large number of families. Since the prior odds that the β hemoglobin locus is within measurable distance of one of the enzymes currently being typed in cell culture are about 1:5, we ask what are the prospects that close linkage of this locus with a co-dominant polymorphic marker will be clinically useful?

It is potentially possible to predict the genotype of the fetus only in the double intercross, *i.e.* when both parents are heterozygous at the marker as well as the hemoglobin locus. Such matings occur 25% of the time if the two alleles at the marker locus have equal frequency, but become increasingly uncommon the farther the marker gene frequencies deviate from equality. In order to use linkage in genetic counseling (see Mayo, 1970, for review), it is desirable to know the phase of linkage in the doubly heterozygous parent. In the postulated situation, with a marker gene frequency of 0.5, the phase will be determinable only 50% of the time even with known genotypes for all four grandparents. Moreover, when the phase of linkage in each parent is not the same, *i.e.* when one is in coupling and the other in repulsion, all three possible fetal β hemoglobin genotypes will still not be distinguishable. Thus, even with close linkage to a marker locus with allele frequencies of 0.5 and genotypes available from both parents and four grandparents, the fetal genotype will be predictable in only 1 of 16 families.

In a fetus with a 25% risk for a sex-linked recessive disorder one can by determining nuclear sex change the risk to either 0 or 50%. Similarly, two equally likely classes of offspring for an autosomal recessive disorder can be distinguished, one which, assuming no recombination, has a 50% chance of having the recessive genotype and one which will not have it. What is interesting and the point I wish to make here is that this desirable refinement in the risks can be made in 15 to 40% of the matings between sickle heterozygotes according to whether the frequency of the variant allele at the marker locus is nearer 5% or 50%. In such a situation between 500–1,500 families per year in the United States alone could benefit from prenatal diagnosis and counseling.

Note added in proof

Isocitrate dehydrogenase and supernatant malate dehydrogenase have been reported syntenic (Shows, 1971), and the locus for α_1-antitrypsin has been added to the Gm linkage group (Gedde-Dahl et al., 1972). The reader is referred to Ruddle et al. (1972) for review of the evidence suggesting that Duffy, congenital zonular cataract, amylase$_1$, amylase$_2$, PGM$_1$, elliptocylosis$_1$, Rh, 6-PGD, and peptidase C are all on chromosome 1.

GEDDE-DAHL Jr, T., FAGERHOL, M. D., COOK, P. J. L. and NOADES, J. (1972): Autosomal linkage between the Gm and Pi loci in man. *Ann. Hum. Genet., 35,* 393.

RUDDLE, F., RICCIUTI, F., MCMORRIS, F. A., TISCHFIELD, J., CREAGAN, R., DARLINGTON, G. and CHEN, T. (1972): Somatic cell genetic assignment of peptidase C and the Rh linkage group to chromosome A-1 in man. *Science, 176,* 1429.

SHOWS, T. B. (1971): Genetics of human/mouse somatic cell hybrids: linkage of human genes for malate dehydrogenase and isocitrate dehydrogenase. In: *Abstracts, IV International Congress of Human Genetics, Paris, 1971,* p. 165. ICS 233, Excerpta Medica, Amsterdam. *Biochem. Genet.*, in press.

REFERENCES

BAGLIONI, C. (1962): The fusion of two peptide chains in hemoglobin Lepore and its interpretation as a genetic deletion. *Proc. nat. Acad. Sci. USA, 48,* 1880.

BANNERMAN, R. M. and RENWICK, J. H. (1962): The hereditary elliptocytoses: clinical and linkage data. *Ann. hum. Genet., 26,* 23.

BLAKE, N. M., KIRK, R. L., LEWIS, W. H. P. and HARRIS, H. (1970): Some further peptidase B phenotypes. *Ann. hum. Genet., 33,* 301.

BODMER, W. F., BODMER, J. G. and TRIPP, M. (1970): Recombination between the LA and 4 loci of the HL-A system. In: *Histocompatibility Testing 1970,* pp. 187–191. Munksgaard, Copenhagen.

BOONE, C. M. and RUDDLE, F. H. (1969): Interspecific hybridization between human and mouse somatic cells: enzyme and linkage studies. *Biochem. Genet., 3,* 119.

CEPPELLINI, R. (1959): Discussion. In: *Ciba Foundation Symposium on Biochemistry of Human Genetics,* pp. 133-138. Editors: G. E. W. Wolstenholme and C. M. O'Connor. Little, Brown and Co., Boston, Mass.

CHALMERS, J. N. M. and LAWLER, S. D. (1953): Data on linkage in man: elliptocytosis and blood groups. I. Families 1 and 2. *Ann. Eugen. (Lond.), 17,* 267.

CHINNICI, J. P. (1971): Polygenic control of crossing over in *Drosophila melanogaster. Genetics, 68, Suppl.,* s11.

CLARKE, C. A., DONOHUE, W. T. A., FINN, R., MCCONNELL, R. B., SHEPPARD, P. M. and NICOL, D. S. H. (1960): Data on linkage in man: ovalocytosis, sickling and the Rhesus blood group complex. *Ann. hum. Genet., 24,* 283.

COOK, P. J. L. (1965): The Lutheran-Secretor recombination fraction in man: a possible sex difference. *Ann. hum. Genet., 28,* 393.

DAS, S. R., MUKHERJEE, B. N., DAS, S. K., ANANTHAKRISHNAN, R., BLAKE, N. M. and KIRK, R. L. (1970): LDH variants in India. *Humangenetik, 9,* 107.

DEMINATTI, M., DELMAS-MARSALET, Y., MENNECIER, M., MARQUET, S., AGACHE, P., and HURIEZ, C. (1968): Étude du linkage probable entre une génodermatose à transmission autosomale dominante et le système de groupe sanguin MNSs. *Ann. Génét., 11,* 217.

DUNN, L. C. and BENNETT, D. (1967): Sex differences in recombination of linked genes in animals. *Genet. Res., 9,* 211.

FELLOUS, F., BILLARDON, C., DAUSSET, J. and FRÉZAL, J. (1971): Linkage probable entre les loces «HLA» et «P». *C. R. Acad. Sci. (Paris), 272,* 3356.

FORD, C. E. and HAMERTON, J. L. (1956): The chromosomes of man. *Nature (Lond.), 178,* 1020.

FRASER, G. R., HARRIS, H. and ROBSON, E. B. (1959): A new genetically determined plasma-protein in man. *Lancet, I,* 1023.

FRASER ROBERTS, J. A. (1970): *An Introduction to Medical Genetics,* p. 136. Oxford University Press, London.

GAGNÉ, R., TANGUAY, R. and LABERGE, C. (1971): Differential staining patterns of heterochromatin in man. *Nature New Biol., 232,* 29.

GEDDE-DAHL, T., Jr, GRIMSTAD, A. L., GUNDERSEN, S. and VOGT, E. (1967): A probable crossing over or mutation in the MNSs blood group system. *Acta genet. (Basel)*, *17*, 193.

GEDDE-DAHL, T., Jr and MONN, E. (1967): Linkage relations of the phosphoglucomutase PGM_1 locus in man. *Acta genet. (Basel)*, *17*, 482.

GEERDINK, R. A., NIJENHUIS, L. E. and HUIZINGA, J. (1967): Hereditary elliptocytosis: linkage data in man. *Ann. hum. Genet.*, *30*, 363.

GIBLETT, E. R. (1969): *Genetic Markers in Human Blood*, p. 581. F. A. Davis Co., Philadelphia, Pa.

GREENWALT, T. J. (1961): Confirmation of linkage between the Lutheran and Secretor genes. *Amer. J. hum. Genet.*, *13*, 69.

HALDANE, J. B. S. (1922): Sex ratio and unisexual sterility in hybrid animals. *J. Genet.*, *12*, 101.

HARPER, P. S., RIVAS, M. L., BIAS, W. B., HUTCHINSON, J. R., DYKEN, P. R. and MCKUSICK, V. A. (1971): Genetic linkage between the locus for myotonic dystrophy and the ABH secretor and Lutheran blood group loci. In: *Abstracts, IV International Congress of Human Genetics, Paris, 1971*, p. 87. ICS 233, Excerpta Medica, Amsterdam.

HARRIS, H. (1959): *Human Biochemical Genetics*, p. 11. Cambridge University Press, Cambridge.

HARRIS, H. (1970): *The Principles of Human Biochemical Genetics*, p. 49. North Holland Publishing Co., Amsterdam.

HENDERSON, S. A. and EDWARDS, R. G. (1968): Chiasma frequency and maternal age in mammals. *Nature (Lond.)*, *218*, 22.

HILL, C. J., ROWE, S. I. and LOVRIEN, E. W. (1972): Probable genetic linkage between human serum amylase (Amy_2) and Duffy blood group. *Nature (Lond.)*, *235*, 162.

JAMIESON, G. A. and GANGULY, P. (1969): Studies on a genetically determined albumin dimer. *Biochem. Genet.*, *3*, 403.

KÁO, F. and PUCK, T. T. (1970): Genetics of somatic mammalian cells: linkage studies with human-Chinese hamster cell hybrids. *Nature (Lond.)*, *228*, 329

KISSMEYER-NIELSON, F., SVEJGAARD, A., AHRONS, S. and STAUB-NIELSON, L. (1969): Crossing-over within the HL-A system. *Nature (Lond.)*, *224*, 75.

KUNKEL, H. G., NATVIG, J. B. and JOSLIN, F. G. (1969*a*): A 'Lepore' type of hybrid γ globulin. *Proc. nat. Acad. Sci. USA*, *62*, 144.

KUNKEL, H. G., SMITH, W. K., JOSLIN, F. G., NATVIG, J. B. and LITWIN, S. D. (1969*b*): Genetic marker of the γA2 subgroup of γA immunoglobulins. *Nature (Lond.)*, *223*, 1247.

LAMM, L. U., KISSMEYER-NIELSON, F. and HENNINGSEN, K. (1970): Linkage and association studies of two phosphoglucomutase loci (PGM_1 and PGM_3) to eighteen other markers. *Hum. Hered.*, *20*, 305.

LAMM, L. U., SVEJGAARD, A. and KISSMEYER-NIELSON, F. (1971): PGM_3: HL-A is another linkage in man. *Nature New Biol.*, *231*, 109.

LAURELL, C.-B. and NILÉHN, J.-E. (1966): A new type of inherited serum albumin anomaly. *J. clin. Invest.*, *45*, 1935.

LEVITAN, M. and MONTAGU, A. (1971): *Textbook of Human Genetics*, p. 370. Oxford University Press, London.

LEWIS, W. H. P. and HARRIS, H. (1967): Human red cell peptidases. *Nature (Lond.)*, *215*, 351.

LOVRIEN, E. W. (1971): Personal communication.

MAYO, O. (1970): The use of linkage in genetic counseling. *Hum. Hered.*, *20*, 473.

MAYR, W. R. and MICKERTS, D. (1971): Another case of recombination within the HL-A system. *Tissue Antigens*, *1*, 47.

MCILREE, M. E., TULLOCH, W. S. and NEWSAM, J. E. (1966): Studies on human meiotic chromosomes from testicular tissue. *Lancet*, *I*, 679.

MCKUSICK, V. A. (1964): *Human Genetics*, p. 47. Prentice-Hall, Inc., Englewood Cliffs, N. J.

MILLER, O. J., COOK, P. R., MEERA KHAN, P., SHIN, S. and SINISCALCO, M. (1971): Mitotic separation of two human X-linked genes in man-mouse somatic cell hybrids. *Proc. nat. Acad. Sci. USA*, *68*, 116.

MISHU, M. K. and NANCE, W. E. (1969): Further evidence for close linkage of the Hb^{β} and Hb^{δ} loci in man. *J. med. Genet.*, *6*, 190.

MOHR, J. (1951): A search for linkage between the Lutheran blood group and other hereditary characters. *Acta path. microbiol. scand.*, *28*, 207.

MOHR, J. (1954): *A Study of Linkage in Man*. Munksgaard, Copenhagen.

MOHR, J. (1964): Practical possibilities for detection of linkage in man. *Acta genet. (Basel)*, *14*, 125.

MORGAN, T. H. (1964): *The Theory of the Gene*, p. 10. Hafner Publications Co., Inc., New York, N. Y.

MORTON, N. E. (1955): Sequential tests for the detection of linkage. *Amer. J. hum. Genet.*, *7*, 277.

MORTON, N. E. (1956): The detection and estimation of linkage between genes for elliptocytosis and Rh blood type. *Amer. J. hum. Genet.*, *8*, 80.

