**Progress in medical genetics. Volume 6 / edited by Arthur G. Steinberg and Alexander G. Bearn.**


**Contributors**

Steinberg, Arthur Gerald, 1912-
Bearn, Alexander G., 1923-2009.

**Publication/Creation**

London : William Heinemann (Medical Books) Ltd., 1969.

**Persistent URL**

https://wellcomecollection.org/works/ur2tg35z

**License and attribution**

You have permission to make copies of this work under a Creative Commons, Attribution, Non-commercial license.

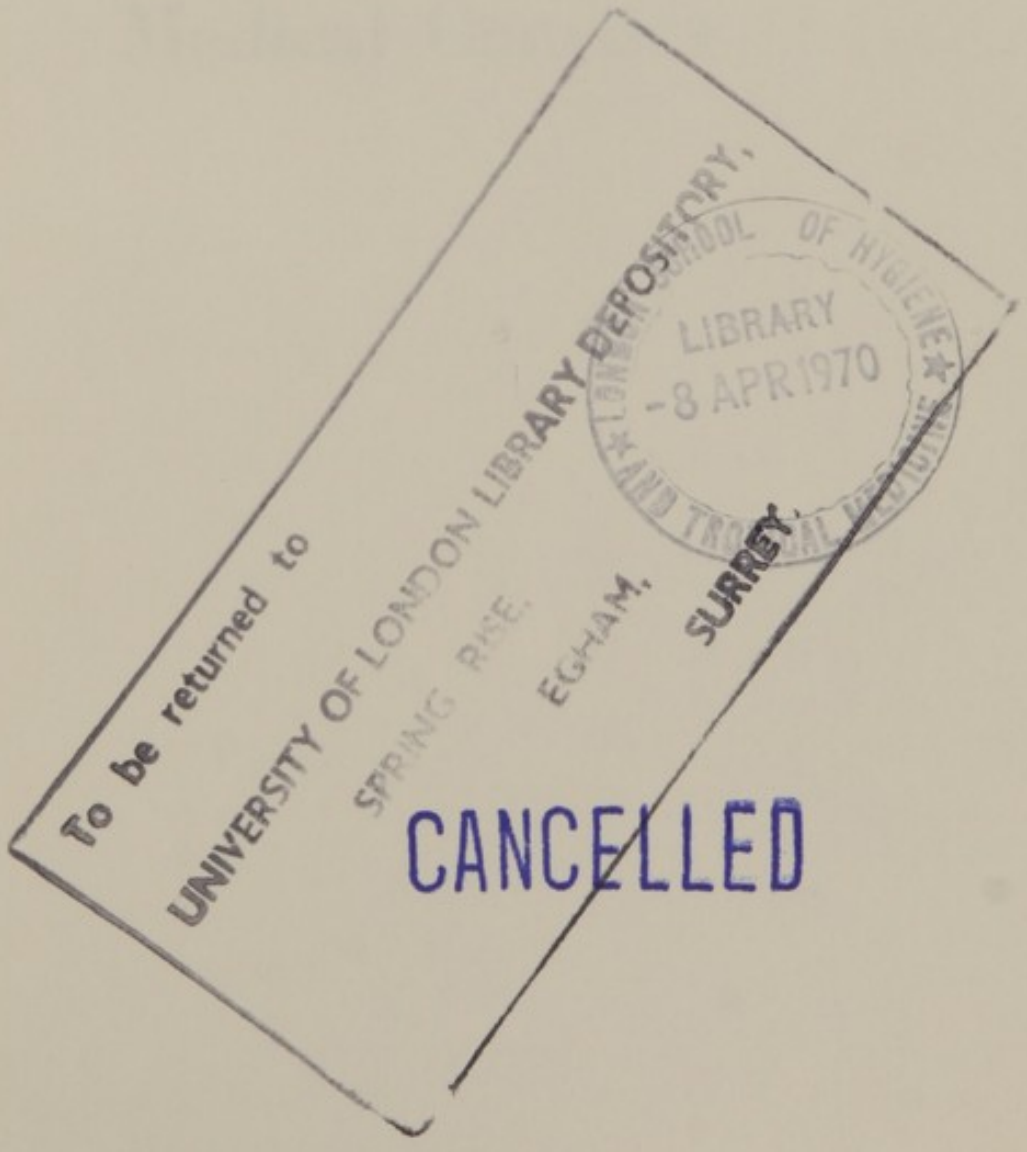
Non-commercial use includes private study, academic research, teaching, and other activities that are not primarily intended for, or directed towards, commercial advantage or private monetary compensation. See the Legal Code for further information.

Image source should be attributed as specified in the full catalogue record. If no source is given the image should be attributed to Wellcome Collection.

wellcome collection

Wellcome Collection
183 Euston Road
London NW1 2BE UK
T +44 (0)20 7611 8722
E library@wellcomecollection.org
https://wellcomecollection.org

22501959066


To be returned to
UNIVERSITY OF LONDON LIBRARY DEPOSITORY,
SPRING RISE, EGHAM, SURREY.
LONDON SCHOOL OF HYGIENE
LIBRARY
-8 APR 1970
CANCELLED

PROGRESS IN

# Medical Genetics

PROGRESS IN

# Medical Genetics

Volume VI

Edited by

**ARTHUR G. STEINBERG, Ph.D.**

Professor of Biology, Department of Biology, and Associate Professor of Human Genetics, Department of Preventive Medicine, Case Western Reserve University, Cleveland, Ohio

and

**ALEXANDER G. BEARN, M.D.**

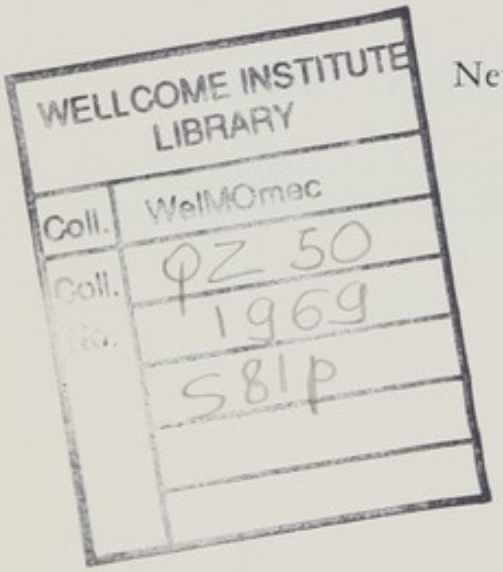
Professor and Chairman of the Department of Medicine, Cornell University Medical College and Physician-in-Chief, The New York Hospital, New York

WILLIAM HEINEMANN MEDICAL BOOKS LTD
LONDON

Library of Congress Catalog Card No. 60-53514

Copyright © 1969

GRUNE & STRATTON, INC.
381 Park Avenue South
New York, New York 10016




Printed in the U. S. A.
(St-A)

# Contents


Foreword vii

1. Chromosomal Abnormalities in Clinical Medicine
*D. H. Carr* 1

2. Genetics and the Gastrointestinal System
*Richard B. McConnell* 63

3. The Genetics of Thyroid Disease
*G. R. Fraser* 89

4. Genetic Aspects of Autoimmunity
*Philip J. Fialkow* 117

5. The Application of Bacterial Genetics to the Study of Human Genetic Abnormalities
*Jean-Claude Dreyfus* 169

6. Histocompatibility in Man—Genetic and Practical Considerations
*Fritz H. Bach* 201

7. The Genetics of Intestinal Carbohydrate Intolerance
*E. Eggermont* 241

Author Index 280

Subject Index 286

# Foreword

SEVEN YEARS AGO when the first volume of *Progress in Medical Genetics* was published, it was not completely clear whether the tempo of discovery would justify an annual review. In the intervening years, it has become apparent that the rate of accumulation of new genetic knowledge and its relevance to man has exceeded all expectations. Indeed, Barton Childs has recently collected data which indicate that the number of papers in genetics has been increasing logarithmically during the past decade. This volume represents our annual attempt to cover some of the more rapidly moving subjects of relevance to students of medical genetics. As in previous years, the contributors have been urged to write a critical essay rather than an exhaustive and comprehensive review.

The past 12 years have witnessed an astonishing advance in our knowledge of human chromosomes. The role of chromosomal abnormalities in clinical medicine is now such that most clinical centers have a laboratory devoted to their study. D. H. Carr presents a timely and incisive review of the theoretical and practical aspects of this important subject (Chapter 1). The genetics of disorders of the gastrointestinal tract are less clear-cut than that of many other organ systems; nevertheless our understanding of these will be of particular value to physicians concerned with these disorders. R. C. McConnell critically reviews this subject (Chapter 2). The genetics of thyroid disease is an area of investigation which has advanced our knowledge of biochemical genetics, as well as clinical medicine; G. R. Fraser discusses these problems (Chapter 3). The genetics of autoimmunity is an increasingly important problem of theoretical and practical interest. P. Fialkow (Chapter 4) presents a thorough review of our knowledge in this field and indicates areas in which further investigation is needed. The far-reaching discovery of the nature of genetic control mechanisms in *E. coli,* by Jacob and Monod, has prompted many to wonder about the applicability of these ideas to a diploid organism such as man. A discussion in which the difficulties of adapting the Jacob-Monod hypothesis to man can be found in the chapter by J. C. Dreyfus (Chapter 5). The chapter by F. H. Bach (Chapter 6) is a survey of our present knowledge of transplantation genetics with particular emphasis on the

histocompatibility locus in man. With the acceleration of organ transplantation in man, this chapter takes on particular importance. In the last chapter of this volume (Chapter 7) E. Eggermont reviews our current knowledge of the genetics of carbohydrate intolerance.

The editors owe considerable gratitude to the authors who, despite increasing demands on their time, agreed to undertake these reviews. It is a pleasure to acknowledge once again our indebtedness and our admiration.

A. G. S.
A. G. B.

CHAPTER 1

# Chromosomal Abnormalities In Clinical Medicine

**D. H. Carr**
McMaster University,
Hamilton, Ontario

OVER 100 DIFFERENT TYPES of human chromosome disorders have been described since the first anomaly was reported in 1959. The first abnormalities disclosed the underlying disorder in 3 well-known syndromes. Previously unrecognized syndromes associated with chromosome anomalies have now been described. The rapid appearance of papers, often reporting single cases, led clinical cytogenetics through a period of chaos. The smoke has now cleared sufficiently to allow a prognosis for the individual suffering from a chromosome anomaly, to relate chromosome anomalies to one another and to predict certain general effects for a particular disorder.

It is my intention to offer not a catalogue of human chromosome anomalies found during the last 8 years, with several thousand references, but a discussion of original discoveries and key contributions to various disorders of clinical importance. Clinical cytogenetics has now reached a point of stability. This does not mean the subject is closed. However, it seems unlikely that many new, clearly-defined syndromes will be described in the future. Any new syndromes are likely to be based on a constellation of signs of increasing subtlety. This is hardly surprising, as syndromes with striking and characteristic features are likely to have been recognized already.

## SEX CHROMOSOME ABNORMALITIES IN PHENOTYPIC MALES

### 1. *Klinefelter's Syndrome*

The way for the discovery of sex chromosome disorders in phenotypic males was paved by the discovery that certain men had sex chromatin in their interphase nuclei. Plunkett and Barr (1956) noted the presence of sex

chromatin in buccal smears of men with Klinefelter's syndrome. They suggested that the presence of sex chromatin may be due to an XXY sex chromosome complex in affected males. This conjecture was confirmed in 1959 by Jacobs and Strong. Since that time, more than a dozen sex chromosome variants have been described in this syndrome.