NABHOLZ, M., MIGGIANO, V. and BODMER, W. (1969): Genetic analysis with human-mouse somatic cell hybrids. *Nature (Lond.)*, *223*, 358.

NATVIG, J. B., KUNKEL, H. G. and GEDDE-DAHL, T., Jr (1967): Genetic studies of the heavy chain subgroups of γG globulin. Recombination between closely linked cistrons. In: *Nobel Symposium 3, Gamma Globulins*, pp. 313–328. Editor: J. Killander. Almquist and Wiksell, Stockholm.

NEEL, J. V. and SCHULL, W. J. (1954): *Human Heredity*, p. 116. University of Chicago Press, Chicago, Ill.

NGUYEN, V. C., BILLARDON, C., PICARD, J.-Y., FEINGOLD, J. and FRÉZAL, J. (1971): Etudes de linkage en culture de cellules. Marqueurs enzymatiques. In: *Abstracts, IV International Congress of Human Genetics, Paris, 1971*, p. 132. ICS 233, Excerpta Medica, Amsterdam.

O'RIORDAN, M. L., ROBINSON, J. A., BUCKTON, K. E. and EVANS, J. E. (1971): Distinguishing between the chromosomes involved in Down's syndrome (trisomy 21) and chronic myeloid leukaemia (Ph^1) by fluorescence. *Nature (Lond.)*, *230*, 167.

PEARSON, H. A. and MOORE, M. M. (1965): Human hemoglobin gene linkage: report of a family with hemoglobin B_2, hemoglobin S, and β thalassemia, including a probable crossover between thalassemia and delta loci. *Amer. hum. Genet.*, *17*, 125.

PONTECORVO, G. (1971): Induction of directional chromosome elimination in somatic cell hybrids. *Nature (Lond.)*, *230*, 367.

POULIK, M. D., ZUELZER, W. W. and MEYER, R. (1961): Proteins in normal and nephrotic sera with filter paper electrophoretic mobility of albumin. *J. Lab. clin. Med.*, *57*, 206.

RACE, P. R. and SANGER, R. (1968): *Blood Groups in Man*, p. 6. Blackwell Scientific Publications, Oxford.

RAPLEY, S., ROBSON, E. B., HARRIS, H. and MAYNARD-SMITH, S. (1967): Data on the incidence, segregation and linkage relations of the adenylate kinase (AK) polymorphism. *Ann. hum. Genet.*, *31*, 237.

REID, D. H. and PARSONS, P. A. (1963): Sex of parent and variation of recombination with age in the mouse. *Heredity*, *18*, 107.

RENWICK, J. H. (1968): Male and female recombination fractions in man. *Bulletin of the European Society of Human Genetics*, *2*, p. 7. (Not published; privately distributed to members of the Conference.)

RENWICK, J. H. (1969): Progress in mapping human autosomes. *Brit. med. Bull.*, *25*, 65.

RENWICK, J. H. (1970): Personal communication.

RENWICK, J. H. (1971): The Rhesus syntenic group in man. *Nature (Lond.)*, *234*, 475.

RENWICK, J. H., BUNDEY, S. E., FERGUSON-SMITH, M. A. and IZATT, M. M. (1971*a*): Mohr's linkage hat-trick confirmed: enables prenatal diagnosis of myotonic dystrophy from secretor phenotype of fetus. In: *Abstracts, IV International Congress of Human Genetics, Paris, 1971*, p. 150. ICS 233, Excerpta Medica, Amsterdam.

RENWICK, J. H., BUNDEY, S. E., FERGUSON-SMITH, M. A. and IZATT, M. M. (1971*b*): Confirmation of linkage of the loci for myotonic dystrophy and ABH secretion. *J. med. Genet.*, *8*, 407.

RENWICK, J. H. and LAWLER, S. D. (1955): Linkage between the ABO and nail-patella loci. *Ann. hum. Genet.*, *19*, 312.

RENWICK, J. H. and LAWLER, S. D. (1963): Probable linkage between a congenital cataract locus and the Duffy blood group locus. *Ann. hum. Genet.*, *27*, 67.

RENWICK, J. H. and SCHULTZE, J. (1965): Male and female recombination fractions for the nail-patella: ABO linkage in man. *Ann. hum. Genet.*, *28*, 379.

ROBSON, E. B. (1971): Personal communication. *cf.* Cook, P. J. L., Noades, J., Hopkinson, D. A. and Robson, E. B. (1972): Demonstration of a sex difference in recombination fraction in the loose linkage, Rh and PGM_1. *Ann. hum. Genet.*, *35*, 239.

ROBSON, E. B., SUTHERLAND, I. and HARRIS, H. (1966): Evidence for linkage between the transferrin locus (Tf) and the serum cholinesterase locus (E_1) in man. *Ann. hum. Genet.*, *29*, 325.

RUDDLE, F. H. (1970): Utilization of somatic cells for genetic analysis: possibilities and problems. In: *Control Mechanisms in the Expression of Cellular Phenotypes. Symposia of the International Society for Cell Biology*, pp. 233–264. Editor: H. A. Padykula. Academic Press, New York, N. Y.

RUDDLE, F. H., CHAPMAN, V. M., CHEN, T. R. and KLEBE, R. J. (1970): Genetic analysis with man-mouse somatic cell hybrids. Linkage between human lactate dehydrogenase A and B and peptidase B. *Nature (Lond.)*, *227*, 251.

RUDDLE, F. H., CHAPMAN, V. M., RICCIUTI, F., MURNANE, M., KLEBE, R. and MEERA KHAN, P. (1971): Linkage relationships of seventeen human gene loci as determined by man-mouse somatic cell hybrids. *Nature New Biol., 232*, 69.

SANTACHIARA, A. S., NABHOLZ, M., MIGGIANO, V., DARLINGTON, A. J. and BODMER, W. (1970): Genetic analysis with man-mouse somatic cell hybrids. Linkage between human lactate dehydrogenase B and peptidase B genes. *Nature (Lond.), 227*, 248.

SCHLEUTERMANN, D. A., BIAS, W. B., MURDOCH, J. L. and MCKUSICK, V. A. (1969): Linkage of the loci for the nail-patella syndrome and adenylate kinase. *Amer. J. hum. Genet., 21*, 606.

SCHNEDL, W. (1971): Unterschiedliche Fluorescenz der beiden homologen Chromosomen Nr. 3 beim Menschen. *Humangenetik, 12*, 59.

SCHULTZE, H. E. and HEREMANS, J. F. (1966): *Molecular Biology of Human Proteins, Vol. I, Section III*, Chapter 1, pp. 402–410. Elsevier Publishing Co., Amsterdam.

SHOWS, T. B. (1970): Human gene expression, linkage relationships. and control properties in human/mouse somatic hybrids. *Amer. J. hum. Genet., 22*, 43a.

SHOWS, T. B. (1972): Genetics of human-mouse somatic cell hybrids: linkage of human genes for lactate dehydrogenase-A and esterase-A_4. *Proc. nat. Acad. Sci. USA, 69*, 348.

SIMCHEN, G. and STAMBERG, J. (1969): Fine and course controls of genetic recombination. *Nature (Lond.), 222*, 329.

SMITH, C. A. B. (1963): Testing for heterogeneity in the recombination fraction values in human genetics. *Ann. hum. Genet., 27*, 175.

SMITHIES, O., CONNELL, G. E. and DIXON, G. H. (1962): Chromosomal rearrangements and the evolution of the haptoglobin genes. *Nature (Lond.), 196*, 232.

STEINBERG, A. G. (1965): Evidence for a mutation or crossing-over at the Rh locus. *Vox Sang. (Basel), 10*, 721.

STERN, C. (1960): *Principles of Human Genetics*, pp. 245–247. W. H. Freeman and Co., San Francisco, Calif.

SUMNER, A. T., EVANS, H. J. and BUCKLAND, R. A. (1971): New technique for distinguishing between human chromosomes. *Nature New Biol., 232*, 31.

SVEJGAARD, A., BRATLIE, A., HEDIN, P. J., HÖGMAN, C., JERSILD, C., KISSMEYER-NIELSON, F., LINDBLOM, B., LINDHOLM, A., LÖW, B., MESSETER, L., MÖLLER, E., SANDBERG, L., STAUB-NIELSON, L. and THORSBY, E. (1971): The recombination fraction of the HL-A system. *Tissue Antigens, 1*, 81.

SZYBALSKI, W. (1970): Cited in Watson, J. D.: *Molecular Biology of the Gene, 2nd ed.*, Chapter 10, p. 310. W. A. Benhamin, Inc., New York, N. Y.

TJIO, H. J. and PUCK, T. T. (1958): The somatic chromosomes of man. *Proc. nat. Acad. Sci. USA, 44*, 1229.

TUCHINDA, S., RUCKNAGEL, D. L., NA-NAKORN, S. and WASI, P. (1968): The Thai variant and the distribution of alleles of 6-phosphogluconate dehydrogenase and the distribution of glucose-6-phosphate dehydrogenase deficiency in Thailand. *Biochem. Genet., 2*, 253.

UCHIDA, I. A. and LIN, C. C. (1971): Genetic segregation of a fluorescent marker on chromosome No. 3. In: *Abstracts, IV International Congress of Human Genetics, Paris, 1971*, p. 180. ICS 233, Excerpta Medica, Amsterdam.

VAN LOGHEM, E., NATVIG, J. B. and MATSUMOTO, H. (1970): Genetic markers of immunoglobulins in Japanese families. Inheritance of associated markers belonging to one IgA and three IgG subclasses *Ann. hum. Genet., 33*, 351.

VESELL, E. S. (1965): Genetic control of isozyme patterns in human tissues. In: *Progress in Medical Genetics, Vol. 4*, Chapter 5, pp. 128–175. Editors: A. G. Steinberg and A. G. Bearn. Grune & Stratton, New York.

VOGEL, F. (1964): A preliminary estimate of the number of human genes. *Nature (Lond.), 201*, 847.

WAHLSTRÖM, J. (1971): Human chromosomes and fluorescence. *Humangenetik, 12*, 77.

WEATHERALL, D. J. (1964): Biochemical phenotypes of thalassemia in the American Negro population. *Ann. N. Y. Acad. Sci., 119*, 450.

WEATHERALL, D. J. (1969): The genetics of the thalassemias. *Brit. med. Bull., 25*, 24.

WEITKAMP, L. R. (1971): Further data on the genetic linkage relations of the adenosine deaminase locus. *Hum. Hered., 21*, 351.

WEITKAMP, L. R., ARENDS, T., GALLANGO, M., NEEL, J. V., SCHULTZ, J. and SHREFFLER, D. C. (1972): The genetic structure of a tribal population, the Yanomama Indians. III. Seven serum protein systems. *Ann. hum. Genet., 35*, 271.

WEITKAMP, L. R., GUTTORMSEN, S. A. and GREENDYKE, R. M. (1970*a*): Genetic linkage between a locus for 6-PGD and the Rhesus locus. *Clin. Res.*, *18*, 396.

WEITKAMP, L. R., GUTTORMSEN, S. A. and GREENDYKE, R. M. (1971*b*): Genetic linkage between a locus for 6-PGD and the Rh locus. Evaluation of possible heterogeneity in the recombination fraction between sexes and among families. *Amer. J. hum. Genet.*, *23*, 462.

WEITKAMP, L. R., GUTTORMSEN, S. A., SHREFFLER. D. C., SING, C. F. and NAPIER, J. A. (1970*b*): Genetic linkage relations of the loci for 6-phosphogluconate dehydrogenase and adenosine deaminase in man. *Amer. J. hum. Genet.*, *22*, 216.

WEITKAMP, L. R., RENWICK, J. H., BERGER, J., SHREFFLER, D. C., DRACHMANN, O., WUHRMANN, F., BRAEND, M. and FRANGLEN, G. (1970*c*): Additional data and summary for albumin-Gc linkage in man. *Hum. Hered.*, *20*, 1.

WEITKAMP, L. R., ROBSON, E. B., SHREFFLER, D. C. and CORNEY, G. (1968): An unusual serum albumin variant: further data on genetic linkage between loci for human serum albumin and group-specific component (Gc). *Amer. J. hum. Genet.*, *20*, 392.

WEITKAMP, L. R., RUCKNAGEL, D. L. and GERSHOWITZ, H. (1966): Genetic linkage between structural loci for albumin and group-specific component (Gc). *Amer. J. hum. Genet.*, *18*, 559.

WEITKAMP, L. R., SHREFFLER, D. C. and SAAVE, J. (1969): Serum albumin variants in New Guinea indigenes. *Vox Sang.* (*Basel*), *17*, 237.

WESTERVELD, A. and MEERA KHAN, P. (1972): Evidence for linkage between human loci for 6-phosphogluconate dehydrogenase and phosphoglucomutase$_1$ in man-Chinese hamster somatic cell hybrids. *Nature* (*Lond.*), *236*, 30.

Elizabeth B. Robson

Medical Research Council Human Biochemical Genetics Unit, The Galton Laboratory, University College London, United Kingdom

Gene assignment

I do not propose to discuss the methods of gene assignment since these have been known, in general principle, for long enough, and in particular I shall not deal with somatic cell genetics since we have already had a whole session on this topic. I should, however, like to deal with each assigned linkage group separately, and in some detail, illustrating the different methods where they have, in practice, been informative.

The first autosomal assignment in man was that of the blood group Duffy to the largest of the autosomes, No. 1. Donahue *et al.* (1968) described a large family in which some members had a first chromosome which was unusually long, apparently because part of the long arm, near the centromere, was relatively uncoiled. This variant can be seen in 0.5% of the population and appears to be under simple genetic control. Donahue and his co-authors postulate the occurrence of something, called non-committally an 'element', which controls the morphology of this region – perhaps a single locus or a small chromosomal rearrangement. Since only one of the two homologous chromosomes is affected it seems likely that this element is situated on chromosome 1 – and probably nearer, rather than further away from the marker region. In this family close linkage with Duffy is apparent and when various other smaller families are taken into account the peak likelihood ratio is 8,000. The final probability of Fy being on chromosome 1 is 0.985. If it is, then the length of the map interval is very short – about 0.05 Morgans.

Duffy had already been shown by Renwick and Lawler (1963) to be very closely linked to the locus Cae for congenital zonular pulverulent cataract – which is consequently elected to chromosome 1, though the order is still unknown.

Kamarýt *et al.* (1971) have investigated a family segregating for the 'uncoiler' chromosome No. 1 which suggests close linkage with the locus for pancreatic amylase, Amy 2. The family has now been extended and has been described by Dr. Brunecky at this Congress. There are one recombinant and nine probable non-recombinants.

These linkages are very close and so it ought to be possible to demonstrate the linkage between Duffy and amylase 2 in normal families. Hill *et al.* (1971) have now done this and get a maximum lod score of 4 at a recombination fraction of 0.04. This suggests that Duffy and Cae may lie between the 'uncoiler' locus and amylase 2.

There are then three gene loci assigned to chromosome 1 – and a possible fourth if further data confirm the suggestion of Nance *et al.* (1970) that the β-δ locus of haemoglobin is fairly loosely linked to Duffy. All this work has been based on conventional family analysis using an identifiable chromosome as an additional marker. The next chromosome which I want to discuss broadens the scope of the investigations a little.

The second autosome to have a locus assigned to it by pedigree analysis was No. 16, and that

was the locus for the α-chain of haptoglobin. Haptoglobin was a candidate for assignment very early in the game. In 1964 Gerald *et al.* described the case of a child with a ring 13 chromosome and the apparent absence of either paternal allele for the α-chain of haptoglobin, and they suggested that the child had lost the paternal haptoglobin allele along with a terminal portion of chromosome 13 during the formation of the ring chromosome. One difficulty in this type of argument is to be sure of the genotype of the apparently homozygous parent. In this case one can be sure only that an individual is producing only one recognisable type of α-chain, but not so sure that he has two Hp^2 alleles. Indeed a 'silent' allele is known to exist in the haptoglobin system. Harris *et al.* (1958) investigated a family in which three apparently homozygous sibs, a man and two women, all had children of apparently incompatible homozygous type. Since then five such families have been published, but the allele is undoubtedly fairly rare, perhaps with a frequency of about 0.003 in England. The chance of the concurrence of a rare chromosomal anomaly and a rare allele can be roughly estimated – in the case of deletions of chromosome 13 and Hp^0 it may be calculated that about 0.6 of a case might have been reported in the world literature, and we have to account for two. Not a great difference in view of the very speculative assumptions upon which the expectation is based.

It is preferable of course to have something better than a statistical probability of the existence of a 'silent' allele and so it may be necessary in these cases to make an extensive study of the family. In the case of Dr. Gerald's patient this was not possible, but later on I had the chance to study a mother and child each with a deletion of the long arm of chromosome 13 (Cook *et al.*, 1969). The mother was Hp 2.2 and the child Hp 1.1 This appeared to confirm the assignment of Hp to chromosome 13, but since we had described the 'silent' allele in the first place we felt obliged to search further in the family and we found that the mother's uncle, who was Hp 1–1, had a child who was Hp 2–2, and both their chromosomes were normal. In the face of this evidence it seemed that two unlikely things had happened together and that consequently haptoglobin was still unassigned.

The firm localisation of haptoglobin to chromosome 16 did not come until 1969, and then it was established by means of conventional pedigree linkage analysis using a balanced translocation as one marker (Robson *et al.*, 1969).

The family in which the linkage was first noticed was segregating for a translocation involving chromosomes 2 and 16. The Hp^1 allele is in coupling with the translocation. There are seven certain and four probable non-recombinants between the Hp locus and one or other break point of the translocation. One cannot tell from such a family, of course, whether 2 or 16 is the chromosome involved. This was sorted out by inspecting other pedigrees involving these chromosomes in different translocations. Those involving chromosome 2 did not favour linkage, but those involving 16 did. One family involving a t (1; 16) shows several probable non-recombinants. Further evidence was obtained from families with marker 16 chromosomes and the simple sum of the lod scores amounted to a maximum of 3.63 when $\theta = 0.1$. In 1970 Magenis *et al.* published a large pedigree in with many people had a No. 16 chromosome which was subject to breaks at a site on the long arm – a so-called 'fragile' site. This character segregated in a simple Mendelian fashion and showed close linkage with the haptoglobin locus. There were only three recombinants out of 33 chances. Together with our data this gives a lod of over 8 where $\theta = 0.1$, thus very definitely siting the haptoglobin locus on chromosome 16.

The location of the haptoglobin locus on the chromosome can be argued from the different chromosomal situations found in the informative families. Both the translocations involved the long arm of 16. In the first t (2;16) the break point has not been determined, but Dr. Jacobs has re-examined the second t (1;16) and finds that the break point is between the middle and the end of the long arm of 16. The marker point used in families showing the extended secondary constriction is in the proximal portion of the long arm and the 'fragile' site of Magenis is described as being at the junction of the middle and distal thirds of the

long arm. This clearly points to the localisation of the haptoglobin locus between the middle and terminal point of the long arm. Using his Bayesian method of map-position analysis, which enables data from different pedigrees to be combined statistically, Renwick (1971*a*) has estimated that the odds on the assignment of haptoglobin to chromosome 16 are over $10^7:1$. The odds in favour of a long arm rather than a short arm location are about a million to one. If the arm is 0.64 M long, this is based on a total map length of $33\frac{1}{3}$ M, with 16q being about 2% of the total, then Hp appears to be 0.37 M or so from the centromere – or about half way along. This analysis does not, however, use the exact cytological information now available on the chromosomal situation in the translocations.

Confirmation of this location is contained in the report of Pergament *et al.* (1970) on a child with a ring chromosome who was heterozygous at the haptoglobin locus. If the terminal portions of a chromosome are lost during the formation of a ring, then the haptoglobin locus is not very near the end of the chromosome. This type of evidence (often called deletion mapping) is, in general, of limited value, since viable deletions are uncommon, but it is possible to construct a chromosome map which shows where genes are not. In itself this is an inadequate way of establishing a location, but the positive evidence obtained by other methods must agree with such findings, and in the case of chromosome 16 and haptoglobin this is the case. No other loci have been shown to be linked to marker 16 chromosomes or to haptoglobin.

A number of assignments have been claimed on the grounds of quantitative evidence. De Grouchy *et al.* (1968) described a child with a deletion of the short arm of a C group chromosome, probably 6, who appeared to have half the normal amount of the Hageman factor, blood coagulation factor XII, whilst its parents were normal, both biochemically and chromosomally. By a similar argument Sparkes *et al.* (1969) suggested that the locus for TPI, triosephosphate isomerase, might be on the short arm of chromosome 5. A good deal of data on the latter claim has now accumulated and does not confirm the findings (Brock and Singer, 1970; Rüdiger *et al.*, 1970). Quantitative tests are always hazardous unless a great deal is known of the normal variation in enzyme activity, and where the critical observation is on a patient with a deletion who is usually not normal, then single cases may be very deceptive. And the very rarity of most cases makes the accumulation of a convincing series very difficult. The association of apparent exceptions to normal inheritance of a well understood character in association with chromosomal abnormalities has been touched on in relation to the haptoglobin story. Evidence of this sort has also been used by German *et al.* (1969) to suggest that the MN locus may be on the long arm of either chromosome 2 or 4. Their propositus had a translocation between the long arms of 2 and 4 and was thought to lack a very small chromosomal segment. He was of type MS while the father was N$\bar{s}$. Dosage tests are possible for this blood group system and they suggested that the child was probably hemizygous whilst the father was homozygous. In a family described by Weitkamp *et al.* (1969) with a pericentric inversion of chromosome 2 there seemed to be free recombination with the MN locus and it therefore appears to be more likely that if MN is on either of these chromosomes, it is on No. 4. One form of tylosis is linked to the MNS locus (Deminatti *et al.*, 1968) so it too may be on chromosome 4.

A claim of a similar type assigned the locus for cystic fibrosis to the short arm of chromosome 5 (Smith *et al.*, 1968) because a child with the disease, normally a homozygous condition, had a parent who did not react positively to the Spock test for heterozygosity. However, another test, that of cellular metachromasia, did give positive results (Danes and Bearn, 1968), and so the normal pattern of inheritance was no longer in doubt. Using this method makes one painfully aware that the chance of discovering that one's knowledge of a system is less than one thought is very much greater than the chance of establishing an assignment.

Perhaps I might just mention here two other approaches to gene assignment which so far have not paid off. Bateman (1960) suggested that the study of genotype frequencies in trisomies might provide useful information. The proportion of homozygotes would be

lower than expected if the locus in question were on the trisomic chromosome, since three, rather than two alleles would have to be the same. There are many theoretical difficulties in evaluating such an analysis, apart from the fact that it is really only for trisomy 21 where one can build up a series of adequate size for such a statistical test. This method was devised mainly with blood groups in mind, but its implications are easier to understand in relation to an electrophoretic enzyme system where the allelic products can be directly examined. In such an instance, with a system like acid phosphatase which has three common alleles, a trisomic might be genotypically AcP^A AcP^B AcP^C and phenotypically recognisable. In an informative family situation one such case could give very strong evidence for localisation. At a less dramatic level one might expect that a system with two common alleles might reveal an abnormal phenotype where an apparent heterozygote showed twice as much of one form of the enzyme as the other. In the haptoglobin system we know that we can detect quite small disproportions of the two α-chain components and with this in mind I tested the only two cases of trisomy 16 which I could find – and they were both apparently of type 2–2, and so uninformative. This reasoning of course is subject to all three loci being active.

Finally, I want to present the evidence for localisation of the newest linkage group – Rh, elliptocytosis (El_1), PGM_1 and PGD. The close linkage of Rh and elliptocytosis was the second autosomal linkage group to be discovered (Lawler, 1954). Weitkamp *et al.* (1970) found linkage between PGD and Rh, with a recombination fraction of about 24%. Analysis of the Galton records by Dr. Renwick confirms this finding, with a recombination fraction of about 20%.

A hint that PGM_1 may be linked to PGD was contained in a paper by Boone and Ruddle in 1969. In studying man-mouse hybrids they examined twelve lines and found between one and five human chromosomes in each. None had human PGM_1 or PGD activity and the authors pointed out that these loci might be linked. Using clones of man-hamster hybrids Westerveld and Meera Khan (1971) have found that there is a high correlation in retention or loss of these two enzymes. This experimental work prompted a search for linkage between PGM_1 and Rh by conventional means, and Renwick (1971*b*) has reported that linkage is indeed present. He estimates that the interval is 0.27 M.

Cook *et al.* (1972) confirmed the linkage on the Galton data and found a most marked sex difference. The most likely recombination fraction in males is 31.3%, but there is no good evidence for linkage in females. This is the first instance where a finding from the experimental side has provided the necessary stimulus to perform the tiresome calculations needed to detect a loose linkage between two common polymorphisms.