The syndrome, as described by Klinefelter, Reifenstein and Albright in 1942 included small testes, gynecomastia, and increased urinary follicle-stimulating hormone. It has now come to have a somewhat different meaning and includes phenotypic males with 2 or more X chromosomes, with or without a number of Y chromosomes. The only other regular finding is testicular atrophy with azoospermia or oligospermia. A modification of the title is sometimes given to men with the same clinical features but a normal male karyotype (46,XY) — " 'false' Klinefelter's syndrome" (Barr, 1966). Other features of Klinefelter's syndrome include diminished facial and body hair, increased body height, increased length of limbs and normal size of the penis. Gynecomastia is found in only 25 per cent of cases (Raboch, 1964).

The incidence of the condition at birth appears to be about 1 in 480 males, according to 5 neonatal buccal smear surveys of over 18,000 infants. (Moore, 1959; Bergemann, 1961; Maclean, Harnden, Court Brown, Bond and Mantle, 1964; Marquez-Monter and Carnevale-Lopez, 1966; Bochkov, 1965). Two other reports are at variance with these five. In one, from India, no chromatin-positive smears were found in more than 2,000 boys examined (Subray and Probhaker, 1962). In the other report, which, like Bergemann's study, came from Switzerland, sections of placenta were used instead of buccal smears (Wiesli, 1962). Only one chromatin-positive male was found among 1,563 and the infant was anencephalic.

A comparison has been made between the incidence in the newborn and various groups of males, especially different categories of mentally retarded subjects and patients with various mental illnesses. The results, including much of his own material, have been discussed by Hambert (1966). There is considerable variation in the findings of different investigators but certain generalizations are possible. In all larger buccal smear surveys of populations of the mentally retarded, the number of chromatin-positive males is higher than it is at birth, varying from twice to 11 times as high. Polysomy-X in the male also appears to be commoner in those with an I.Q. above 50 than it is in the more severely retarded. Hambert also concludes, from his own studies and those of others, that chromatin-positive Klinefelter's syndrome is increased among epileptics and patients admitted to mental hospitals with a variety of diagnoses.

Returning to the relationship between mental retardation and Klinefelter's syndrome, the commonest variety (XXY) must be considered first. The

incidence of mental retardation in this type is probably above 25 per cent. Raboch and Sipova (1961) found that 1 in 4 chromatin-positive men attending an infertility clinic were mentally retarded. This is probably a low estimate overall because the more severely retarded would not be found in such a population. It seems probable that an increase in the number of X chromosomes above 2 and perhaps also Y chromosomes above 1 increases the risk of retardation. Most subjects with multiple X and Y chromosomes have been found during buccal smear screening of patients in institutions for the retarded. The only unbiased way to assess the effect of these anomalies on development is by follow-up of subjects found to have sex chromosome anomalies during routine surveys of the newborn (Valentine and Sergovich, 1967).

The cytogenetic spectrum of Klinefelter's syndrome, as defined above, has been investigated in several surveys (Table 1). In all published reports, re-

TABLE 1. — *Surveys of Chromatin-positive Males*

| Author | Sex Chromosomes | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | XXY | XXXY | XXXXY | XXYY | XX | XX/XXY | XY/XXY | Others |
| Court Brown et al., 1964* | 99 | 4 | 1 | 3 | 2 | 2 | 16 | 6 |
| Barr et al., 1964 (and unpublished data) † | 17 | 3 | 2 | 3 | 0 | 0 | 2 | 0 |
| Hambert, 1966* | 32 | 2 | 0 | 2 | 0 | 0 | 3 | 1 |
| Makino, 1966* | 29 | 1 | 1 | 1 | 0 | 1 | 0 | 0 |
| Zuppinger et al., 1967‡ | 23 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Froland, 1967* | 66 | 2 | 4 | 0 | 2 | ? | ? | 2 |

* Various sources
† Three institutions for the retarded
‡ General Hospital

gardless of the source of the subjects studied, 47,XXY is by far the commonest sex chromosome complex. Some rare karyotypic variations have only been reported in single cases and bring the total variants to fourteen or so.

The phenotype in this syndrome is related in part to the cytogenetic picture. Mosaicism can presumably modify the effect, as a fertile male with XXY/XY karyotype has been reported (Court Brown, Harnden, Jacobs, Maclean and Mantle, 1964). For this reason, the effect of the sex chromosome anomalies on development can only be judged if mosaicism is absent. It appears that the XXY anomaly does not increase the risk of early intrauterine death (Carr, 1967). However, although this disorder has not been

described in spontaneous abortions, it does appear to have an increased incidence in stillborn infants. MacLean, Harnden, Court Brown, Bond and Mantle (1964) found 4 chromatin-positive males among 212 cases of neonatal death, 3 of whom were stillborn and 1 who died a day after birth. Bochkov (1965) reported 2 chromatin-positive buccal smears among 57 stillborn males and 5 among 211 male infants who died within the first month. Though the numbers are small this suggests an increased perinatal mortality. Perhaps related to this is the observation by Gautier (1966) of approximately 1 per cent of XXY males among a series of children with congenital heart disease.

The development of the gonads is undoubtedly affected at puberty but the microscopic findings in the testes of 47,XXY infants is variable. Germ cells may be moderately reduced (Ferguson-Smith, 1959; Mosier, Scott and Cotter, 1960), markedly reduced (Conen, Erkman and Jeffs) or absent (Lawrence and Yuceoglu, 1961; Khorsandi, Alexander, Bryans and Haust, 1964). Peritubular fibrosis may be seen as early as 7 years of age and spermatogenesis is rarely seen in the testes of adults with Klinefelter's syndrome (Barr, Shaver, Carr and Plunkett, 1960). On the few occasions that sperm have been found in the ejaculate of these patients, oligospermia was extreme (Futterweit, 1967). There has been one very well documented example of fertility in a 47,XXY male and it was concluded that the probability of the man being the progenitor of his offspring was greater than 5 to 1 (Warburg, 1963).

In discussing the effect of mosaicism on the phenotype, a serious problem arises because there is great bias in selection of subjects. Those with symptoms and in need of medical advice will present themselves at clinics. There may on the other hand be many normal men with a proportion of their body cells having an XXY sex chromosome complement. The commonest mosaicism in Klinefelter's syndrome is 46,XY/47,XXY. When this complement is found in a typical case, the phenotype, by definition, is not modified. At the other extreme, the XY cell line may lead to the development of normal testes and the patient is thus eliminated from the category of Klinefelter's syndrome (Barr, Carr, Morishima and Grumbach, 1962).

Increasingly complex sex chromosome complements in males produce certain phenotypic variations. In the XXYY anomaly, first described by Muldal and Ockey (1960), the individuals tend to be of above average height (Barr, Carr, Soltan, Wiens and Plunkett, 1964), and they apparently have a predisposition to develop stasis dermatitis (Peterson, Gorlin, Peagler and Bruhl, 1966).

In a study of 942 males in two institutions for the "hard-to-manage" male, Casey, Segall, Street and Blank (1966) found 21 chromatin-positive subjects,

7 of whom proved to have an XXYY sex chromosome complex. In one of our own studies in institutions for the retarded, in which 1,215 male inmates were studied specifically excluding psychotic or violent patients, it turned out that of the 10 chromatin-positive males there, 3 had XXYY complements (Barr, Carr, Soltan, Wiens and Plunkett, 1964). Although our series had a much lower percentage of chromatin-positive males, the proportion of XXYY individuals was almost the same. But leaving that aside, the findings of Casey, Segall, Street and Blank (1966) led to a chromosome study of "hard-to-manage males" which suggested a relationship between extra Y chromosomes and aggressive behavior. This will be discussed in detail below.

The most complex sex chromosome constitution in chromatin-positive males is XXXXY. This disorder has been recorded, in pure or mosaic form, some 40 times since its original description by Fraccaro, Kaijser and Lindsten (1960a). This plethora of reports, which exceeds those for any other sex chromosome complex except XXY, must surely reflect a fascination with high numbers, both by authors and editors. It is inconceivable that the XXXXY anomaly is as common a variant in Klinefelter's syndrome as suggested by its reporting. Court Brown, Harnden, Jacobs, Maclean and Mantle (1964) noted this anomaly (or a mosaic with XXXY) 3 times in 134 cases from different sources. Our own findings among 2,500 males in various institutions for the retarded are shown in Table 1.

Zaleski, Houston, Pozsonyi and Ying (1966) have reviewed the literature on 30 cases of the XXXXY anomaly. In no case did the I.Q. exceed 60 and in many instances it was much lower. The testes were very small and in one third they were undescended. Skeletal anomalies were found in the elbow and wrist regions of all patients who had x-rays. Eight of 19 had radioulnar synostosis, a fusion of the two bones of the forearm. Other developmental anomalies included flattening of the nasal bridge and curving of the little finger. At the microscopic level, absence of Leydig cells contrasts with the excessive prominence of these cells in the testes of patients with an XXY sex chromosome complex. Thus, the XXXXY variant has features which separate it from other types of the broadly defined Klinefelter's syndrome.

Finally, some male subjects with clinical and pathological features often indistinguishable from regular Klinefelter's syndrome have only a normal female complement (46,XX). Examples of this condition were described simultaneously from three centers and others have been reported since (de la Chapelle, Hortling, Niemi and Wennström, 1964; Therkelsen, 1964; Court Brown, Harnden, Jacobs, Maclean and Mantle, 1964). The development of a testis in the absence of a Y chromosome requires explanation. Hecht, Antonius, McGuire and Hale (1966) found a very low proportion of XXY cells in a patient originally thought to have only female cells. Ferguson-

Smith (1967) has suggested a reciprocal translocation between parts of the X and Y as a source of testis-determining genes in the XX male. de la Chapelle, Hortling, Wennström, Niemi and Johansson (1965) reported Xga studies in 2 males with 46,XX chromosomes. In each family, the XX patients were Xg(a–) while their father was Xg(a+). This could be explained by assuming that both X chromosomes came from the mother and that no sex chromosome came from the father. This requires a non-disjunctional event in both parents, which seems an unlikely occurrence. Ferguson-Smith suggests that a reciprocal translocation between the X and Y chromosomes could explain both phenomena. Suppose the Xg locus on the X were translocated to the Y and male determining genes on the Y exchanged to the X chromosome. This would allow one X chromosome of the patient to have come from the father while explaining the presence of testes and the Xg findings.

*2. The XYY Anomaly*

The first report of an XYY sex chromosome complex resulted from the investigation of a man who had a 50 per cent incidence of abnormal progeny from 2 marriages (Hauscha, Hasson, Goldstein, Koepf and Sandberg, 1962). Following this, a number of reports of XYY males appeared in the literature, without clear-cut identifying features but including mental retardation, hypogonadism and congenital abnormalities (Kosenow and Pfeiffer, 1967).

During the last 2 years, this disorder has taken on new importance and significance. As stated earlier, 7 XXYY males were found among 21 chromatin-positive patients in an institution for the mentally subnormal requiring special security (Casey, Segall, Street and Blank, 1966). On the other hand, in a buccal smear survey of 2,607 men and boys in ordinary institutions for the retarded, only 2 of 28 chromatin-positive males had an XXYY sex chromosome complex (Maclean, Mitchell, Harnden, Williams, Jacobs, Buckton, Baikie, Court Brown, McBride, Strong, Close and Jones, 1962). This discrepancy between the 2 series suggested that the presence of extra Y chromosomes might be associated with unusually aggressive behavior (Jacobs, Brunton, Melville, Britain and McClemont, 1965). These workers therefore studied the chromosomes of 197 individuals who were "mentally subnormal male patients with dangerous, violent or criminal propensities." Among these 197 patients, 12 were found to have chromosome disorders, including 7 who were 47,XYY and one with an XXYY sex chromosome constitution. There was also a significant increase in height between this group and those with normal male chromosomes. This supported the suggestion made earlier for XXYY males that extra Y chromosomes lead to an increase in height. It was concluded by these investigators that a male in this category of aggressive behavior measuring over 72 inches in height had about a 50

per cent chance of having an XYY sex chromosome constitution. This high incidence of the XYY anomaly in "hard-to-manage" males has been confirmed (Casey, Blank, Street, Segall, McDougall, McGrath and Skinner, 1966). These workers studied 5 groups of chromatin-negative males over 6 feet in height. They found 12 XYY males among 50 who were detained for antisocial acts and were also mentally subnormal, and 4 among 50 who were detained for the same reason and were also mentally ill. Welch, Borgaonkar and Herr (1967) studied "defective delinquents" in an institution. The only combination of antisocial acts and increased height which yielded an XYY male was in a group of 21 inmates who were over 74 inches tall. (It should be noted that one other member of this group had an extra chromosome indistinguishable from members of the D group.) Their results do not support the findings reported above, and other similar populations require study.