On what little information we have it appears that the distance between PGM_1 and PGD is large. It seems most likely therefore that Rh lies between PGD and PGM_1. The sum of the two distances is apparently well over half a Morgan, and it seems unlikely that there will ever be sufficient data on which to get a decent estimate, though this would in theory provide a good test situation to determine the strength of interference in man.

Although the clue to this linkage group came from cell hybrids, the chromosomal analysis of the cells has not yet led to the identification of the actual chromosome involved, but there is some other evidence available. Conover and Hirschhorn (1969) have suggested that PGM_1 is located on a C group chromosome. They found that human PGM_1, of heterozygous pattern, was present in three clones of man-mouse hybrids. The only common chromosome, or group of chromosomes, was group C. PGD activity was not found in these clones, which is surprising in view of the linkage. It is perhaps also rather surprising that both homologous chromosomes containing the PGM locus were retained when the majority of the human chromosomes were lost. Pedigree analysis has not yet come up with a definite location. There is some deletion evidence. Callender *et al.* (1971) have described a very interesting case where a chromosomal change in a patient was accompanied by apparent change of Rh type. A man with polycythaemia vera who, at the onset of his disease had chromosomally normal cells in the bone marrow and Rh blood group CDe/cDE, later

developed a major abnormal clone of cells which was 45, XY, B—, C—, 16+, and a minor line which was 45, XY, 2+, 3—, C—, whilst his red cells showed two populations, one CDe/cDE, that is unchanged, and the other CDe/CDe which can be interpreted as CDe, hemizygous, although the distinction cannot be made serologically. So the locus for Rh may be on one of the chromosomes involved in the abnormal clones, either the long arm of a B, or a C. There are a number of cases of Rh mosaicism in the literature but only two are associated with haematological disorders, and chromosomal studies were made in only one – again a case of polycythaemia vera. The major cell population was hyperdiploid with additional C group chromosomes, but some of the smaller Cs were rather unusually small and might suggest that several chromosomal rearrangements had taken place. The red cell lines were cDE/cde and cde/cde, in keeping with the idea of loss of genetic information.

The final observations I want to describe support the linkage of PGD and Rh but not the C group assignment. They are found in the description by Fialkow *et al.* (1972) of a case with the Philadelphia chromosome who had lost one allele for PGD in her red cells, though not in her skin, and who appeared to be R_1R_1, but handed on r to her children. Seemingly the clone of cells giving rise to her red cells was hemizygous at both the Rh and PGD loci. However, a great many other cases of chronic myeloid leukaemia have been studied and no abnormalities of PGD or Rh reported. This implies that the deletion in the special case was different from the usual one affecting chromosome 22, or was due to a second chromosomal deletion which could not be detected – though the authors suspected the involvement of an E group chromosome. Neither of these suggestions supports a C group assignment, but the new localisation evidence is not particularly convincing in itself.

Another locus has been added to this linkage group at this Congress. Dr. Frézal and his colleagues have reported that their studies on man-mouse hybrids suggest that peptidase C may be linked to PGM. As yet they have no chromosomal evidence to help with the assignment.

REFERENCES

Bateman, A. J. (1960): Blood group distribution to be expected in persons trisomic for the ABO gene. *Lancet, i,* 1293.

Boone, C. M. and Ruddle, F. H. (1969): Interspecific hybridization between human and mouse somatic cells: enzyme and linkage studies. *Biochem. Genet., 3,* 119.

Brock, D. J. H. and Singer, J. D. (1970): Red cell triosephosphate isomerase and chromosome 5. *Lancet, ii,* 45.

Callender, S. T., Kay, H. E. M., Lawler, S. D., Millard, R. E., Sanger, R. and Tippett, P. A. (1971): Two populations of Rh groups together with chromosomally abnormal cell lines in the bone marrow. *Brit. med. J., 1,* 131.

Conover, J. H. and Hirschhorn, K. (1969): A system for the localisation of autosomal traits in a somatic cell hybrid. Abstract. *Amer. Soc. hum. Genet.,* 23.

Cook, P. J. L., Gray, J. E., Brack, R. A., Robson, E. B. and Howlett, R. M. (1969): Data on haptoglobin and the D group chromosomes. *Ann. hum. Genet., 33,* 125.

Cook, P. J. L., Noades, J., Hopkinson, D. A., Robson, E. B. and Cleghorn, T. E. (1972): Demonstration of a marked sex difference in recombination fraction in a loose linkage, Rh and PGM_1. *Ann. hum. Genet., 35,* 239.

Danes, B. S. and Bearn, A. G. (1968): Localisation of the cystic fibrosis gene. *Lancet, ii,* 1303.

De Grouchy, J., Veslot, J., Bonnette, J. and Roidot, M. (1968): A case of ? 6p-chromosomal aberration. *Amer. J. Dis. Child., 115,* 93.

Deminatti, M., Delmas-Marsalet, Y., Mennecier, M., Marquet, S., Agache, P. and Huriez, C. (1968): Etude du linkage probable entre une génodermatose à transmission autosomale dominante et le système de groupe sanguin MNS-s. *Ann. Génét., 11,* 217.

Donahue, R. P., Bias, W. B., Renwick, J. H. and McKusick, V. A. (1968): Probable assignment of the Duffy blood group locus to chromosome 1 in man. *Proc. nat. Acad. Sci. USA, 61,* 949.

Fialkow, P. J., Lisker, R., Giblett, E. R., Zavala, C., Bryant, J. I., Cobo, A. and Detter, J.

(1971): Genetic markers in chronic myelocytic leukaemia: evidence opposing autosomal inactivation and favouring 6PGD-Rh linkage. *Ann. hum. Genet., 35,* 321.

GERALD, P. S., WARNER, S., SINGER, J. D., CORCORAN, P. A. and UMANSKY, I. (1964): Possible identification of the chromosome bearing the haptoglobin locus. *J. clin. Invest., 43,* 1297.

GERMAN, J. L., WALKER, M. E., STIEFEL, F. H. and ALLEN, F. H. (1969): Autoradiographic studies of human chromosomes. II. Data concerning the position of the MN locus. *Vox Sang. (Basel), 16,* 130.

HARRIS, H., ROBSON, E. B. and SINISCALCO, M. (1958): Atypical segregation of haptoglobin types in man. *Nature (Lond.), 182,* 1324.

HILL, C. J., ROWE, S. I. and LOVRIEN, E. W. (1971): Probable genetic linkage between the human serum amylase (AMY_2) and Duffy blood group. *Nature (Lond.), 235,* 162.

KAMARÝT, J., ADÁMEK, R. and VRBA, M. (1971): Possible linkage between uncoiler chromosome Un 1 and amylase polymorphism Amy 2 loci. *Humangenetik, 11,* 213.

LAWLER, S. D. (1954): Family studies showing linkage between elliptocytosis and the Rhesus blood group system. *Caryologia, 6, Suppl. Part II,* 1199.

MAGENIS, R. E., HECHT, F. and LOVRIEN, E. W. (1970): Heritable fragile site on chromosome 16: probable localization of haptoglobin locus in man. *Science, 170,* 85.

NANCE, W. E., CONNEALLY, M., KANG, K. W., REED, T., SCHRODER, J. and ROSE, S. (1970): Genetic linkage analysis of human hemoglobin variants. *Amer. J. hum. Genet., 22,* 453.

PERGAMENT, E., PIETRA, G. C., KADOTANI, T., SATO, H. and BERLOW, S. (1970): A ring chromosome no. 16 in an infant with primary hypoparathyroidism. *J. Pediat., 76/5,* 745.

RENWICK, J. H.(1971*a*): Assignment and map-positioning of human loci using chromosomal variation. *Ann. hum. Genet., 35,* 79.

RENWICK, J. H. (1971*b*): The Rhesus syntenic group in man. *Nature (Lond.), 234,* 475.

RENWICK, J. H. and LAWLER, S. D. (1963): Probable linkage between a congenital cataract locus and the Duffy blood group locus. *Ann. hum. Genet., 27,* 67.

ROBSON, E. B., POLANI, P. E., DART, S. J., JACOBS, P. A. and RENWICK, J. H. (1969): Probable assignment of the alpha locus of haptoglobin to chromosome 16 in man. *Nature (Lond.), 223,* 1163.

RÜDIGER, H. W., PASSARGE, E., HIRTH, L., GOEDDE, H. W., BLUME, K. G., LÖHR, G. W., BENÖHR, H. C. and WALLER, H. D. (1970): Triosephosphate isomerase gene not localised on the short arm of chromosome 5 in man. *Nature (Lond.), 228,* 1320.

SMITH, D. W., DOCTER, J. M., FERRIER, P. E., FRIAS, J. L. and SPOCK, A. (1968): Possible localisation of the gene for cystic fibrosis of the pancreas to the short arm of chromosome 5. *Lancet, ii,* 309.

SPARKES, R. S., CARREL, R. E. and PAGLIA, O. E. (1969): Probable localization of a triosephosphate isomerase gene to the short arm of the number 5 human chromosome. *Nature (Lond.), 224,* 367.

WEITKAMP, L. R., GUTTORMSEN, S. A., SHREFFLER, D. C., SING, C. F. and NAPIER, J. A. (1970): Genetic linkage relations of the loci for 6-phosphogluconate dehydrogenase and adenosine deaminase in man. *Amer. J. hum. Genet., 22,* 216.

WEITKAMP, L. R., JANZEN, M. K., GUTTORMSEN, S. A. and GERSHOWITZ, H. (1969): Inherited pericentric inversion of chromosome number two: A linkage study. *Ann. hum. Genet., 33,* 53.

WESTERVELD, A. and MEERA KHAN, P. (1971): In: *Enzyme Studies in the Interspecific Somatic Cell Hybrids with Special Reference to the Mapping of the Human X-Chromosome.* Beugelsdijk, Leiden.

DISCUSSION

A. D. MERRITT, Indianapolis

We feel that pancreatic amylase and salivary amylase are two discrete enzymes controlled by at least 2 autosomal dominant loci. Further, Dr. Rivas and I have recently submitted a paper showing the close linkage of these loci controlling the expression of pancreatic and salivary amylase. There have been no recombinants thus far; the maximum lod score is over 4 at the recombination fraction 0.0.

We have also examined linkage with other markers, in particular the Duffy system. A few points are worthy of note: (1) The peptide structure of human salivary and pancreatic amylases is not yet defined; (2) It is possible that they share, in common, a polypeptide from another locus distinct from the loci just mentioned; therefore only families in which the salivary amylase and pancreatic amylase variant alleles are in apparent repulsion (and whose variant enzymes have been clearly demonstrated to occur alone) have been included in our

linkage analyses; (3) All members of our informative Duffy sibships were tested twice for the 'a' and 'b' antigens.

Among our 23 sibships the present maximum lod occurs between the recombination fractions 0.20 and 0.25. The lod for pancreatic amylase and Duffy is 0.8, and for salivary amylase and Duffy, 0.6. Because of the close linkage of the two amylase loci it is reasonable to pool the lods. Thus the highest combined lod between the specific amylase loci and the Duffy locus is 1.4.

Chapter XI Genetics – past, present and future

L. Gedda

Institute of Medical Genetics, University of Rome, Rome, Italy

Réflexions sur la génétique

Il est permis d'imaginer, à l'issue de ce Congrès dans la métropole de l'élégance, que la Génétique puisse se regarder dans un miroir, pour chercher sur son visage les témoignages du passé, les caractères du présent, et les signes annonciateurs de l'avenir. Plus encore, elle tenterait de percevoir les exactes significations de son rôle dans l'histoire de la pensée humaine, de la culture et de l'évolution sociale.

Le généticien, isolé par son milieu et par son langage, est souvent presque coupé du reste du monde, mais il doit savoir qu'en étudiant l'information du phage, les structures des nucléotides ou le code d'un enzyme, il fait de la culture, de la civilisation et même de l'histoire.

L'homme d'aujourd'hui, qui considère notre science du dehors est en droit, à son tour, de savoir que la Génétique n'est pas l'antre de la Sibylle, mais qu'elle est un métier sur lequel la navette de la recherche tisse une nouvelle toile sur l'ourdi de la sagesse antique.

Même avec la Génétique, le savant n'a pas mission de créer les lois, c'est-à-dire les constantes universelles de la vie qui sont naturellement offertes à sa vigilance; mais il les découvre, les examine, les interprète avec les mystérieux paramètres de l'absolu qu'il porte en soi.

Parallèlement à cela, il y a l'homme en proie à son propre mystère, l'homme assoiffé de vérité, l'homme qui questionne et qui exige, et aucune génération ne le sait mieux que la nôtre, et elle le déclare dans le langage de son art et de sa philosophie par un mot qui revient, harcelant: l'angoisse.

Et voici que la Génétique vient au secours des philosophies, de l'art et des moeurs pour apaiser l'angoisse de l'homme. Ses arguments sont nouveaux et solides, et le roman de la vie lui est familier.

Une rivière symbolique, le fleuve immense de la vie, qui coule sur notre planète depuis des temps reculés, quand ce nouveau phénomène de la matière apparut; lancé par-dessus les eaux du fleuve, pour unir les deux rives du savoir, la biologie et la médecine, apparaît alors un pont qui a nom Génétique Humaine.

Cette image ne manque pas de logique, mais elle s'avère insuffisante; la Génétique va plus haut, va plus loin que l'image qui lui est prêtée: modèle pour la technique, référence pour l'élaboration et pour l'application des lois, elle est à la fois témoin et juge des connaissances et des oeuvres humaines.

Cette position de proue qu'occupe la Génétique dans l'assemblée des sciences modernes, n'est pas due seulement aux découvertes par lesquelles l'homme est allé au-delà de lui-même, mais à la manifestation d'un sentiment à travers lequel la Génétique nous apparaît comme la vérité qui nous faisait défaut: le lien entre l'homme et l'homme; entre l'homme d'hier, celui d'aujourd'hui et celui de demain.

N'est-ce pas la Génétique qui nous apprend que dans nos gènes revivent les gènes de l'homme

des premiers âges? que dans nos cellules travaillent en combinaisons très variables les gènes de nos contemporains, gènes qui préparent les gènes de nos enfants, de ceux-là mêmes qui vivront sur les astres?

Avec la Génétique, l'humanité retrouve son unité fondamentale, son inévitable solidarité, jusqu'à son avenir inexorablement commun.

La Génétique procède à pas si rapides que ce soir, quand nous aurons clôturé ce dernier Symposium de notre Congrès, nous saurons parfaitement qu'à l'aube prochaine naîtra un savoir nouveau.

Claudius Francis Mayer va nous parler de ce passé tout récent mais qui est long comme un âge du monde.

Jérôme Lejeune, aux mérites de première grandeur dans la cytogénétique moderne, nous dira ce souffle nouveau qu'apporte la Génétique à la médecine qui a soin de la vie avant même qu'elle n'existe.

Pour avoir eu l'honneur de présider le Congrès de Rome, je me trouve dans l'agréable devoir de présenter les rapporteurs de ce dernier Symposium et de les remercier très vivement au nom de tous les présents; c'est-à-dire au nom du Congrès de Paris.

Claudius F. Mayer

5513 39th Street N.W., Washington, D.C., U.S.A.

From Plato to Pope Paul

Pages from the history of population problems and utilization of human genetic knowledge throughout the ages*

Man kann nichts Kluges, nichts Dummes denken,
Was nicht die Vorwelt schon gedacht.

GOETHE

While the history of genetics as a whole is a fascinating field of study, historical investigation into *human* genetic practices is, of course, the most alluring and the most rewarding. I appreciate it therefore very much that your Congress invited me to participate in a presentation trilogy aimed at a panoramic display of human breeding practices in the past, the present, and the imaginary future.

My assignment was to leaf through the pages of the past, and to gather information on the ways and means the human race employed in modifying the size and trait of its progeny through the ages. Reports of historians, works of ancient, medieval and modern scholars, physicians, playwrights, poets, philosophers, anthropologists, sociologists, travellers, politicians, and codices of laws were our sources in finding the practices of civilized peoples. Backwards, toward the dawn of history, however, the years run in an infinitely long row, and, since the ordinary sources of history do not stretch that far, other methods have to be used, such as observations of primitive peoples, still living in their paleolithic or neolithic period, to reconstruct the breeding practices of the human race as far back as perhaps the *Pithecanthropus*.

Soon after the start of this investigation, it became clear to me that most of the means and genetic practices which twentieth century individuals use for the modification of the size and/or quality of their progeny, just as well as the various population checks set up by modern states, the intricate marriage prohibitions elected, the latent and overt oppressions and persecutions of national, ethnic, racial or religious minorities by pan-nationalistic genocidal governments, have their predecessors in prehistoric times. As man-made aids of natural selection they have continued to operate throughout the history of mankind in weeding out the worst and preserving the fittest.

* This is a shortened version of the study as it was read at the Congress. It is also presented for publication at the request that it would be more appropriate to include the shorter version in the *Proceedings*. The documented original study, prepared for the Congress, has many supplementary annotations and references to source material. Its publication *in extenso* as a special monograph is being considered.

Infanticide, abortion, contraception, castration, celibacy, nuptial age regulations, family grants, tax reductions for fecundity, job security, euthanasia for the unfit, genocide for national planning, and other selective breeding practices have a very long history. The collection of data, fairly representative for the human genetic practices of all ages, would require many years of reading and studying the world literature, and large volumes could not hold all the related findings. Lack of time and lack of space force me therefore that – keeping in view the Polybian advice about historical truth – I present my material as historical samples of the genetic ways of the human race, in a manner as Madách, Hungarian playwright, used just scenic fragments of history to illustrate the fate of mankind in his '*The Tragedy of Man*'.

PREHISTORIC AND PRIMITIVE RACES

The social and sexual behavior of early man in the Pleistocene is a matter of speculation. Stone Age races had many population problems. Although the primitive groups needed extra fighters and producers, they were also afraid of extra mouths to feed. To increase tribal population, killing the males captured in war and retaining the females as secondary mates has been therefore a common practice throughout the centuries. About preventive checks of the paleo- and neolithic populations some idea may be derived from observation of analogous customs existing a few decades or centuries ago among primitive peoples. Most of such observations are found in the reports of travellers and Christian missionaries in the XVIIth, XVIIIth, and early XIXth centuries at their first contact with uncivilized peoples (supposedly still in their Stone Age period) all over the globe.

Among primitive peoples these checks included castration, coitus interruptus, mechanical contraception, urethral surgery (Mica), abortion, infanticide and infant cannibalism, delayed lactation, and gerontocide. By analogy we may assume that these practices date from prehistoric times. Induced abortion, either as an egotistic or as a group policy, has been a universal phenomenon in savage life. It is one of the earliest efforts of man, in an immediate and brutal way, to ward off the burden of children. In spite of taboos, laws, and later even death penalties, induced abortion remained a characteristic of mankind.

Infanticide and infant cannibalism remind us of the habit of carnivorous animals which devour their brood as soon as they are born, a form of self-limitation of population. Neolithic graves in England have contents which serve as evidence of infanticide practiced in England. The killing of deformed children was common, and indeed the practice of infanticide has been almost universal; therefore, it can be supposed that during former times it prevailed much more extensively. The wide spread of infanticide can be demonstrated by the New Zealand Maoris among whom women were found who slaughtered 4 to 7 of their children, mostly females.

The custom of killing the old and the sick for group welfare was connected with the nomadic nature of existence among hunting and fishing races. All these primitive genetic practices became the inheritance of mankind, and, in spite of laws and regulations, they remained cherished tradition of civilized societies.

ANCIENT CIVILIZATIONS; CASTES AND RACES

Among all Indo-European races worship of the dead and worship of the ancestors by offerings was a universal custom. It was the same also in ancient *India*. The lawbook of Manu stated that a man of rejected origin would inherit the bad quality of his father or of his mother, and could never deny his origin. This view was at the basis of the ancient Hindu caste system, and it resulted in a hereditary stratification of occupations and classes.

It was the law that if a person was born with a disease or defect, or became sick before the

start of puberty, he was rejected from the caste. Thus, castes partially protected against physical and mental degeneration. The traditional population checks continued to be still in use. The custom of female infanticide among Hindus arose largely from their low valuation placed upon baby girls.

The criminal and civil laws of *Assyr-Babylonia*, especially the oldest law as codified in 1751 B.C. in the cuneiform writings of Hammurabi, had 68 of 280 articles related to marriage and family life, with some reference to such population checks as abortion, infanticide. The planning genius of Assyrian statesmen was revealed by one measure – the transfer of large groups of people as a means of solidifying the empire.

The *Persians*, who considered themselves the greatest race on earth, superior to all others, followed the precepts of Zend-Avesta, the sacred book of Zoroaster, favoring all conditions which encourage marriage and make their race productive. The Avesta has a formal interdiction on abortion. Sterility was considered a terrible misfortune, and Persian kings used to reward fecundity by annually distributed premiums to those who had many children.

Ancestor worship also developed in the Far East in ancient *China*, probably still in the Bronze Age, first in the royal and the noble families. Nevertheless, preventive genetic practices were also reported early. Since about 500 B.C., Taoism and Buddhism condemned infanticide and abortion.

The ancient *Egyptians*, just as the Jews of biblical times, thought of themselves as a specially chosen people of God. They felt they were superior to all others, yet they could not preserve the purity of their race. Such foreign races as the Israelites became too many and too strong, so that the Egyptian king planned genocide as a precautionary measure, by killing the Hebrew baby boys, but letting the girls live. Egyptians believed in the immortality of the soul, and therefore they rarely practiced abortion and infanticide.

The *Phoenicians* developed infanticide as a religious rite for Baal Hammon, their chief god, which they practiced until the complete razing of their Carthage in 146 B.C. The genetically important fact is that the selective slaughter of the offspring of the upper classes was just as deleterious on the Phoenician gene pool as sterilization of the leading families would have been.

The ancient *Jews* are the most interesting people for the study of inbreeding and race mixture. Their principle of inbreeding is based upon a spiritual basis, and not upon geographic situations. Their dispersion in early history would facilitate mixture, but for the religious laws they had since Moses and Ezra until now. Their history is almost nothing but a list of calamities, oppressions, persecutions. Their Exodus can be considered a large-scale eugenic experiment, and Moses could be called the greatest practical 'biological engineer' of all times.

CLASSICAL ANTIQUITY – GREEK CITIES AND ROMAN EMPIRE

Numerous original documents and many modern studies of classical Antiquity show that in Greece and Rome population problems were just as numerous and their practical solutions – with the genetic knowledge of families, groups, and rulers – were just as ingenious and variegated as they are in our times.

In the time of fables the *Greeks* desired large families, many descendants who would perpetuate the family for ancestor worship. The Greeks recognized the inconsistency in their breeding animals and men, as this has been most effectively pointed out by Theognis, and commented on by Stobaeus. The ancient Greek system of eugenics was built on infanticide, which radically solved their problems of heredity. It was the most important negative eugenic measure in Sparta. The Spartan discipline aimed at the production of supermen; it made citizens fit chiefly for war only, and made the women beautiful and self-controlled.

Quantitative measures for the maintenance of the number of Spartan citizens were prevention of emigration, penalties for (or prohibition of) celibacy, rewards for fertility, enfranchise-

ment of aliens, establishment of the caste system, wife-lending to approved young men. Nevertheless, depopulation threatened Sparta in the IIIrd and IInd centuries B.C., because of its declining birth rate; it threatened Athens because of the Persian wars. Indeed, whole Hellas was afflicted in the IInd century B.C. with low birth rate. People were unwilling to marry or to beget.

The theoretical aspects of eugenics were systematically described by Plato in the IVth century B.C. on the basis of the living and still successful Spartan community. He suggested that the State should select suitable mates, and pairing should be regulated so that there be no over- or underpopulation. Two children, one of each sex, were supposed to be sufficient. The Hellenic dream state was further developed by Aristotle.

In his population policies Alexander the Great might have been influenced by his teacher, Aristotle. In his empire building he promoted miscegenation and looked forward to it as a potent instrument for realizing his dreams. Such population checks as exposure and abortion were extensively practiced in classical Antiquity, also for eugenic reasons. Aristotle remarked that elderly women after 40 years of age should abort, and have no children.

Among the ancient *Romans* family and married life was originally highly esteemed. To have children and to rear legitimate offspring was regarded a duty of all citizens. Yet, patrician families began to decline rapidly, and the State had to introduce various penalties and taxes on bachelors. Perpetuity of the State was the only object of Augustus. To this end, he passed laws against celibacy, and for prolific families. He aimed at the regeneration of the Italian stock, the stimulation of the birth rate. Subsequent emperors followed his exemplary policies.

Destruction of conspicuously deformed infants was already authorized by the Roman Twelve Tables. Partly for eugenic reasons the practice of infanticide continued. Exposure, abandonment of defective infants was common in the lower classes. Abortion became a universal vice in all classes of Roman society.

In the Roman Empire persecution of the Christians was a prominent example of genocide, although Rome generally resented the intrusion of any new religion. Emperors, concerned about depopulation, used to order the movement and transfer of various peoples. In the IInd and Ist centuries B.C. and in the IIIrd century A.D. several unsuccessful attempts occurred to establish an idealistic state in imitation of Lycurgos and Plato.