Price and Whatmore (1967) studied a group of XYY males and found them to be different from those in the same population of aggressive delinquents whose chromosomes were normal. The men with a 47,XYY complement began their criminal activities earlier, resisted conventional treatment, had negative family histories and displayed less violence against persons.

In retrospect, it is interesting to review the earlier reports of the XYY disorder, referred to previously. Aggressive behavior was apparently not a striking feature in these cases, nor even in a 5-year-old boy with 48,XYYY chromosomes (Townes, Zeigler and Lenhard, 1965).

## Females with X-Polysomy

The commonest sex chromosome anomaly in the female is the presence of one extra X chromosome. Its effect is not obvious, as no specific syndrome is caused by an XXX sex chromosome complex. There were 2 principal features in the first human "super female": mild mental retardation and secondary amenorrhea (Jacobs, Baikie, Court Brown, MacGregor, Maclean and Harnden, 1959). A large number of XXX females have been reported since then and there seems little doubt that the proportion of mental retardation in reported cases is increased. However, gynecological complaints such as infertility and amenorrhea proved to be unusual.

Duplicated sex chromatin, indicating the presence of 3 X chromosomes, was found in 12 of 10,000 newborn female infants. (Maclean, Harnden, Court Brown, Bond and Mantle, 1964). Chromosome studies in 9 of the 12 infants in which culture was successful, confirmed the XXX sex chromosome complement. It is clear that this anomaly is compatible with normal development and fertility (Close, 1963; Baikie, Garson, Weste and Ferguson, 1966; Malvaux, van den Berghe and Visscher, 1967).

The majority of patients with the XXX anomaly have been discovered during buccal smear surveys of institutions for the retarded. This, of course, introduces a bias in judging the significance of mental retardation in this disorder. However, the results of 12 surveys of the mentally retarded show the XXX sex chromosome complex to be 3½ times higher than its incidence at birth (Table 2). This is almost the same as the increase in Klinefelter's syndrome among the retarded when compared with the newborn. The total number of males examined in 19 series quoted by Hambert (1966) was 13,379, including 114 who were chromatin-positive. This figure of 1 in 120 among the retarded is about 4 times the incidence at birth. If we can extrapolate, therefore, it seems probable that about the same proportion of XXX females exhibit mental retardation as do chromatin-positive males. This figure was set at about 25 per cent or a little higher for the latter. If these deductions are valid, it means that the vast majority of triple-X females are members of the normal population. As they remain mostly undetected, their reproductive capacity is important.

The majority of XXX females are free of serious physical defects and as far as is known have no increased prenatal mortality. However, triple-X females with serious anomalies have been described, including major congenital heart disorders (Lévêque and Guérineau, 1965; Gilgenkrantz, Gilgenkrantz, Faucher and Pierson, 1966; Gautier, 1966).

There is information about 27 progeny from 9 triple-X females. The sex chromosome complex is known for 4 male and 2 female children of XXX women (Fraser, Campbell, MacGillivary, Boyd and Lennox, 1960; Close, 1963; Day, Larson and Wright, 1964; Rosenkranz, 1965; Anderson, 1965). One of these is a male Klinefelter's syndrome with XXY sex chromosome complex. In addition, there are 9 male and 4 female offspring of triple-X women whose sex chromatin findings are normal (Barr and Carr, 1960; Court Brown, Harnden, Jacobs, Maclean and Mantle, 1964). Finally, a triple-X woman had 10 children, 8 of whom are living fertile males (Baikie, Garson, Weste and Ferguson, 1966). The fertility of these 8 males is presumptive evidence against the presence of XXY sex chromosomes. We can also add one XXY male, the child of an XXX/XX mosaic woman (Rosenkranz, 1965). Thus, 28 progeny of XXX females includes only 2 males in whom the extra X chromosome was passed from the mother (secondary non-disjunction). This is quite different from the situation in women with mongolism (triple-21 syndrome) who have had children.

As in males with extra X chromosomes, so in females, there is suggestive evidence of an increase in psychosis with X polysomy. Olanders (1967) found duplicated sex chromatin in buccal mucosal smears of 6 out of 1,589 unselected females in mental hospitals in Sweden. This corresponds to a

prevalence of 0.38 per cent, or 3 times as high as in the newborn. Other relevant supporting data are reviewed by Olanders.

Tetrasomy and pentasomy X in females appears to be much rarer than their male counterparts. Tetrasomy-X appears to have been reported in only 4 subjects (Carr, Barr and Plunkett, 1961; Bergemann, 1962; Day, Larson and Wright 1964). Even more striking is the contrast between 40 reports of the XXXXY anomaly in males against 2 cases of XXXXX females (Kesaree and Wooley, 1963; Brody, Fitzgerald and Spiers, 1967). The reason for this difference is not clear.

## Gonadal Dysgenesis and its Chromosomal Variants

As in Klinefelter's syndrome in the male, an indication of a sex chromosome anomaly in phenotypic females preceded the development of techniques to prove it. In 1954, 3 groups of workers independently demonstrated the absence of sex chromatin in individuals with Turner's syndrome (Decourt, Sasso, Chiorboli and Fernandes, 1954; Polani, Hunter and Lennox, 1954; Wilkins, Grumbach and van Wyke, 1954). As a result of color-blindness studies, Polani, Lessof and Bishop (1956) suggested that these chromatin-negative females had an XO sex chromosome complement. This observation was confirmed 3 years later by chromosome analysis of a girl with Turner's syndrome (Ford, Jones, Polani, de Almeida and Briggs, 1959).

In 1955, Swyer described 2 chromatin-negative girls, who were tall and free from congenital defects. He believed they were suffering from an undescribed form of male pseudohermaphroditism. Hoffenberg and Jackson (1956) suggested that these cases were a variant of gonadal dysgenesis. We now know that the 2 proposed diagnoses were not far removed from one another and that the sex chromosome complexes in each are similar though variable.

Since 1959, more than a dozen chromosomal variants have been described in association with different types of gonadal failure in phenotypic females.

### *1. XO Gonadal Dysgenesis*

The cytogenetic spectrum of gonadal dysgenesis from six surveys is shown in Table 3. Each of the 6 series were of patients with signs of dysgenesis of the gonads, though other features varied. In 51 per cent of the patients the sex chromosome complex was XO, without signs of mosaicism. In another 34 per cent, there was an XO cell line in a subject showing mosaicism. Apart from the dysgenesis of the gonads, the greatest common factor related to height. Only one of the 121 patients with an XO sex chromosome complex measured over 150 cm. in height and many were considerably shorter. In 4 of the series there was no selection for shortness of stature, whereas in the other 2, only short patients with certain congenital defects were included

(Lindsten, 1963; Lamy, Josso, Grouchy and Bitan, 1965). In Lindsten's series there were no women with normal male karyotype 46,XY. However, Lamy, Josso, Grouchy and Bitan (1965) reported this chromosome complement in an apparently typical case of Turner's syndrome. The lack of growth is manifest *in utero* among XO fetuses (Singh and Carr, 1966) and in neonates with this disorder (van der Verff ten Bosh, 1960).

Abnormalities are common in females with a 45,X chromosome constitution. About 90 per cent have skeletal abnormalities, often minor, the most characteristic being shortness of the fourth metacarpal or metatarsal and deformity of the medial tibial condyle (Lindsten, 1963; Kosowicz, 1959). Anomalies of the urinary tract are found in 50 to 60 per cent of XO females, horseshoe kidney being the most serious (Anderson and Probst, 1963; Jeune, Bertrand, Deffrenne and Forget, 1963). Cardiovascular abnormalities vary in this syndrome according to the age at which patients are studied. In a pediatric population of 25 XO females, there were 9 cases of coarctation of the aorta and 4 other cardiovascular anomalies (Lemli and Smith, 1963). Lindsten (1963), studying XO patients of all ages, found only 2 out of 35 with coarctation of the aorta. In other series of individuals with a 45,X chromosome complement, cardiac anomalies were found in 9 of 44 patients (Lamy, Josso, Grouchy and Bitan, 1965) and in 4 of 38 subjects studied for a variety of reasons (Court Brown, Harnden, Jacobs, Maclean and Mantle, 1964). A history of lymphedema was mentioned in 20 out of 24 young patients with an XO sex chromosome complex ( Lemli and Smith, 1963). Cervical lymphangiomata have been noted frequently in XO fetuses (Singh and Carr, 1966) and occasionally in the newborn (Verger, Battin, Kermarec and Vital, 1962; Faint and Lewis, 1965). It seems probable that webbing of the neck and laxity of posterior neck skin are the end result of resorbing lymphangiomata. Although anomalies of the lymphatic system are common in association with a 45,X complement, it is apparently responsible for only a minority of cases of primary lymphedema. Benson, Taylor and Gough (1967) made a cytogenetic study of 26 phenotypic females with this disorder. Six had a chromosome anomaly: 2 were XO, 1 XO/XX mosaic, 2 had normal male karyotypes and 1 had a ring G chromosome.

The occurrence of mental retardation in 45,X females is difficult to assess. It is almost certainly lower than in Klinefelter's syndrome. Using the same 12 surveys as in Table 2, there were 9 females with XO or XO mosaicism mentioned by the authors. It is possible that some investigators reported only the X-polysomies, so this is a minimal figure. However, if this is valid, the proportion of 9 in 10,250 patients is about double that in neonates with the same anomaly (Maclean, Harnden, Court Brown, Bond and Mantle, 1964). It was noted previously that Klinefelter's syndrome is about 4 times as com-

Table 2. — *Triple-X Females in Institutions for Mentally Retarded*

| Investigator | Country | No. Examined* | *Double chromatin-positive* No. | % |
|---|---|---|---|---|
| Fraser et al. 1960 | Scotland | 595 | 4 | .68 |
| Ferguson-Smith 1962 | U. S.A. | 827 | 3 | .36 |
| Maclean et al. 1962 | Great Britain | 1907 | 8 | .42 |
| Hamerton et al. 1962 | England | 196 | 1 | .51 |
| Breg et al. 1963 | U. S. A. | 727 | 5 | .68 |
| de la Chapelle 1963 | Finland | 1256 | 2 | .16 |
| Hienz et al. 1963 | Germany | 769 | 2 | .26 |
| Ridler et al. 1963 | England | 735 | 2 | .27 |
| Day et al. 1964 | U. S. A. | 1088 | 6 | .55 |
| Anderson et al. 1964 | S. Africa | 899 | 4 | .44 |
| Nakagome et al. 1966 | Japan | 253 | 0 | 0 |
| Pfeiffer et al. 1967 | Germany | 1008 | 6 | .60 |
| | | 10,250 | 43 | .42 |

* Some were mosaic on chromosome analysis

mon in mentally retarded populations as it is at birth. An estimate was also made that about 25 per cent of subjects with Klinefelter's syndrome are mentally retarded. If these figures are applied to females who are XO we find that the condition is only twice as common among the retarded as it is at birth. This suggests that perhaps 12.5 per cent of XO infants show mental retardation. The figure is close to an estimate based on clinical data by Haddad and Wilkins (1959). In addition to an increased risk of frank retardation, patients with XO Turner's syndrome appear to possess a specific cognitive defect in space-form perception (Money and Alexander, 1966).

The most constant disorder in patients with the XO anomaly is defective ovarian development, by far the majority having only white linear streaks of connective tissue. The exceptions are few; and in order not to be misled by mosaicism, only those having an XO cell line will be considered. Monardo (1965) reported an XO female who had menstruated for 17 years, while a 14-year-old girl had "one possible primordial follicle" in an ovarian biopsy (Court Brown, Harnden, Jacobs, MacLean and Mantle, 1964). In another large series, 3 of 45,XO subjects had menstruated (Lamy, Josso, Grouchy and Bitan, 1965). One had some follicles in an ovarian biopsy, and another had large ovaries on culdoscopy which were not biopsied. The third had streaks with ovarian stroma only. Conclusive proof of ovarian function in an XO adult is provided by a patient who had a normal male child (Bahner, Schwarz, Heinz and Walter, 1960).

Gonadal histology in the XO newborn, as in the XXY newborn, varies from a normal condition (Conen and Glass, 1963; Shine and Corney, 1966),

through gross reduction (Froland, Lykke and Zachu-Christiansen, 1963; Carr, Haggar and Hart, 1967) to complete absence of germ cells (Court Brown, Harnden, Jacobs, Maclean and Mantle, 1964). A normal looking oocyte from the ovary of an XO infant who died at 8 days of age is shown in Fig. 1.

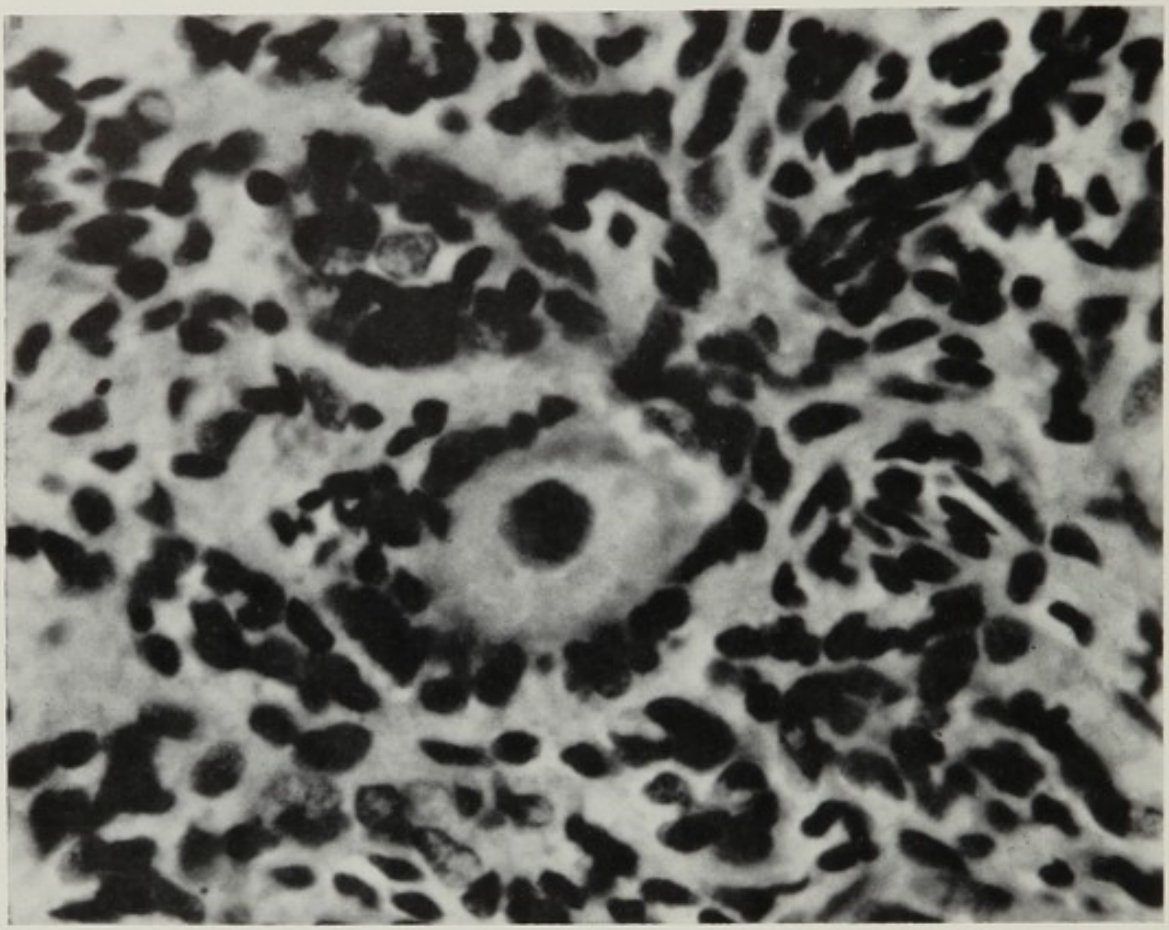


FIG. 1—Oocyte in ovary of XO infant.

Singh and Carr (1966), found normal-appearing ovaries containing germ cells in 9 embryos and fetuses, though there seemed to be a reduction in germ cells and an increase in connective tissue septa in older specimens.

Gonadal development in males with 47,XXY and in females with 45,XO chromosome complements seem to be comparable. The prenatal gonadal development in XXY males is unknown, but at birth the prevalence of germ cells is variable in both the XXY and XO anomalies. They both apparently undergo reduction at a stage which is normal for their sex but excessive in degree (Carr, Haggar and Hart, 1967) and there is a possibility of fertility in both conditions, without evidence of mosaicism (Warburg, 1963; Bahner, Schwarz, Hienz and Walter, 1960).

The relationship between chromosome anomalies and ovarian function has been approached in a different way. Three research groups from different

countries studied 150 cases of primary amenorrhea and 48 per cent had a chromosome anomaly. This is considerably lower than in the 237 patients studied because of "gonadal dysgenesis." The commonest chromosomal defect in both groups is the 45,X complement. Patients with normal female chromosomes, though identical chromosomally, differ among themselves in the degree and type of ovarian failure (Kinch, Plunkett, Smout and Carr, 1965).

The XO sex chromosome complement is uncommon among female newborn infants. In a survey of 10,000 females, only 4 were found to be chromatin-negative. Three were XO and one was an XO mosaic (Maclean, Harnden, Court Brown, Bond and Mantle, 1964). In another survey, 3 chromatin-negative female neonates were found in 1,516 smears (Marquez-Monter and Carnevale-Lopez, 1966). In the absence of chromosome studies, this may represent a local concentration of XY females. In the absence of further details, the figure of 4 in 10,000 will be accepted as the incidence of female neonates with 45,X chromosome complement. This contrasts markedly with the finding of 5 per cent XO anomaly in 227 spontaneous abortions of both sexes (Carr, 1967). If 15 per cent of human pregnancies end in spontaneous abortion it can be calculated that only about 1 in 40 zygotes, lacking a sex chromosome, go to term.

The reason for the high mortality in the XO anomaly is not clear. Most, if not all, of the congenital defects found in infants with Turner's syndrome are compatible with live birth. It may be that the X chromosome, when hemizygous, leads to high prenatal mortality because of lethal recessive genes. If this is so, it requires the acceptance of some function for the Y chromosome which makes the hemizygous male different. It has been shown that the Y chromosome in *Drosophila* suppresses the expression of X-linked recessive lethal genes (Lindsley, Edington and Von Halle, 1960) and the situation may be similar in man.

On the basis of Xg blood group studies of the parents of XO females, it appears that in about three quarters of them, the single X is maternal in origin (Edwards, Ferguson-Smith, Froland, Lindsten, Polani, Race and Sanger, 1966).

*2. Ovarian Failure and Chromosome Mosaicism*

As already mentioned, it is difficult to judge the modifying effect of a normal cell line in a percentage of body cells of someone carrying a chromosome anomaly. This is because the ascertainment of cases is through symptoms or abnormal signs. Starting from this anomalous position, there is evidence that a patient with XO/XX mosaicism does not show more favorable clinical features than one with 45,X cells alone (de la Chapelle, 1962; Jones, Ferguson-Smith and Heller, 1963). Lindsten (1963) found atretic ovarian

TABLE 3. — *Chromosome Studies in Gonadal Dysgenesis and Primary Amenorrhea*

| Authors | Sex chromosomes | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | XO | XO/XX | XXqi | XO/XXqi | XO/XXf | XO/XXp− | XO/XXq− | XO/XXr | XX | XY | XO/XY | Other | Total | % Chromosomes abnormal for phenotype |
| *Gonadal dysgenesis:* | | | | | | | | | | | | | | |
| de la Chapelle 1962 | 10 | 6 | 0 | 2 | 1 | 0 | 1 | 0 | 2 | 1 | 0 | 0 | 23 | 91 |
| Lindsten 1963 | 35 | 7 | 2 | 9 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 57 | 98 |
| Ferguson-Smith et al. 1964 | 7 | 8 | 1 | 5 | 1 | 0 | 1 | 1 | 3 | 0 | 0 | 3 | 30 | 90 |
| Lamy et al., 1965 | 46 | 4 | 0 | 4 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 2 | 59 | 96 |
| Engel and Forbes 1965 | 16 | 0 | 4 | 7 | 0 | 0 | 2 | 0 | 6 | 0 | 1 | 1 | 37 | 84 |
| Greenblatt et al. 1967 | 7 | 0 | 0 | 4 | 1 | 0 | 2 | 1 | 7 | 1 | 5 | 3 | 31 | 77 |
| *Totals:* | 121 | 25 | 7 | 31 | 3 | 1 | 6 | 3 | 21 | 3 | 6 | 10 | 237 | 91 |
| *Primary amenorrhea* | | | | | | | | | | | | | | |
| Jacobs et al. 1961 | 6 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 15 | 2 | 2 | 3 | 32 | 53 |
| Philip et al. 1965 | 12 | 4 | 0 | 0 | 1 | 0 | 0 | 0 | 60 | 18 | 2 | 3 | 100 | 40 |
| Jagiello et al. 1966 | 8 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 2 | 2 | 18 | 83 |
| *Totals:* | 26 | 8 | 1 | 0 | 1 | 1 | 1 | 0 | 78 | 20 | 6 | 8 | 150 | 48 |

follicles within a normal ovarian stroma of a patient with XO/XX mosaicism. However, in 6 other mosaic patients who had ovarian biopsies, no evidence of germ cells was found. Ferguson-Smith, Alexander, Bowan, Goodman, Kaufman, Jones and Heller (1964) found no primordial follicles in the ovaries of 6 patients who were mosaic for 45,X and another cell line. On the other hand, normal ovaries have been found in a 4-year-old child with XO/XX mosaicism (Mikkelsen, Froland and Ellebjerg, 1963). In addition, 2 women of proven fertility were found to be sex chromosome mosaics, one XX/XO (Mackay, Cockburn and Biggs, 1967) and the other XO/XX-/XXX (Armenderes, Buentello, Sanchez and Ortiz, 1967).

Ferguson-Smith (1965) reviewed the literature on gonadal dysgenesis with a view to correlating the chromosomal and phenotypic findings. It is possible to make some generalizations but they are certainly no more than a guide. The presence of normal female (46,XX) or triple-X cells in addition to an XO cell line may change the phenotype in the direction of normality, both for systemic effects and ovarian development. It may well be that XO/XX mosaicism is present in some perfectly normal fertile females. Deletion of the short arm (Xp–) rarely, if ever, alters the phenotype from that associated with XO sex chromosome complex. On the other hand, deletion of the long arm (Xq–) may result in gonadal failure in a woman of normal height (Ferguson-Smith, 1965). This does not appear to be invariably true, since a patient with deletion of the long arm of the X-chromosome may also be short (Conen, 1967).