MIDDLE AGES – CHRISTIANS, MOSLEMS, HERETICS

Few data remained about the genetic practices of the new tribes and races which emerged at the fall of the Roman Empire and at the transition to the Middle Ages. In the old German law infanticide was treated as murder of a relative, and the guilty mother was buried alive in a sack (together with all kinds of scratching animals). The primitive usages of the Teutons also included exposure of infants.

By the existing medieval laws procreation was considered a duty of the people who had to provide inhabitants for the country. Thus, the laws promulgated by Alfonso X (1263–1348) called for early marriage, healthy mates, and gave eugenic advice for avoiding the begetting of defective children.

The Crusades, feudal wars, and epidemics devastated many countries, including England and France. Sometimes the wars assumed a truly genocidal character. Justinian persecuted the Samarites. Genocide resulted also from superstition of the population concerning the origin of epidemics. Persecution of the Jews continued throughout the whole Middle Ages in all European countries.

The traditional genetic practices continued. Abortion, abandonment of children, exposure of infants, infanticide, castration remained with the medieval man, just as contraception, regardless of the laws and religious instructions. Abortion was sometimes recommended for

therapeutic purposes. Although the Gospel showed the way towards practical eugenics, early Church laws and the Christian fathers considered infanticide and induced abortion a murder.

The Middle Ages developed some institutions which had an ultimately dysgenic effect – monasticism, celibacy, and humanitarianism. The knowledge of heredity shows a great advancement in the works of Albert the Great. A favorite topic of patristic and scholastic theology was the time of fetal animation. For the suppression of heritable diseases the ancient Scots practiced castration, while Thomas of Aquino held that even the life of a newborn of a leper is better than no life.

The first thought of 'human engineering' evolved in the Middle Ages. Experimental human breeding was mentioned by William of Aubergne (=Alvernus), bishop of Paris. It was also suggested that the Holy Land should be changed into an ideal state where wise women would marry princes and celibate priests to improve the human race.

NEW WORLDS, NEW POWERS – STATE PLANNERS AND REVOLUTIONARIES

The discovery of America gave opportunity to Europeans to study the genetic customs of newly contacted peoples, the population policies of the Aztecs in Mexico, of the Incas in Peru. During the Renaissance and afterwards, induced abortions, abandonment, infanticides, and the use of preservatives (linen sheaths) increased in frequency. Civilian laws prescribed various (usually capital) punishments in induced abortions according to fetal viability.

With the rise of State power, the private life, and its propagative and genetic aspects became public concern. According to the new political philosophy the State was the supreme end, and the individuals only breeders and workers for it. There was hardly a government without making efforts at (1) placing disabilities on celibates, (2) encouraging marriage directly, (3) encouraging fecundity, (4) providing asylums for foundlings, etc. Colbert elaborated a policy for France and sent boatloads of French girls to colonial soldiers.

In Colonial America the typical family had 7-8 children. To have many children was considered a blessing. Premiums and tax exemptions were offered in many countries for numerous offspring. Some believed that celibacy was better, though others advocated punishment of celibacy and of sterility by increased taxation.

Dream States were described by many in this period. The best known such works are those of More, Campanella, and Bacon; they discuss mate selection, optimum number of children, provisions for population expansion, etc. The effect of these utopistic ideas upon actual State politics and laws has not yet been sufficiently evaluated, but they definitely provided material for sectarians and revolutionaries, as More for the anabaptists, Campanella for the Jesuit state in Paraguay, Morelly for the 'Club of Equals' in the French Revolution. Transmigration of nations as a breeding experiment was suggested by Burton in the XVIth century as a refreshment of the old stock.

Religious persecution with genocide occurred in France against the protestants, in Spain against the Moors. Bavaria invented a new genocidal method against Jews at the end of the XVIIIth century by restricting their marriages. The attitude of the puritanical English settlers in America toward native Amerindians was no better than genocide.

Depopulation was widespread in many countries in this era. After the Thirty Years' War Germany was so devastated that two centuries were hardly able to restore normal conditions. Poverty was known to lead to death, disease and depopulation. In Bavaria for the control of pauperism the marriage of proletars was restricted.

The knowledge of genetics and heredity was purified from many superstitions in the XVIth to XVIIIth centuries. Practical advices were given about who should mate and how to procreate wise sons. In the XVIIth century it was suggested that deaf-mutes should not marry because they beget children like themselves. Consanguineous marriages were considered disadvantageous for heredity, and in his '*Anatomy of Melancholy*' Burton brought up again the

old argument in favor of human eugenics. A kind of eugenic breeding was the effort of the XVIIIth century Prussian Army to recruit and to mate giants for bodyguards.

The idea of an asexual laboratory production of man has been entertained for many centuries. In the hermetic writings of Paracelsus (1520) a procedure was described for the generation of 'homunculi', while Bacon's '*Nova Atlantis*' depicted Saloman's House, a scientific research center where experiments would be made with artificial variations of the species. Some scholars proposed that environmental influences (nurture) may also have an influence upon inherited characteristics in the sense that one might wear out any passion from a family by culture, as skilful gardeners blot a color out of a tulip that hurts its beauty.

POPULATION WORRIES – OVERPOPULATION, DEPOPULATION, DEGENERATION

Human population had its ups and downs throughout history, especially in the last centuries, characterized by intensive and excessive warring. Population problems involved many phases of individual lives and human genetics, and stimulated publications all over the world. Rapid increase in people caused proletarization and unreasonable increase in vice and crime. The birth rate of the lower levels of society was the highest. Even the French Revolution was alarmed by an excessive population.

Remedies for overpopulation have been suggested by many in the past three centuries. The following were suggested: (1) positive checks: wars, pestilence, famine, unwholesome occupations, etc., and (2) preventive or negative checks: moral restraint (of Malthus), birth control by neo-Malthusian means. Other traditional population checks continued to exist also in the XVIIIth to XIXth centuries, such as infanticide, abortion, prolonged abstention from intercourse in marriage, postponement of marriage, celibacy, coitus interruptus, castration, infibulation.

The relation of population to food had already been studied in 1756 by Mirabeau who found that "men will multiply as rats in a granary if it is full with means". In his 1798 '*Essays on the Principles of Population*' Malthus made the conclusion that population is increasing faster than the means of subsistence. Yet, as it is known, geometric increase of population is not a rule in Nature, merely a potentiality.

The use of mechanical and other devices to prevent conception in marriage dates back to Antiquity, but it was vigorously advertised after 1818 until now. This social and genetic movement, generally called 'neo-Malthusianism', had a very turbulent history, and it was attacked by States, churches, social groups, including anarchists, communists, and the Catholic Church.

The view that the population of specific countries had fallen since ancient times was widespread in the XVIIth to XIXth centuries. The depopulation of XVIII/XIXth century France ('*oliganthropie française*'; Dumont) was asserted by some, denied by others. It was especially keenly felt since 1870 and France was considered a 'dying nation'. The number of its recruitable youth gradually diminished, their quality also became inferior.

Since 1890 we heard everywhere much of 'race decadence' and 'race suicide'. Various organizations were established to study the causes of depopulation and to find remedies for nationwide increase in natality. Neither was the qualitative aspect of human population forgotten. It was found that the XIXth century wars, not to mention the XVIIIth century revolution, took much valuable material away from propagation, and a gradual degeneration ensued. War eliminates the strongest and healthiest of the population, leaving the weaklings at home to be fathers of the next generation.

Another dysgenic factor was found in charity, the great paradox of civilization. It induces decay because it saps the vital qualities of men and women. Pauperism was felt the result of physical and moral degeneration of the individual to which charity was often a contributing cause. Various measures were suggested for improving the health and quality of population,

and those who blamed modern civilization for the general degeneracy of mankind suggested a return to Nature, to the savages and wild animals.

HUMAN BREEDING EXPERIMENTS – SLAVES, SECTARIANS, VANISHING PEOPLES

Laboratory attempts at creating single human beings date back to the early medieval periods. Mass selective breeding of people was practiced in certain states of Antiquity for improvement of the native stock. It was often restricted to special castes. A typical example of selective caste breeding is slave breeding in Antiquity, and in modern times. In the Roman Empire slaves were encouraged by their masters to propagate. After the discovery of the New World, already the first Negroes who were imported to America were used for breeding, just as were domestic animals.

Selective human breeding for race improvement was tried in the US by some sectarians in the XIXth century. It was the group of 'perfectionists' at Oneida, N.Y., whose founder (Noyes) preached free love or 'complex marriage', a combination of polygyny and polyandry, male continence (1844), and aimed at 'scientific propagation or stirpiculture' (1875), by pairing off those for sexual intercourse whom he thought to be the most advanced in health and perfection. This matching plan was inaugurated in 1869 and 58 experimental children were produced.

An immensely interesting field of human genetics is the study of the extinction of races and the scrutiny of genetic practices and cultural factors of the peoples who are about to vanish. Several races, or a great number of them, have disappeared within historical times.

EUGENIC AWAKENING – PROSPER LUCAS AND EUGENIC THINKING

At the beginning of the XIXth century heredopathology was considerably advanced in theory, and physicians also offered some practical advice for proper mate selection in order to avoid hereditary diseases. But, in general, this kind of literature was of a very modest volume in the first half of the XIXth century. Still struggling with the new problems created in natural philosophy by the discoveries of the preceding two centuries, the period was also a politically and socially very stormy, turbulent era, with continuous wars and recurrent revolutions in most European countries.

This was the state and set for the pioneer work of a French psychiatrist, Prosper Lucas (1808–1885) whose '*Philosophical and Physiological Treatise on Natural Heredity*' (Paris, 1847–1850, 2nd Vol.) served as the basic inspiration for many mid-XIXth century students of human genetics and eugenics, so much so that Prosper Lucas ought to be resurrected and placed upon a pedestal as the *true founder of human genetics*.

Prosper Lucas was born in St. Brieuc in 1808. During his student years he wrote a paper (1831) on '*The Freedom of Education*', and graduated as a medical doctor in Paris in 1833 with the thesis '*Contagious Imitation, or Sympathic Propagation of Neuroses and Monomanias*'. Afterwards, he worked as a psychiatrist in the Bicêtre and in the Ste. Anne asylum. The publication of his main work (which includes about 14 years of experience) became the starting point and source for all other studies that appeared on the question of heredity, including the publications of Moreau (de Tours), Morel (1857), Ribot, Francis Galton, etc.

Prosper Lucas was the first who tried to establish that the psychological and mental qualities of man are also hereditary phenomena. He used a method to prove that, in addition to physical features, intellectual and moral qualities are also (at least partly) heritable in man. He showed that all types of defectives may be a product of heredity, yet he did not believe in the heredity of acquired characters which will disappear, according to him, with the cessation of original causes.

In his book he devoted a special section to the 'rules of treating morbid heredity'. The treatment included (1) prevention, and (2) repression. His rule was: 'Never cross diseases' ('Ne jamais croiser les maladies'; p. 909).

One field of knowledge which was fostered by the work of Lucas was the study of abnormal states which Morel called degeneracies, and which was developed into a discipline of 'morbid anthropology', the precursor of the Lombroso school.

Extensive studies in the 1880s in America and elsewhere (Germany, France) on hereditary degeneration and insanity brought up again the desirability of various eugenic legalized population checks (restriction of marriage, life-long asylum, castration, sterilization). The first compulsory law was enacted in Indiana, USA, on 9 March 1907. Similar laws soon followed in European countries, in Denmark (1929), in Germany (1934), in Sweden (1935), etc.

Race betterment, racial hygiene, eugenics came into the center of attention of individuals, governments, churches. At the end of the XIXth century the deep contradiction which exists between modern medicine and racial welfare had been also recognized, and it was pointed out that the principle of Natural Selection – this positive eugenic check of Nature – is suppressed or inhibited in its full operation, by keeping the morbid stock in circulation through medical measures. Dreamers again proposed to produce a new superior race by exchange of Italian and German boys and girls for breeding. Genealogical studies were also made and conclusions drawn that talent and degeneracy are inherited (Galton launched such studies in 1869).

The so-called 'eugenics movement' became stronger and stronger during the later XIXth and early XXth century years, and numerous positive and negative, qualitative and quantitative measures were suggested for race betterment. By 1915 eugenics reached the dimensions of a dangerous fad in America, and began to influence national planning and immigration policies everywhere.