In several patients with primary amenorrhea, one cell line, or more rarely all cells, have one normal X chromosome and a fragment of chromosome material too small for identification. Cohen, Sandberg, Takagi and MacGillivary (1967) have studied the replication pattern of this fragment by autoradiography in 5 cases in which the chromosome complex was mosaic with an XO cell line. In 2 of the 5 patients the small element proved to be a ring and this was late replicating. In the other 3 patients, the fragment was not late replicating. This does not exclude the possibility that the fragment was derived from an X-chromosome, since in isolation the pericentric region may not be late replicating.

It is of some practical importance to establish whether or not the fragment is derived from a Y-chromosome. Phenotypic females carrying Y-chromosomal material, even in mosaic form, seem to be prone to develop malignant gonadal tumors (see below).

The XO/XY type of chromosome mosaicism requires separate consideration. Each cell type alone would give rise to an individual of different sex, either sterile female (XO) or normal male (XY). It is not surprising therefore that this chromosome mosaicism has been associated with a variety of

phenotypes, including female XO gonadal dysgenesis, "male Turner's" syndrome, true hermaphrodite, male pseudohermaphrodite, mixed gonadal dysgenesis and pure gonadal dysgenesis (Jackson, Hoffman and Makda, 1966; Pfeiffer and Pawlowitzki, 1967; Greenblatt, Byrd, McDonough and Mahesh, 1967). It should be noted that each of these clinical categories can be associated with sex chromosome complexes other than XO/XY mosaicism.

*3. The XY Female*

The most characteristic condition in which a female phenotype is associated with a normal male karyotype (46,XY) is the testicular feminization syndrome. This condition is particularly associated with the names of Pettersson and Bonnier (1937) and Morris (1953). The sex chromosome complex is usually XY (Jacobs, Baikie, Court Brown, Forrest, Roy, Stewart and Lennox, 1959; Zourlas and Jones, 1965a) but there have been reports of this condition in which the sex chromosome complement was identical to that in Klinefelter's syndrome (German and Vesell, 1966; Botella Llusia, Clavero-Nunez and Nogales, 1964; Uozumi, Nozaki, Matsumoto, Seki and Furuyama, 1967).

Suffice it to say that testicular feminization is genetic in origin and usually a clearly defined syndrome. The testes produce normal male amounts of androgen but the male secondary sex characters fail to develop. On the contrary, the individual usually undergoes feminization at puberty (Zourlas and Jones, 1965a; Dewhurst, 1967; Rivarola, Saez, Meyer, Kenny and Migeon, 1967). There are, however, atypical cases in which breast development is accompanied by some masculinization, especially clitoral enlargement (Philip and Trolle, 1965; Teter and Boczkowski, 1966).

More recently, developments have suggested that other, less sharply defined, types of male pseudohermaphroditism are genetic in origin and related to a type of gonadal dysgenesis in which the chromosomes are normal male (46,XY). The latter combination of findings was first observed in a 19-year-old girl with primary amenorrhea (Harnden and Stewart, 1959).

Excluding typical cases of testicular feminization, 46,XY (or sometimes XO/XY mosaicism) has been described in 3 categories of predominantly phenotypic females: pure gonadal dysgenesis, "mixed" gonadal dysgenesis, and male pseudohermaphroditism. A relationship between these conditions was suggested when Alexander and Ferguson-Smith (1961) reported 9 cases of male pseudohermaphroditism, 4 of whom had a testis on one side and a streak gonad on the other. The latter type has since been separated from other male pseudohermaphrodites and called atypical or mixed gonadal dysgensis (Bergada, Cleveland, Jones and Wilkins, 1962; Sohval, 1964; Zourlas and Jones 1965b). The evidence that XY gonadal dysgenesis is genetic in origin comes from reports of its occurrence in sisters, twins, and in 3 dif-

ferent sibships of the same family (Cohen and Shaw, 1965; Brogger and Strand, 1965; Frasier, Bashore and Mosier, 1965; Sternberg and Barclay, 1967).

Having established a relationship between male pseudohermaphroditism and mixed gonadal dysgenesis and the familial occurrence of XY gonadal dysgenesis, the triangle was completed by the report of male pseudohermaphroditism and pure gonadal dysgenesis in sisters (Barr, Carr, Plunkett, Soltan and Wiens, 1967). In describing this family, the authors noted the frequency with which testicular remnants are found in the streak gonads of patients with XY gonadal dysgenesis.

On clinical grounds there is another reason for grouping females with XY gonosomes in the same category, and that is their predisposition to malignant gonadal tumors. This phenomenon has been stressed by several authors (Philip and Teter, 1964; Scott, 1965; Fathalla, Rashad and Kerr, 1966; Dewhurst, 1967). This tendency to develop tumors even in "streak" gonads is apparently dependent upon the presence of a Y chromosome. There is no obvious increase in risk for patients with XO gonadal dysgenesis, unless an XY cell line is also present (Greenblatt, Byrd, McDonough and Mahesh, 1967; Teter and Boczkowski, 1967; Goldberg and Scully, 1967).

TABLE 4. — *Prevalence of Chromosal Variants in Trisomy-21 Syndrome*

| Variant | Maternal Age: Under 30 Number | % | Over 30 Number | % | Unselected Number | % |
|---|---|---|---|---|---|---|
| Trisomy 21 | 658 | (91.1) | 646 | (97.8) | 1028 | (95.2) |
| Mosaic | | | | | 21 | ( 1.9) |
| D/G translocation | 33 | ( 4.6) | 7 | ( 1.1) | 16 | ( 1.5) |
| G/G translocation | 31 | ( 4.3) | 7 | ( 1.1) | 15 | ( 1.4) |
| *Totals:* | 722 | | 660 | | 1080 | |

## MONGOLISM (21-TRISOMY)

The commonest chromosome anomaly compatible with live birth in man is that associated with 21-trisomy. Lejeune, Gautier and Turpin (1959) demonstrated the presence of an extra small acrocentric chromosome, identified by them as number 21. The subject has been thoroughly reviewed by Lejeune (1964), and the material to follow will be mainly a discussion of data related to translocations and mosaicism in this disorder.

Soon after the discovery of an extra chromosome in mongolism, cases were

described in which the extra material was translocated to another member of the complement. Translocation was noted between chromosome 21 and a member of the D group (Polani, Briggs, Ford, Clark and Berg, 1960) and also between chromosome 21 and a member of its own group, G (Fraccaro, Kaijser and Lindsten, 1960; Penrose, Ellis and Delhanty, 1960). The following year, a patient suspected of having trisomy-21 syndrome, was found to have a mixture of normal cells and cells containing an extra chromosome 21 (Clarke, Edwards and Smallpiece, 1961).

As a result of surveys of patients with the trisomy-21 syndrome, reported from many countries of the world, it is now possible to judge the relative frequency of the main chromosomal variants of this disorder. Wright, Day, Muller and Weinhouse (1967) have collected data from 20 published and unpublished series of cytogenetic studies in trisomy-21 syndrome. Their analysis of information for 1,382 patients is shown in the first two columns of Table 4. It shows that by far the majority of patients with trisomy-21 syndrome have an extra, free chromosome 21, regardless of maternal age at the time of their birth. It also illustrates that within each maternal age group, the prevalence of D/G and G/G translocations is equal. Further, it appears that translocations are about 4 times as common in infants with trisomy-21 syndrome born to women under 30 than in those of the older group.

When translocation in trisomy-21 syndrome was first reported, an impression was created that the abnormal chromosome was usually transmitted from a parent. The parent, apparently normal, had 45 chromosomes due to translocation of one chromosome 21 on to another member of the complement. As unselected series of trisomy-21 syndrome began to appear in the literature it became clear that translocation associated with the disease is usually a *de novo* event in the affected progeny. However, there is a great difference in the ratio of *de novo* to inherited translocations between the D/G and G/G type. The results from published papers are summarized in Table 5 and taken from

TABLE 5.—
*Prevalence of D/G and G/G Translocations in Relation to Maternal Age*

| Maternal age | *de novo* Number | % | *Inherited* Number | % | *de novo* Number | % | *Inherited* Number | % |
|---|---|---|---|---|---|---|---|---|
| < 30 | 16 | (59) | 11 | (41) | 23 | (92) | 2 | (8) |
| > 30 | 5 | (71) | 2 | (29) | 6 | (100) | 0 | |

data collected by Wright, Day, Muller and Weinhouse (1967). The figures do not equal those in Table 4 because in some instances it was not known whether the translocation was inherited from a carrier parent or not. It can

be seen from Table 5 that D/G translocations are much more commonly inherited than are those of the G/G type.

If the data from unselected series of patients with trisomy-21 syndrome are collected, the relative frequencies of translocation and mosaicism can be ascertained. Huang, Emanuel, Liao and Hsu (1967) have recently surveyed the results of 1,080 cases of trisomy-21 syndrome published by 11 investigators in 7 different countries. The results are listed in the third column of Table 4. Over 95 per cent of all cases of trisomy-21 syndrome have regular trisomy 21 and another 2 per cent have trisomy 21 with mosaicism. Less than 3 per cent have translocations and this is almost equally divided between the D/G and G/G type. Not more than 1 in 4 of these would be inherited. To put this in terms of the practicing physician: he may expect to deliver one newborn infant with trisomy-21 syndrome every 10 years, but to see one of these with an inherited translocation he would practice on an average 1,000 years!

The translocations which have been mentioned as predisposing to trisomy-21 syndrome in the offspring are of the so-called "centric fusion" type. In this, almost all of the long arm of one acrocentric chromosome becomes transferred to the short arm of another acrocentric chromosome (Fig. 2b.). The other product is very small and, although containing a centromere, is usually lost. Because of this, there is incomplete synapsis at meiosis between the product of centric fusion and its 2 normal homologues (Fig. 2c.). At diakinesis, a "chain of three" will form and the products of anaphase will depend on the behavior of the elements of this chain (Fig. 2d.). The translocation chromosome alone (ii) or its 2 normal homologues alone (i and iii) will be associated with a normal phenotype. The first will result in a carrier of the translocation and the second in a chromosomally normal individual. However, the chain may break in such a way that the translocation chromosome and the smaller homologue (i and ii) enter one cell, the alternative being a gamete containing the other homologue alone (iii). On fertilization the first product would be trisomic for chromosome 21 and the second would be monosomic and presumably inviable.

In view of the above facts, carriers of a D/G translocation have, on theoretical grounds, a 1 in 3 chance of producing normal children, carriers and children affected with trisomy-21 syndrome. In addition, a number of unbalanced gamates would be expected to result in abortions. From 27 reports in the literature, the results of 262 pregnancies from D/G translocation carrier females are known. The results are summarized in Table 6, line 1. Most of the subjects in the column "unknown" had normal phenotypes; some were dead and others could not be studied. The majority would presumably be divided between carriers and chromosomally normal subjects. In

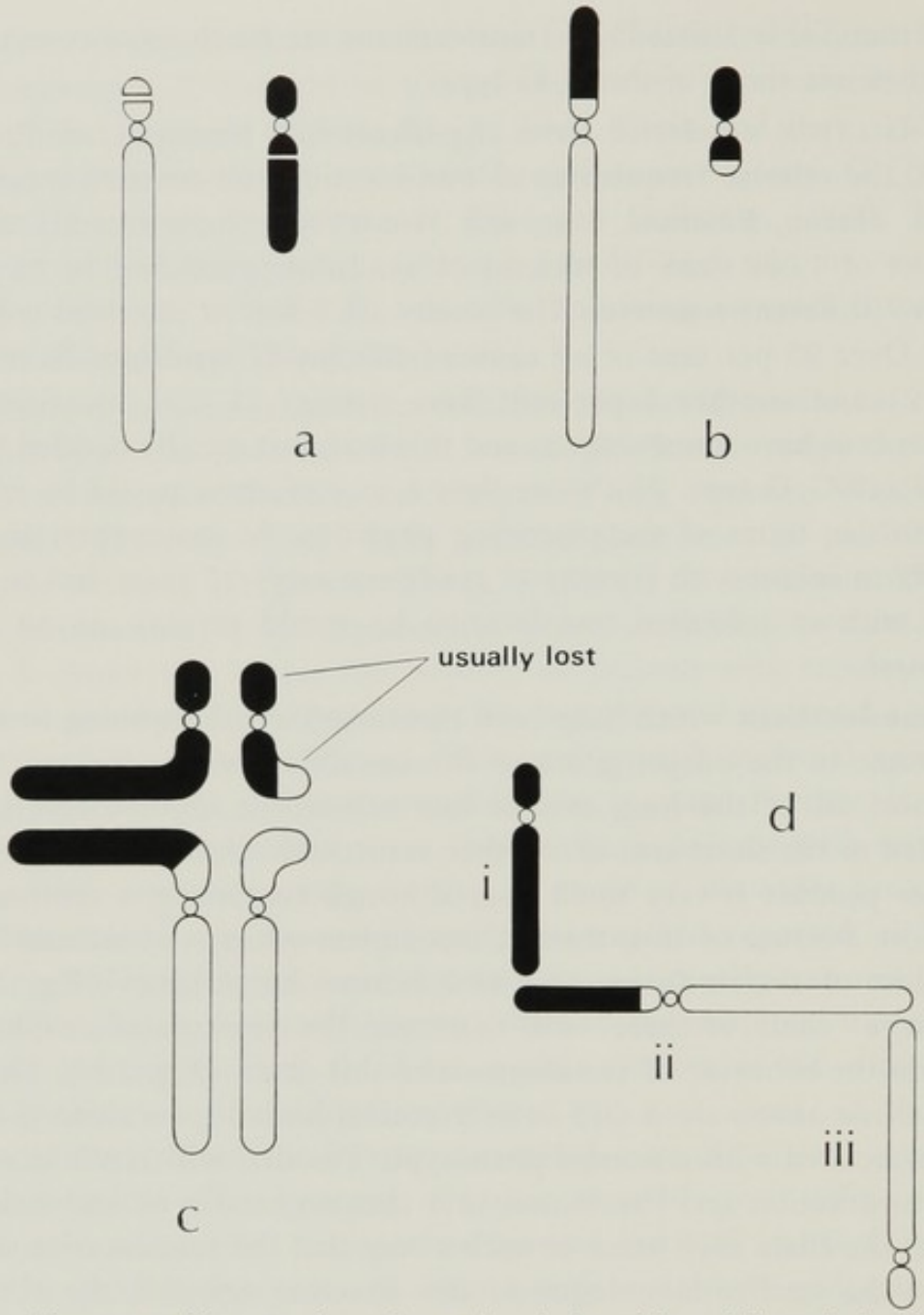



FIG. 2—Diagram to illustrate formation and meiotic pairing of "centric fusion" type of reciprocal translocation products.

any case, the other figures cannot be taken at face value without comment. In the first place, the figures have not been corrected for ascertainment. In the majority of families, the presence of a child with trisomy-21 leads to genetic consultation. The published figures thus exclude any families with carriers who have never had affected progeny. The figure for the occurrence of trisomy-21 syndrome is therefore too high. The second point to notice in the recorded results of pregnancies is that very early abortions may be missed. The prevalence of abortions from female D/G translocation carriers is 18 per cent, which is only slightly higher than that recorded for all pregnancies in women.

TABLE 6. — *Progeny of D/G and G/G Translocation Carriers in Relation to Trisomy-21 Syndrome**

| Translocation | Families | Normal | Carriers | Trisomy-21 syndrome | Abortions | Unknown | Total |
|---|---|---|---|---|---|---|---|
| D/G translocation carrier females | 26 | 50 | 71 | 73 | 46 | 22 | 262 |
| D/G translocation carrier males | 8 | 32 | 48 | 5 | 10 | 14 | 109 |
| 21/22 translocation carrier females | 4 | 5 | 6 | 6 | 9 | 0 | 26 |
| 21/22 translocation carrier males | 4 | 11 | 9 | 4(3)† | 7(5)† | 5 | 36 |

* In compiling this table, mosaic translocation carriers have been omitted.
† Family reported by Forssman and Lehmann (1962) see text.

Atkins, O'Sullivan and Pryles, 1962; Aula and Hjelt, 1964; Beçak et al., 1963; Biesele et al., 1962; Brandt et al., 1963; Breg, Miller and Schmickel, 1962; Buckton et al., 1961; Carter et al., 1960; Day and Wright, 1965; Forssman and Lehmann, 1962; German, de Mayo and Bearn, 1962; Giannelli, Hamerton and Carter, 1965; Grouchy and Roubin, 1965; Hamerton et al., 1961; Hayashi, 1963; Heinz, 1965; Huang et al., 1967; Hustinx, 1966; Jackson and Ashford, 1967; Macintyre et al., 1962; Mikkelsen, 1966; Penrose and Dehanty, 1961; Pfeiffer, 1963; Porter, Brown and Mele, 1966; Sergovich, Soltan and Carr, 1964; Shaw, 1962; van der Hagen, 1966; Warkany et al., 1964; Yunis, Hook and Mayer, 1965a.

In counselling, one may use the data from many families, as listed here. However, when individual pedigrees are considered there is wide variation in the results of pregnancies from different carriers. For instance, families reported by certain workers had high abortion rates (Macintyre, Staples, Steinberg and Hempel, 1962; Aula and Hjelt, 1964; Yunis, Hook and Mayer, 1965), while in the D/G translocation family described by Shaw (1962), the abortion rate was nil. It is true that variations from family to family may be due to chance but there are other possible factors including genetic influences on meiotic behavior. Therefore, though the collective behavior of D/G translocations is an average, the translocation chromosome may segregate differently in some families.

A simple way to correct for ascertainment is to deduct one case of trisomy-21 syndrome for each family listed. This reduces the expectation of producing the anomaly by female D/G carriers to 47 in 262, or 18 per cent. Thus, though the theoretical risk is 1 in 3, based on corrected data it is actually closer to 1 in 5. This is almost identical to the figure given by Hamerton (1966), using a more sophisticated method of correction for ascertainment. Hamerton (1966) also noted that after correction, the proportion of carrier to normal individuals is close to a 1:1 ratio.

There are fewer progeny from male D/G translocation carriers, but one is immediately struck by the very small number of offspring with trisomy-21 syndrome (Table 6, line 2). In none of these families has more than one affected child appeared. If one corrected for ascertainment the risk would drop even further.

Just as extraordinary is the very small number of abortions, about 9 per cent, or one third less than in normal people! The reason for this is quite obscure. The only advice which one could give a D/G translocation male carrier is that the risk of producing a child with trisomy-21 syndrome is relatively small — probably 1 or 2 per cent (Hustinx, 1966). On the other hand the risk of producing carriers remains, and if they are female they have a greatly increased risk of conceiving children with trisomy-21 syndrome.

The majority of subjects with trisomy-21 syndrome associated with a translocation between two G-group chromosomes arise *de novo,* the progenitors having normal chromosomes (Table 5). Families in which G/G translocations have been transmitted from parent to offspring are of two types: 21/21 and 21/22. The former either pass the unpaired anomalous chromosome to the zygote or they do not pass it on. In the first instance, fertilization results in trisomy-21 and in the second instance in monosomy-21. Therefore, only 2 products of pregnancy result, affected infants with trisomy-21 syndrome and abortions (Mukherjee, Miller and Breg, 1962; Dallaire and Fraser, 1964; Hard, Eisen and Stanage, 1965; Gedda, Calabresi, Del Porto, Del Porto-

Mercuri, Alfieri, Torrioli-Riggio and Romei, 1967). In view of this, G/G translocations transmitted through carriers with normal phenotypes must be of the 21/22 type. They behave at meiosis in a comparable way to D/G translocations, producing therefore 4 types of progeny: normal, carriers, those affected with trisomy-21 syndrome, and abortions. Families with this type of translocation are few and it is important to consider one family separately. Forssman, and Lehmann (1962) presented a pedigree of a G/G carrier in which it was inferred, but not proven, that the translocation had been passed through two generations. If this is so, the product was obviously 21/22, but its male carrier fathered only affected children, the other 5 pregnancies ending in abortion. It is included in Table 6, line 4 as 21/22 male transmission in brackets. Three of the 4 infants with trisomy-21 syndrome and 5 of 7 abortions came from this family. Otherwise, male transmission of trisomy-21 syndrome appears to be considerably less than female transmission, as in D/G translocation carriers. In female carriers the ratio of normal : carriers : affected is equal, judging by the small numbers presently available, but this does not take into account correction for ascertainment.

The phenotype of trisomy-21 syndrome may be modified if the subject is a chromosomal mosaic, including cells with a normal cell line. The first such patient to be described had an I.Q. of 100 and her photograph is not a striking example of trisomy-21 syndrome (Clarke, Edwards and Smallpiece, 1961). It has since been found that the phenotype in individuals mosaic for trisomy 21/normal cells varies from completely normal, to extremely retarded, typical cases of trisomy-21 syndrome (Finley, Finley, Rosecrans and Tucker, 1966).

The practical aspect of mosaicism is that if it is found in a subject who has trisomy-21 syndrome, the prognosis is not necessarily improved and should be guarded. If the patient is clinically atypical and has mosaicism (trisomy-21/normal) a more optimistic but still guarded prognosis may be justified. Finally, the finding of cells with trisomy-21 in the mother of a child with trisomy-21 syndrome increases the risk of recurrence of the condition in future pregnancies but the degree of risk is not predictable.

Penrose (1961), has suggested that about one quarter of the cases of trisomy-21 syndrome are independent of maternal aging effect. Beside mosaicism and translocations directly involving chromosome-21, it appears that other unrelated chromosome anomalies in a parent predispose to non-disjunction of chromosome-21 in the gametes (Sergovich, Luce and Carr, 1967; Uchida, Ray and Ursel, 1967).

The question of whether an infant with trisomy-21 syndrome is ever chromosomally normal is still a subject for debate. The first question arising is, what is necessary to establish the diagnosis of the disease? Various authors

have suggested cardinal signs and tried to weigh their significance (Oster, 1953; Gustavson, 1964; Hall, 1964). Trisomy-21 syndrome may be mistakenly diagnosed in the newborn (Meadow, 1967). As already mentioned, the phenotype is variable in mosaicism and the number of abnormal cells may be small. Hall (1962) described a patient which he regarded as typical, in whom the chromosomes were normal. A small piece of chromosome-21 could be translocated to any large chromosome in the complement without detection by present techniques. Day and Miles (1965) described a family in which a woman had 3 miscarriages, unlike-sexed twins and 3 infants with trisomy-21 syndrome. One of the miscarriages and one of the trisomy-21 syndrome offspring were by different fathers. Evidence of translocation was presented by the occurrence of a minute, centric fragment in the mother, normal male twin son and one of the children with trisomy-21 syndrome. Two other infants with trisomy-21 syndrome had completely normal karyotypes. This family presents the strongest evidence to date that the disorder can be associated with an apparently normal karyotype.

There have been some 15 well-documented reports on the offspring of women suffering from trisomy-21 syndrome, 6 of them with full cytogenetic study (Tagher and Reisman, 1966; Uchida, 1967). Nine of the progeny were normal and 6 had trisomy-21 syndrome. This is much closer to the theoretical 1:1 ratio than is found in the progeny of triple-X females. From studies in lower forms, secondary nondisjunction should be closer to 50 per cent when the parent is trisomic for a larger chromosome. The reason for the unusual result in man has not been explained.

The identification of the extra chromosome in trisomy-21 syndrome is important. In G/G translocation carriers, no prognosis for future pregnancies can be given without establishing whether the abnormal element is 21/21 or 21/22. This cannot be done on morphological grounds, but Yunis, Hook and Mayer (1965) believe that identification by autoradiography is possible. Many other investigators disagree and maintain that the replication pattern of the chromosomes in group G is quite irregular and unsuitable for identification (Back, Dormer, Baumann and Olbrich, 1967; Fraccaro, Lindsten and Tiepolo, 1967; Hecht and Weleber, 1967).