MODERN EUGENIC POPULATION POLICIES – ADVENT OF RACISM AND GENOCIDE BY STATE

Our last chapter embraces the first half of the present century which is loaded with two world wars, many colonial wars, and has witnessed the rise of diverse totalitarian systems of government. The chief characteristic of these years was (and it still is) State control of both the quantitative and the qualitative aspects of practical genetics at both the family and the national level.

The controversial doctrines of the XIXth century on primitive and progressive human races announced principles which were gradually assimilated by politicians, national planners who then introduced them into the consciousness of vast majorities. It was said that higher races were destined to push away lower ones; that miscegenation of different types would lead to physical and mental degeneration and that it would be the seed of peoples' decline.

At the beginning of the XXth century in America the races of southern and eastern Europe were considered inferior, and immigration from these areas was thought to pervert the 'bloodstream' of Anglo-Saxon America. Immigrants now enter by a national-origin quota system. Exclusions from the US are still only phenotypical and not according to the genotype, although Theodor Roosevelt used to say that 'the blood and the traditions of the old stock' should be perpetuated. In Brazil in 1943 a similar quota system of immigration was introduced as the US system, and it is aimed at the preservation of the character of 'Brasilidade'.

Forced migrations occurred after the two world wars. Wilson's principle of self-determination resulted in mass expulsion of minority populations from East European countries after World War I. The same transfer principle was applied in the next world war, and in the postwar period by Germans, Russians, and others to deal with their eugenically undesirable elements. The Third Reich was planned on the principle that 'common blood belongs in a com-

mon Reich' (Hitler). This was the type of uniforming selection which, though of a laudable intention, meant high-grade eugenics for the preferred, genocide for the rejected.

Meanwhile, all the ancient, primitive genetic traditional practices, including abortion, contraception, coitus interruptus, voluntary sterilization, infanticide, etc., still remained at an uncontrollable potential reach of individuals. In the past two decades abortion has been legalized in many countries, contraceptive methods were encouraged, and 'eugenic sterilization' was introduced in Japan. In China the communist government admitted that overpopulation is not a problem peculiar only to capitalist societies.

For his utopistic dream State a modern German eugenist (Ploetz, 1895) suggested that euthanasia ('sanfter Tod'), perhaps by a small dose of morphine, should be used to eliminate weak or abnormal infants. In the Third Reich cases of state-controlled euthanasia continued to occur. Mercy killing is still one of the great problems of western society, and rather lately it has been promoted as a means for killing deformed babies and useless old men.

CONCLUSION – FUTURE GENERATIONS: FAITH OF POPE PAUL VI

Turning the pages of history, and focusing upon population problems and practical human genetics we gathered bits of information on the breeding practices of the human race from the Stone Age to our times. The quantitative and qualitative population checks of primitive peoples ripened into permanent devices and institutions which became the tradition of all civilized peoples and which were used by families, tribes, peoples, States throughout the entire course of history.

Did these qualitative and quantitative checks themselves, as such, have any substantially favorable effect upon the development of mankind, of society? Or do they have a share as contributing factors in human evolution? Although we are inclined to answer both questions rather with yes, it seems that, in his propagation and breeding habits, man is still there where he was, let us say, 500 generations ago. *Homo sapiens* is more refined, more civilized, more educated than he has been in the past, but his germ plasm, his genotype remains eternally the same as it was in the Pleistocene, except for some mutations and recombinations.

With all our differences and diversities today we are just as our ancestors were yesterday, forming a line of unity in the same population, in the same human race, at the same organic level, without any superiority, without any substantial improvement, in spite of organized gigantic efforts of State planners and governments in the past. Eugenic practices were not able to save society from the unfit, neither do they offer hope for producing future generations of supermen. Indeed, if the present system of social services ('euthenics') continues in operation, it must be highly injurious to the race of man, resulting in genetic deterioration.

Our quantitative population problems and differential fertility rates played regional and world-wide see-saw throughout history. In nearly all ages populations have seemed to many too large for existing resources, while to others they seemed too small for the country's defense. Neither did science find yet a formula for population optimum. But it appears that no principle emerges which would make it impossible for man to cater for his growing numbers. With the faith of Pope Paul VI we should rather 'multiply the bread that is to be shared than diminish the number of guests'.

SUMMARY

Natural selection, negative and positive eugenic measures, active since the dawn of civilization, helped to weed out the worst and to preserve the fittest in the human race. Man pursued his instinct of propagation ('crescite et multiplicamini...') and encountered many difficulties and problems in replenishing Earth.

For population check past centuries applied various means that are still favored today. Since the laws of Manu matrimony has been an object of legislation (State, Church); regulation and encouragement of marriage remains a vital element in empire building. Plato and many others depicted ideal States with provisions to breed a strong progeny. National economists of later years viewed with alarm the ebbs and tides of historic population flow, and offered suggestions to monarchs and governments.

Meanwhile, by gathering observations on normal heredity and inheritance of disease, medical men developed a new science. The true father of the new discipline is Prosper Lucas (1808–1885), French psychiatrist, whose fundamental work published in Paris (1847–1850) appeared almost twenty years before Galton and was followed by a deluge of studies in hereditary pathology.

The proposals of dreamers and physicians were adopted by sectarian groups and growing states (US, Brazil, France, Germany) for experiments in human breeding, nation planning, and race betterment. They are also ingredients in modern family planning and genetic counseling.

Although for the human race self-limitation of population (by contraception, abortion, infanticide, celibacy, polyandry, polygyny) has been a tradition of many centuries, euthenics now nurses the worst in society along with the best. Betterment of future generations, however, demands relentless artificial selection (by sterilization, segregation, etc.) guided by moral eugenic policy. Events of yesteryears show that, when fostered by fanatics, intermarriage of eugenics with politics will beget chimeras of the most grotesque kind.

ACKNOWLEDGEMENTS

Although the field is inexhaustible, a huge amount of source material was made available for my research in original publications, microfilms, and photostats by the kind cooperation of the largest Washington libraries (Library of Congress, D.C. Public Library, National Medical Library), and particularly by the enthusiastic help of the Army-Library's Interlibrary Loan System and its devoted staff in the Pentagon. Needless to say that I owe them many thanks for their professional services.

Jérôme Lejeune

Hôpital des Enfants Malades, Paris, France

Du bon usage de la génétique

Infléchir la destinée des hommes est un redoutable pouvoir. Entre le passé qu'évoquait à l'instant le Professeur Mayer et le futur que scrutera tout à l'heure le Professeur Dubinin, le présent nous assiège confrontant la génétique de l'homme à la souffrance des déshérités.

Devant le caractère inexorable des maladies héréditaires l'usage le plus ancien de notre discipline fut de prévoir ces arrêts du destin. D'où le conseil génétique, permettant de peser avec soin les risques que courrait un éventuel enfant et donnant aux futurs parents les éléments complets d'une décision prudente; soit qu'ils décident finalement de protéger une vie nouvelle, soit encore qu'ils renoncent à la procréation.

Toutes les découvertes rapportées à ce Congrès, qu'il s'agisse du déchiffrage nouveau des chromosomes humains, du dépistage précis d'un trouble biochimique, ou d'une prévision statistique sur des populations entières, peuvent et doivent concourir à ce but, prévenir au maximum les risques génétiques qu'auront à supporter les hommes à venir.

Et pendant deux tiers de siècle cet usage de prudence fut pratiquement le seul.

Très récemment pourtant, un perfectionnement technique, l'analyse de cellules du foetus, prélevées par amniocentèse, a conduit certains à préconiser et mettre en oeuvre un autre usage de nos connaissances: la destruction sélective des foetus qui seraient reconnus porteurs d'une maladie que nous ne savons pas guérir.

Mesurer le droit à la vie selon l'âge ou la taille ou la santé d'un être humain quelconque ne peut être objectivement discuté; une telle métrique de l'homme n'étant point accessible à la logique scientifique.

Le plus étrange dans un pareil usage c'est qu'il serait désespéré. Car seul le désespoir pourrait pousser des médecins à supprimer des malades pour lutter contre une maladie et nous savons de reste que ceux qui vainquirent et la peste et la rage, n'étaient pas ceux qui brûlaient les pestiférés dans leurs maisons ou étouffaient les enragés entre deux matelas.

De la prévision des malheurs à l'aggravation délibérée des peines, de Cassandre à Carabosse, l'usage de la génétique serait-il à ce point réduit?

La génétique de l'homme est une science comme les autres, mais c'est devant le malade qu'elle peut se montrer humaine.

Et il est vrai cependant que notre insuffisance est proprement intolérable. Que pouvons-nous par exemple contre l'affection la plus grave, la plus spécifique de l'homme car elle n'atteint que lui, et la plus inhumaine aussi puisqu'elle lui interdit d'être pleinement lui-même, la débilité de l'intelligence?

La difficulté majeure, qui rend toute recherche si difficile et décourage hélas un grand nombre de laboratoires est que la débilité mentale est bien une maladie pour le patient et sa famille, mais qu'elle n'est qu'un symptôme pour le biologiste. Toute la génétique de l'homme est là pour nous montrer qu'une multitude d'erreurs, des aberrations chromosomiques les plus

variées, aux dérèglements chimiques les plus localisés, sont chacune susceptibles de la même conséquence, l'amputation de l'esprit.

Devant ce terrible catalogue, la tentation est grande de tout abandonner faute d'un modèle logique qui puisse nous permettre d'ordonner ce fatras.

Et pourtant ce modèle existe, même si la nature ne nous l'a pas fourni, puisque le génie de l'homme a su le fabriquer – la machine à simuler certains effets de l'intelligence.

Depuis les roues dentées de Pascal jusqu'aux ordinateurs les plus perfectionnés la seule fonction de ces engins a été de réaliser et même souvent de surpasser certaines des performances de notre esprit.

Prendre l'ordinateur pour modèle n'est nullement prétendre que le support de notre intelligence soit construit comme lui et fonctionne selon les mêmes lois.

La complexité tout d'abord est d'un tout autre ordre de grandeur, le réseau des neurones étant des millions de fois plus compliqué que celui des plus puissantes machines. Et même les propriétés de la matière qu'utilisent ces engins, n'ont guère de rapport avec le fonctionnement biochimique qui doit être celui de notre cerveau.

Il est d'ailleurs remarquable que des machines d'efficacité comparable puissent être fabriquées en utilisant des phénomènes physiques extrêmement différents. Alors qu'une calculatrice mécanique ne fait appel qu'à l'invariance géométrique d'une matière solide, façonnée en roues et tringlerie, l'ordinateur utilise un flux dirigé d'électrons, tandis que des dispositifs parfaitement efficaces exploitent simplement la dynamique des fluides dans les engins à jet contrôlé, des effets photoniques dans des machines à laser, ou encore la migration surveillée d'une bulle magnétique.

Mais cette diversité même nous est d'un précieux enseignement. En effet, quelque soit l'anatomie et la physiologie (si l'on peut dire) de l'engin considéré, on remarque immédiatement que tous satisfont à des nécessités apparemment impératives.

Tout d'abord un réseau, extrêmement complexe et rigoureusement établi – ensuite des composants ayant une action ponctuelle, étroitement déterminée – enfin une multitude de connexions orientées transmettant les effets d'un composant à d'autres en évitant soigneusement toute diffusion parasite.

Il est dès lors possible que les conditions reconnues nécessaires au bon fonctionnement d'une machine soient nécessaires aussi à l'épanouissement de l'intelligence, à côté de beaucoup d'autres que nous ignorons entièrement. Il se pourrait ainsi que les 'pannes' susceptibles de gêner le fonctionnement des machines puissent nous indiquer par simple analogie le point d'impact des conditions génétiques qui font d'un malade un débile mental.

La constatation la plus élémentaire pourrait être qu'il manque une partie du réseau. Par exemple l'arrhinencéphalie de la trisomie 13, l'agénésie du corps calleux de la trisomie 18, voire l'anencéphalie complète correspondraient à un défaut de fabrication. Ici le dépanneur remplacerait la partie manquante par une pièce de rechange et remettrait la machine en marche. Pour le généticien l'idée de réactiver les étapes embryonnaires qui ne se sont pas produites relève de la science future, mais il n'est pas exclu que les processus de régénération ne puissent être un jour contrôlés.

Dans cette même catégorie se rangeraient les atteintes secondaires, que le réseau soit partiellement détruit par une infection, une hémorragie ou une ischémie ou simplement laminé par la pression hydraulique d'une hydrocéphalie.

Un second type de panne pourrait atteindre les isolants qui protègent les éléments du réseau contre les court-circuits ou les diffusions indésirables. Ces affections des gaînes sont hélas bien connues, la maladie de Tay-Sachs, la leucodystrophie métachromatique, la maladie de Niemann-Pick ou celle de Gaucher. Dans tous les cas, l'impossibilité de régler efficacement l'une des étapes biochimiques de la fabrication des substances 'isolantes' conduit à l'effondrement des performances du réseau et leur accumulation peut même détruire les composants.

Mais sur un réseau correctement cablé et dont les isolants seraient en bon état, un troisième

type de panne pourrait se présenter, une défaillance des connexions. Tout amateur de radio sait bien que le plus beau montage peut se trouver ruiné par le grésillement d'une soudure.

Et justement, la majorité des débilités mentales, peut être neuf sur dix, correspondent à ce type, un réseau en état, apparemment normal, et qui fonctionne à très bas rendement.

Ici toute analogie s'écroule, car si dans l'ordinateur les connexions sont des soudures elles ne sont que des pièces disjointes dans notre cerveau. Entre le bouton terminal d'un axone et la cupule réceptrice d'une dendrite il n'y a que le vide ou plutot le milieu intercellulaire, issu du flux sanguin au travers de la barrière hémoméningée. La synapse est ouverte à toute molécule qui viendrait à passer. Et cette ouverture est d'ailleurs tout à fait nécessaire puisqu'il faut que le médiateur chimique soit secrété par le 'bouton' et vienne frapper la cupule, pour que l'influx d'une cellule soit transmise à une autre, pour qu'un circuit s'établisse à l'intérieur du réseau.

Dès lors imaginons qu'une molécule parasite, ressemblant plus ou moins au médiateur chimique par ses dimensions et sa configuration électronique vienne parasiter induement la cupule réceptrice. Sans entrer dans le détail d'effets compétitifs entre molécules on peut simplement dire que le médiateur aura plus de mal à faire son office, qu'il en faudra de plus grandes quantités et, au bout du compte que, la connexion, la synapse réagira moins vite. Le temps de parcours des circuits se trouvant allongé, la vitesse d'interrogation sera diminuée et finalement l'information primaire qu'il fallait analyser puis comparer avec des résultats antérieurs risque d'être déjà évanouie avant que cette comparaison soit possible.

Pour citer un exemple bien connu, qui parut dans tous les journaux, l'ordinateur de bord de la fusée lunaire fut frappé lors de l'arrivée d'une véritable débilité mentale parce qu'il ne parvenait pas à analyser en temps utile les informations qui lui étaient fournies.

Ces phénomènes de saturation correspondent à l'évidence à des constatations familières; la lenteur de l'idéation et la viscosité de l'élocution sont des troubles classiques chez les débiles mentaux.

Pour lutter contre cet encombrement, le réseau central peut débrancher certains circuits qu'il contrôle ordinairement, comme par exemple l'homme attentif qui reste bouche bée et langue pendante parce qu'il utilise toute sa capacité à l'admiration d'une chose nouvelle ou à la réalisation d'un travail délicat. Ainsi du débile mental qui tire la langue sitôt qu'il ne la rétracte pas volontairement, tant il doit utiliser l'ensemble de son peu efficace réseau au moindre travail d'attention. Enfin la lenteur de l'apprentissage, la difficulté de mise en réserve d'une information délicate (nécessitant une analyse par de nombreux circuits) contraste spectaculairement avec la conservation de la mémoire quotidienne qui enregistre sur des circuits très simples, les faits de tous les jours. De même la préservation si remarquable des sentiments artistiques et moraux qui par leur nature même sont au delà de l'analyse.

On voit immédiatement que dans cette hypothèse simplifiée, les débiles mentaux seraient véritablement des 'drogués', c'est-à-dire souffriraient d'une intoxication légère mais continue, par une substance qu'ils accumuleraient du fait d'un blocage enzymatique dans une maladie récessive, ou d'un excès d'activité enzymatique dans une maladie par excès chromosomique.

Un exemple théorique, schématique à l'extrême, peut être proposé, bien que sa réalite ne puisse être actuellement confirmée ou infirmée par l'expérience (Lejeune, 1970).

L'absence d'hydroxylation en 4 de la phénylalanine conduit à l'accumulation, entre beaucoup d'autres substances, de phényléthylamine. Cette substance possède une action pharmacologique faible, mais de type adrénergique et l'on peut penser qu'elle puisse entrer en compétition avec la noradrénaline ou des dérivés voisins qui sont connus pour être des médiateurs chimiques dans les synapses cérébrales.

A l'inverse, si l'organisme fabriquait un excès de tyrosine, des faux médiateurs chimiques du type adrénergique aussi pourraient parasiter à leur tour des sites équivalents. Dans ces deux cas l'excès d'activité ou le blocage d'une étape biochimique simple pourraient avoir le même effet, une débilité mentale. En poursuivant cette induction purement théorique, on pourrait penser qu'une autre étape chimique pourrait unir deux à deux des dérivés de la

tyramine en les joignant par leur hydroxyle et l'on peut démontrer expérimentalement que de tels dérivés bloquent la plaque motrice du muscle par un effet semblable à celui du curare. Dans le cas de blocage de la 4 hydroxylase cette substance manquerait alors qu'elle serait en excès dans les maladies par excès d'enzymes. Enfin les sujets soumis à l'effet d'un faux médiateur du type adrénergique devraient avoir une sensibilité particulière aux médicaments agissant sur le système autonome.

On voit ainsi que les malades souffrant d'un blocage de l'hydroxylation en 4 de la phénylalanine seraient des débiles mentaux frappés d'une raideur caractéristique, et qu'au contraire ceux qui auraient un excès d'activité de cette réaction et des suivantes, du fait de l'excès de plusieurs gènes consécutifs, seraient eux aussi débiles mentaux, mais seraient mous, lents et anormalement sensibles à des drogues comme l'atropine et l'éphédrine.

Il est clair que ces deux tableaux sont ceux de l'idiotie phénylpyruvique d'une part et de la trisomie 21 d'autre part.

Ceci ne prouve nullement que le schéma discuté ici soit correct, mais montre simplement que le raisonnement peut amener à rechercher l'existence des mécanismes biochimiques précis, c'est-à-dire guider la recherche selon une démarche heuristique.

Pour dépasser ce stade il faudrait connaître la constitution chimique des synapses et plus précisément, la configuration moléculaire exacte du récepteur qui reçoit le médiateur chimique et est modifié par lui.

Ici un modèle, lui aussi purement théorique, pourrait être envisagé. Pour qu'un flux d'ions puisse traverser une paroi à certains moments et non à d'autres, ce qui se passe dans une synapse excitée ou au repos, il faut nécessairement que cette paroi possède une porte et que cette porte soit ouverte ou fermée. Ici le médiateur chimique joue le rôle du 'démon de Maxwell' pour ouvrir ou fermer la trappe, et l'apparition de ce démon n'est guère surprenante puisque en dernière analyse un système qui extrait l'information du monde extérieur doit bien être capable de remonter le cours de l'entropie.

Une hypothèse élémentaire serait alors de remplacer la porte par un trou, par exemple celui d'un polypeptide cyclique, le trou étant spontanément bouché par la charge négative de la couronne des carbonyles.

L'ajustement du médiateur, ici l'acétylcholine, serait déterminée par la configuration électronique des acides aminés formant la margelle du puits (ici une histidine) si les charges du site et celles du récepteur sont exactement opposées pour chacun des atomes en contact (ce qui est le cas dans ce modèle). Ainsi la charge positive de l'azote terminal se trouverait au contact de la couronne de carbonyles et annulerait la barrière de charge; autrement dit ouvrirait le trou.

Il est clair qu'un tel modèle ne prétend pas décrire la réalité puisque la configuration du récepteur cholinergique, le plus simple de tous, est encore inconnue. Par contre ce type d'investigation permettra peut-être de guider la recherche sur une voie qui cerne de plus près le mécanisme intime de la débilité mentale. Et il est inutile d'insister sur le fait que si la substance parasite était connue, il suffirait de l'éliminer, soit par le régime soit par un antidote pour ramener le réseau à un fonctionnement normal.

Cet exemple de la débilité mentale nous a mené bien loin de notre propos et cette longue parenthèse sur le fonctionnement possible du substratum de l'intelligence nous a bien écartés semble-t-il du titre de cette communication 'du bon usage de la génétique'.

Et pourtant, il n'en est rien je crois. A côté de l'usage prudent qui fut toujours celui de notre discipline et à l'encontre de l'usage désespéré que j'ai évoqué au début, le bon usage de la génétique est de la rendre vraiment humaine, c'est-à-dire de la mettre au service des malades au lieu d'utiliser nos connaissances partielles à instruire des jugements que le progrès scientifique rendra ridicules demain.

En toute vérité il importe finalement assez peu que les représentations moléculaires dont nous discutions il y a un instant soient une approximation satisfaisante de la réalité. Ce qui importe vraiment, c'est de rassembler nos forces pour ébranler le mur de l'igno-

rance qui déjà se lézarde, c'est de forcer une brèche, là ou nous ne rencontrons qu'obstacles.

Devant l'énormité de la tâche et la formidable nécessité de réussir, le bon usage de la génétique humaine se résume en une seule certitude: 'nous n'abandonnerons jamais'.

BIBLIOGRAPHIE

LEJEUNE, J. (1970): Sur un modèle de recherche dans certains cas de débilité mentale. *C.R. Acad. Sci. (Paris)*, *271*, 2068.

L. Gedda

Institute of Medical Genetics, University of Rome, Rome, Italy

Remarques concluantes

Mayer et Lejeune nous ont présenté la Génétique comme la protagoniste d'une révolution, la plus importante que l'humanité ait connue au cours de son histoire: la révolution biologique.

Mayer en a tracé le profil dans son développement scientifique; depuis son départ – botanique – dans le monastère de Brno, jusqu'à son arrivée – moléculaire – en Amérique, en Angleterre et enfin dans le monde entier; avec ses vicissitudes, les polémiques, les heurts surgis des rencontres avec les idées de Lamarck, de Darwin, de Lysenko et d'autres; enfin, avec ses rayonnements dans le délicat secteur du comportement éthique et de la pensée religieuse.

Lejeune a ouvert toutes grandes les portes de la médecine moderne. Il prête à la Génétique les vertus de l'ange gardien, pour le médecin généraliste aussi bien que pour le spécialiste, pour la médecine individuelle comme pour l'épidémiologie, pour la médecine curative comme pour la médecine préventive, pour le conceptus comme pour l'enfant qui n'est pas encore conçu et que les époux attendent; la Génétique préventive ou eugénique est concernée.

Certes, nous ne pouvons encore rien dire sur le plan des faits du mystérieux passage de l'inorganique à l'organique, de l'organique à l'homme; mais, dès maintenant, nous avons l'exaltant pouvour de déceler dans l'architecture de notre univers une ligne unique: une spirale d'innombrables êtres allant s'élargissant dans le devenir du temps physique et s'épanouissant en harmonieuse séquence, à l'image du coup de pinceau qui était la signature de Giotto.

Dans cette construction, l'homme a le rôle de résumer le tout, non seulement au moyen des gènes qui, dans le temps biologique, qui est leur attribut, reflètent les phases du temps cosmique, mais en vertu de l'intelligence qui perce la matière et cherche la cause.

La perspective historique de Mayer et celle médicale de Lejeune nous ont présenté la Génétique Humaine de cette manière complète et totale, souhaitée par Maurice Lamy et ses collaborateurs, auxquels nous avons le plaisir et le devoir d'exprimer notre admiration et notre reconnaissance pour le noble travail, la longue espérance et pour le brillant succès.

Interprète des sentiments et des voeux des représentants nationaux, aussi bien que de tout le Congrès, j'ai l'honneur d'annoncer que le Comité Permanent a élu à la charge de Président, pour les cinq années à venir, le Professeur Maurice Lamy.

Beaucoup de peuples, un seul peuple. Aucune science plus que la Génétique n'a, je le crois, le droit, et la raison, d'affirmer cette vérité à la face du monde.

Subject index

Prepared by J. B. Bijlsma, M.D., Amsterdam

Index of authors

Human Genetics

- *An abstract journal published since 1963*
- *1 volume yearly in 10 issues*
- *Subscription price: Dfl. 205.00 (ca. $64.00) per volume (plus postage)*

Excerpta Medica brings the world's biomedical literature to your desk. Quick reading, accurate and comprehensive, each issue of *Human Genetics* contains hundreds of abstracts of recently published articles important to your speciality.

First published in 1963, *Human Genetics* currently contains approximately 4,000 abstracts a year, published in volumes of ten issues each. A computer-collated subject/author index is provided with each issue and at the end of each volume.

Excerpta Medica