The intrauterine mortality of trisomy-G is high (Carr, 1967). Because it is impossible to identify the 2 pairs of chromosomes in group G, it is not known whether one or both pairs are involved in abortions with this trisomy. It is known that G-trisomy abortions, like trisomy-21 syndrome, show a maternal age effect. If trisomy-21 were responsible for all G-trisomy abortions, liveborn infants with trisomy-21 syndrome would represent about 30 per cent of trisomy-21 zygotes (Carr, 1967).

## 18-trisomy Syndrome (Edward's)

In 1960, a new syndrome was described associated with multiple congenital anomalies and an extra chromosome in group E (Edwards, Harnden, Cameron, Crosse and Wolff, 1960). Since then, well over 100 examples of this syndrome have been described. The anomalies found involve virtually every system, and the pattern which has emerged is summarized for 119 cases by Taylor (1967).

Infants with 18-trisomy are born at term but their birthweight is reduced to a mean of 4.9 lb. (2,200 Gm.). This retarded physical development becomes even more marked if the child survives the first year. The prevalence of this anomaly in newborn infants has been estimated as 1 in 4,500 (Conen and Erkman, 1966a) and 1 in 6,500 (Taylor, 1967) live births. The mean age at death is a little over 10 weeks, but there is a difference in survival for males and females. An extraordinary feature of this syndrome is the great excess of females affected, 150:42 (Weber, 1967). This author has shown that there is a greatly increased mortality among affected males in the first 3 months. The discrepancy in sex ratio is reduced if one considers only those surviving to 14 days. This increased male mortality may also exist prenatally.

Table 7. — *Principal Features of Three Syndromes Associated with Chromosome Anomalies*

| Syndrome | Signs present in over 90% cases | Signs present in 75-89% cases |
|---|---|---|
| Edward's syndrome (Trisomy-18) | Developmental retardation<br>Failure to thrive<br>Low set malformed ears<br>Micrognathia<br>Flexion deformity of fingers<br>Arches on 3 or more fingers<br>Congenital heart disease | Elongated skull<br>Shield chest and/or short sternum<br>Limited hip abduction<br>Short dorsiflexed big toe<br>Hypertonia |
| Patau's syndrome (trisomy-13) | Developmental retardation<br>Twitching and apnea<br>Deafness<br>Malformed ears<br>Microphthalmos or coloboma<br>Single palmar crease<br>Distal axis triradius<br>Undescended testes | Microcephaly often with scalp defect<br>Cleft lip and/or palate<br>Polydactyly<br>Hyperconvex fingernails<br>Arch fibular in hallucal area of sole |
| *Maladie du cri du chat* | Mental retardation<br>Failure to thrive<br>Microcephaly | Hypertelorism of eyes<br>Transverse palmar crease<br>Epicanthus<br>Low set ears<br>Anti-mongoloid slant of the eyes |

This is difficult to judge as E trisomy in spontaneous abortions usually involves chromosome 16 (Carr, 1967).

The anomalies which are present in more than 75 per cent of infants with 18-trisomy are listed in Table 7. The presence of arches on the finger tips may be observed with a magnifying glass and are most helpful in the diagnosis. Although at least 3 arches are always present in this disorder it is common to find this pattern on 9 or 10 fingers (Uchida and Soltan, 1963). Almost all infants with 18-trisomy have congenital heart disease, usually ventricular septal defect with or without patent ductus arteriosus.

The 18-trisomy syndrome may be missed at birth. In 4 of 5 cases there is a small placenta and single umbilical artery. These findings indicate the need for further study.

As in other clinical syndromes regularly associated with a chromosome anomaly, an occasional clinically typical case is reported to have normal chromosomes. Various investigators have studied infants with all the major features of trisomy-18 syndrome but with normal chromosomes (Grouchy, 1965; Hook and Yunis, 1965; Gilgenkrantz, Sapelier, Thiriet, Hahn and Pierson, 1967).

The identification of the extra element in Edward's syndrome has been uncertain. The extra chromosome was originally thought to be number 17 (Edwards, Harnden, Cameron, Crosse and Wolff, 1960). On the other hand, Smith, Patau, Therman and Inhorn (1962) believed the extra element was Number 18. Autoradiographic study of replication patterns suggest that the latter interpretation is correct. (Yunis, Hook and Mayer, 1964a).

As in trisomy-21 syndrome it appears that the maternal age of the progenitors of infants with 18-trisomy is bimodal, the overall mean maternal age being increased (Taylor, 1967). In contrast, trisomy-16, the commonest trisomy in spontaneous abortions, is not associated with increased maternal age (Polani, 1966; Carr, 1967). One way in which trisomy-18 differs from the other 2 trisomy syndromes is that it rarely results from a translocation in a carrier parent with normal phenotype (Brodie and Dallaire, 1962). The reason for this is not clear, as other anomalies requiring breaks in chromosome 18 have frequently been reported (see below).

### 13-TRISOMY (BARTHOLIN-PATAU)

In 1657, Thomas Bartholin described a child born with no eyes, a deformed mouth with a fleshy tumor beneath the nose and polydactyly of both hands and one foot. This account was reproduced by Warburg in 1960. At almost the same time, Patau, Smith, Therman, Inhorn and Wagner (1960) found an extra D-group chromosome in an infant with multiple congenital anomalies including anophthalmia, cleft lip and palate and polydactyly.

In a review of 55 cases of Patau's (D-trisomy) syndrome, Taylor (1967) has outlined the most constant features. These are listed in Table 7. As in Edward's syndrome, dermatoglyphic findings are most valuable diagnostic aids (Uchida and Soltan, 1963). The characteristic patterns are easily recognized and include transverse palmar crease and distal axis triradius on the palm and an arch fibular, sometimes S-shaped, in the hallucal area of the sole. The importance of the dermatoglyphic features is illustrated in a case reported by Marshall, Newnham, Rawstron, Ellis and Stevens (1964). This infant presented all the major features of the 13-trisomy syndrome except the dermatoglyphic patterns. Chromosomes were normal in all cells cultured in peripheral blood and skin.

The malformation of the external ear consists of a deformity of the helical pattern. The eyes may be almost absent, small, or present a structural abnormality such as coloboma. Congenital heart disease is less common in trisomy-13 than in trisomy-18. Dextroposition of the heart and ventricular septal defect are the most commonly encountered anomalies.

Infants with trisomy-13 are born close to term with a mean birth weight of 5.6 lbs. (2,550 Gm.). Single umbilical artery is found in only 50 per cent of cases. Two estimates of the population incidence of this disorder differ widely. Conen and Erkman (1966) give an estimate of 1 in 14,500 births, whereas Taylor (1967) reported 7 cases in 4 surveys totalling 32,500 births, or 1 in 4,600. Judging from the literature, unless the rate of reporting is different, 18-trisomy is commoner than 13-trisomy syndrome. Taylor's figures suggest the reverse. The mean survival time for 55 cases was 14 weeks, which is longer than in 18-trisomy (Taylor, 1967). However, the initial mortality is high, more than 1 in 4 being dead by the end of the first week and 82 per cent within the first year (Magenis and Hecht, 1966).

The sex ratio in Patau's syndrome is not so distorted as in Edward's syndrome but still shows an excess of females, 84:73 males (Magenis and Hecht, 1966). The mean maternal age at the birth of affected offspring is apparently raised as it is for D-trisomy abortions (Magenis and Hecht, 1966; Carr, 1966). As the 3 pairs of chromosomes in group D have not been separated on morphological grounds, it has not been established whether the extra chromosome in abortions is the same as that in Patau's syndrome. It has been shown by autoradiography that the extra element is the D chromosome with the replication pattern known as $D_1$ or chromosome 13. This is the chromosome with a late replicating segment over most of the long arm of the chromosome (Yunis, Hook and Mayer, 1964b; Giannelli, 1965).

As in trisomy-21 syndrome, trisomy-13 may result from meiotic nondisjunction in the germ cells of a parent carrying a D/D translocation. Among 9 cases of 13-trisomy studied by Conen and Erkman (1966b) there were 4

associated with D/D translocation in a carrier parent (44 per cent). This contrasts with a survey of the literature at that time when only 3.6 per cent of D trisomies were the result of a D/D translocation. Studies by Hustinx (1966) and Hamerton (1966) indicate a risk for 13-trisomy among D/D translocation carrier parents of just over 1 per cent.

Although trisomy-13 and trisomy-18 produce quite characteristic syndromes, there is some overlap in features. Goodman and Kaufmann (1962) reported a child with clinical features of 18-trisomy who had D-trisomy on chromosome examination and Taylor (1967) has noted the reverse association.

Trisomy-D for chromosome pairs 14 or 15 may be lethal when in complete form, resulting in abortion. Beçak, Beçak and Andrade (1964) reported D-trisomy mosaicism in several subjects with congenital anesthesia. In another study of similar clinical cases, no chromosome defect was found (Blau and Mutton, 1967).

## Loss of Chromosome Material

The majority of disorders due to a chromosome anomaly are the result of extra chromosome material, mainly a single element resulting in trisomy. The reverse situation, in which a chromosome is missing from the complement (monosomy) is rare. The only monosomy which can be regarded as at all common in man is the 45,X complement, already discussed.

Only one example has been described of apparently complete monosomy for an autosome in group G (Al-Aish, de la Cruz, Goldsmith, Volpe, Mella and Robinson, 1967). There was nothing very characteristic about the spectrum of signs. The features were compared by the authors with 7 other patients who lacked some of the chromosome material of a G-group chromosome. These included G-monosomy/normal mosaics and subjects with deletion of one of their G-group chromosomes. There did not appear to be any indication of a consistent syndrome.

The case mentioned above appears to be the only living example of complete monosomy of an autosome, but partial monosomy has been described frequently. It may result from deletion of the short arm of a chromosome, deletion of its long arm, or both. If there is deletion of portions of both arms of a chromosome, the damaged ends become joined, forming a ring chromosome.

## "Cri du chat" Syndrome

The most striking syndrome associated with loss of chromosome material was named by its discoverer *maladie du cri du chat* (Lejeune, Lafourcade, Berger, Vialatte, Boeswillwald, Seringe and Turpin, 1963). The disorder was so named because of the peculiar plaintive, high-pitched cry of affected infants.

This most diagnostic feature of the syndrome disappears in older subjects. In almost all cases there is demonstrable loss of chromosome material from the short arm of a member of the B group, now identified as number 5.

Even in older subjects who no longer have the characteristic cry, the spectrum of signs is sufficiently characteristic that observers aware of the condition have identified many adult cases. Breg, Miller, Steel and Warburton (1966) found 12 affected individuals between the ages of 12 and 31 years. The most characteristic features from 50 cases are listed in Table 7 (McGavin, Cant, Ferguson-Smith and Ellis, 1967). Other common features include round face, micrognathia and strabismus. Mental retardation is usually severe with an I.Q. less than 30 (Breg, Miller, Steel and Warburton, 1966). Congenital heart disease is less common than in the trisomy syndromes but has been reported in 9 of 33 cases (Lejeune, Lafourcade, Berger and Réthoré, 1966).

There is no increase in mean parental age. Though affected infants are born at term they have low birth weight (2,590 Gm. mean). There is a significant excess of females to males (39:21) and except for cases with congenital heart disease survival is not obviously reduced (Lejeune, Lafourcade, Berger and Réthoré, 1966).

The chromosome with short arm deletion associated with *maladie du cri du chat* is usually the member of group B which completes replication early (German, Lejeune, Macintyre and Grouchy, 1964). In an autoradiographic study of 15 cases of "cri du chat" syndrome the deleted chromosome was one of the early replicating pair in 13 cases, but one of the late replicating pair in the other 2 (Miller, Breg, Warburton, Miller, Firschein and Hirschhorn, 1966). These authors feel that in the majority of cases the deleted chromosome, the early replicator, is the shorter of the 2 pairs in group B and therefore number 5. They discuss possible reasons why, in a minority of cases, the deleted element has the characteristics of chromosome 4. The most probable explanation, they believe, is that the symptoms produced are quite non-specific and could be due to deletion of different chromosomes. It is not known whether the patients with deleted number 4 chromosome had had the characteristic cry in childhood.

A child has been described who exhibited all the characteristic features of the "cri du chat" syndrome in whom no chromosome deletion could be demonstrated (McGavin, Cant, Ferguson-Smith and Ellis, 1967).

## Deletion of Chromosome 18

Sixteen cases of short arm deletion of chromosome 18 (18 p–) have been described (Grouchy, Bonnette and Salmon, 1966; Migeon, 1966; Pfeiffer, 1966; Nitowsky, Sindhvananda, Konigsberg and Weinberg, 1966). In 2 in-

stances, the defect was associated with cyclops malformation. However, this disorder has been associated with other unrelated chromosome anomalies or with a normal karyotype (Cohen, 1966; Pfitzer, 1967).

No clear-cut syndrome has emerged in association with deletion of the short arm of chromosome 18, and the associated disorders are non-specific. Chromosome 18 is almost acrocentric and the loss of the short arms of the acrocentric chromosomes in groups D and G apparently does not cause regular phenotypic disorders (Shaw, 1962; Neu, Leão and Gardner, 1966; Elmore, Nance, McGee, Montmollin and Engel, 1966; Jagiello and Faiman, 1967; Starkman and Shaw, 1967). It seems probable that chromosome material in the pericentric region may have a variable gene content or function and that therefore its loss would result in irregular phenotypic effects.

On the other hand, 14 cases of deletion of the long arm of chromosome 18 (18q–) appear to present a recognizable syndrome. The characteristic anomalies are: severe mental retardation, microcephaly, hypotonia, retraction of the middle part of the face, prominent chin, a very marked antihelix and rolled helix of the ear, atresia or narrowing of the external auditory meatus, shoulder and hand dimples, spindle shaped fingers and a high frequency of whorls on the fingers (Grouchy, Royer, Salmon and Lamy, 1964; Wertelecki, Schindler and Gerald, 1967; Lejeune, Berger, Réthoré, Lafourcade, Dutrillaux, Canlorbe and Labrune, 1967; Insley, 1967; Destine, Punnett, Thovichit, Di George and Weiss, 1967). The mean birth weight (for 8 cases) was 2,590 Gm. and the sex ratio, 8 females to 6 males.

## RING CHROMOSOMES

The problem of relating phenotype to genotype is more difficult in the case of ring chromosomes than in the case of simple deletions. In the case of a ring chromosome, material has been lost both from the short and long arms. The degree of deletion from either end of the chromosome may be quite variable from case to case. On the other hand, genes located near the end of the chromosome may be lost with great regularity, producing constant phenotypic effects.

Ring chromosomes have been described in pure or mosaic form for at least one member of each of the 7 groups in the complement. A B-group ring chromosome has been detected because the presenting phenotype was the "cri du chat" syndrome (Rohde and Tompkins, 1965; Steele, Breg, Eidelman, Lion and Teryakis, 1966).

From one or two cases, it is obviously impossible to assess the relationship between the phenotype and the finding of a ring chromosome unless the subject conforms to a known deletion syndrome. There have been clinical descriptions of 6 subjects with a ring of chromosome 18 (Palmer, Fareed

and Merritt, 1967). It might be expected that in some of these cases there would be features found in long arm deletion of chromosome 18. Apart from mental retardation there is no striking feature in common. One characteristic found in half the subjects with long arm deletion of chromosome 18, atresia of the external auditory meatus, was found in only 2 of the 6 patients with a ring-18. Grouchy (1965a) has drawn a tentative map of segments on chromosome 18 which when deleted are responsible for various clinical features. The author has emphasized that this is not a "genic" map.

The largest number of ring chromosomes for any one group come from the large acrocentric elements. As there are 3 pairs in this group, different chromosomes may be involved from one case to the other. In 3 of the reported patients with ring D chromosomes, the thumbs are absent (Bain and Gauld, 1963; Adams, 1965; Sparkes, Carrel and Wright, 1967). The last named authors have identified the chromosome involved as $D_2$ (14) by autoradiographic studies. Each of these 3 patients also had ventricular septal defect, with or without other anomalies and abnormal dermatoglyphic findings. Two of the infants were grossly underweight although post-mature. Details were not given for the other patient. It is noteworthy that a long arm deletion of a D-group chromosome was associated with short and abnormally implanted thumbs and a severe congenital heart defect with cyanosis (Laurent, Cotton, Nivelon and Freycon, 1967). The other 7 patients with ring-D chromosomes do not have striking features in common though details of many are lacking. Four of the 7 have short fifth fingers (Turner, 1962; Jacobsen, 1966; Gerald, Warner, Singer, Corcoran and Umansky, 1967; Teplitz, Miller, Hansson and Rundall, 1967). In 2 patients, abnormal inheritance of haptoglobin has been demonstrated, and in each instance the ring chromosome was identified as developing from $D_1$ or number 13 (Bloom, Gerald and Reisman, 1967; Reisman, Darnell and Murphy, 1965; Gerald, Warner, Singer, Corcoran and Umansky, 1967). There were no obvious clinical features in common in these 2 patients. Neither had absent thumbs. Bias and Migeon (1967) studied a family with short arm deletion of $D_1$ (number 13) and excluded this segment as the site of the haptoglobin locus. The other 2 cases of ring D chromosome did not have physical characteristics which would allow identification (Wang, Melnyk, McDonald, Uchida, Carr and Goldberg, 1962; Macintyre, Hempel, Staples and Stenchever, 1964).

## Translocations

One type of translocation, widely known as centric fusion, has already been discussed in connection with the trisomy-21 and trisomy-13 syndromes. In this variety, one of the exchange products is small and frequently lost. This affects the behavior of chromosomes at meiosis.

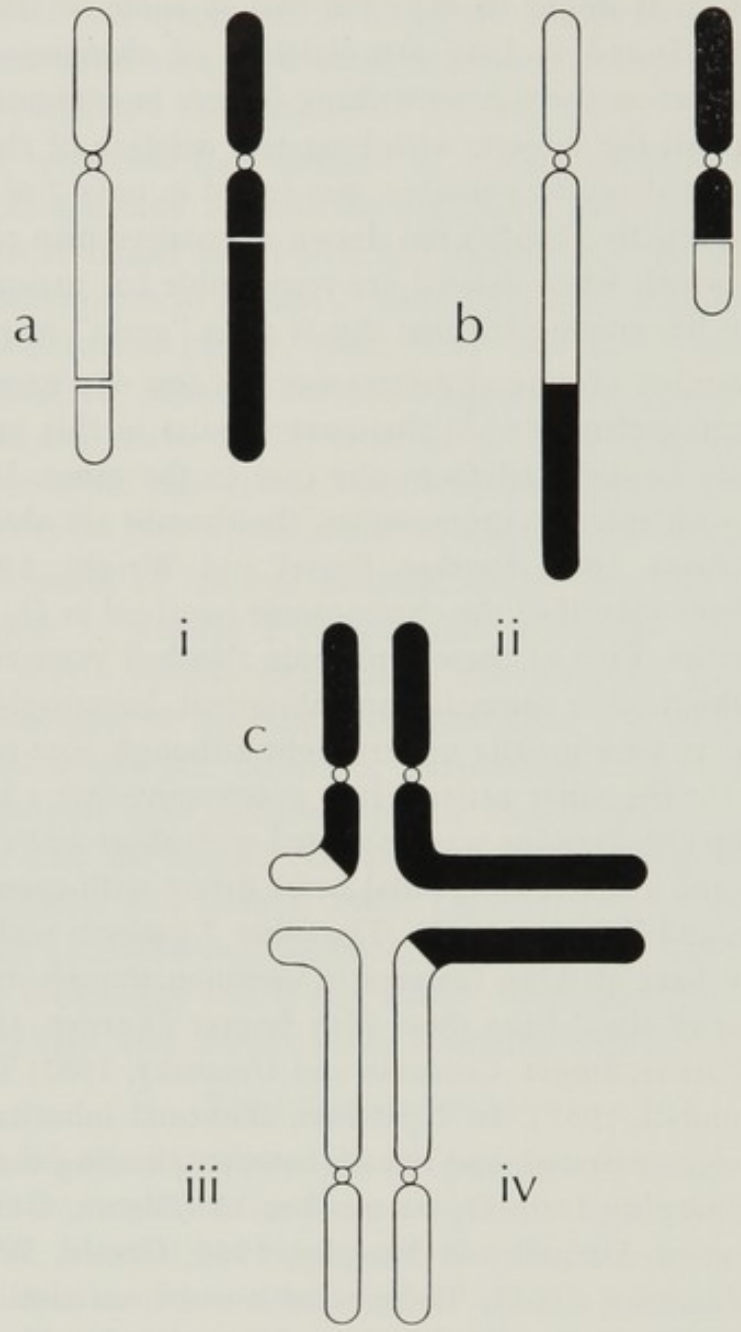



FIG. 3—Diagram to illustrate formation of reciprocal translocations, involving unequal exchange, and the meiotic pairing between these chromosomes and their normal homologues.

In other types of translocation, material is exchanged between two members of the complement, due usually to a single break in each chromosome. The exchanged segments may be equal or unequal in length. At meiosis, homologous chromosomal segments undergo synapsis and, for complete pairing of a translocation heterozygote, a cross-like figure is formed at pachytene by the two translocation chromosomes and their normal homologues (Fig. 3). Chiasmata are usually formed in each of the pairing arms, and as the chromosomes separate and terminalization of chiasmata occurs at diakinesis,

a ring of four chromosomes is formed. However, if one translocation involves only a short segment of the chromosome, chiasmata may fail to form in one arm and a chain, instead of a ring of four, would result at diakinesis.

When a ring of four chromosomes separates at anaphase, several arrangements are possible. If the separation results in chromosomes i and iv entering one cell and ii and iii the other, the product will be genetically balanced (alternate segregation). The presence of ii and iii chromosomes in the germ cell will yield a chromosomally normal zygote on fertilization, while i and iv will produce a balanced translocation carrier. However, the chromosomes may also separate i-iii and ii-iv, which is known as adjacent I segregation; or i-ii and iii-iv, which is known as adjacent II segregation. In either case, adjacent segregation will lead to products which are chromosomally unbalanced with some segments represented in duplication while others are deficient. Finally the ring may separate in such a way that the normal chromosomes ii and iii enter one cell along with another chromosome (i or iv). This is the result of non-disjunction and is especially likely to occur if one of the translocation products is small.

A number of families have been studied in which there is presumptive evidence of reciprocal translocation in parents, who were identified by the birth of abnormal offspring. The resulting phenotypes are too variable to be described in detail, though occasionally they have led to investigation because of the recognition of a known duplication or deficiency syndrome. The clinical significance of translocations can be discussed in various categories:

1) When the segment exchanged between 2 chromosomes is equal or nearly so, it is impossible to detect the defect in mitotic preparations. Though chromosomes would look alike, duplication-deficient gametes may be produced during meiosis. Suggestive evidence would be male infertility in the presence of spermatogenesis, recurrent abortion or repeated abnormal offspring sometimes with different phenotypes, not only within but between generations (Bateman, 1964). Such translocations could be detected only by meiotic studies. There is evidence that such anomalies exist in man from the result of a study of 50 subfertile males. Two among the 50 studied had a multivalent in meiotic preparations from testis while mitotic chromosomes were normal (McIlree, Price, Court Brown, Tulloch, Newsam and Maclean, 1966). The authors considered reciprocal translocation as the most likely cause of the abnormal meiotic figure.

2) Parents who have balanced translocations, as illustrated diagrammatically in Fig. 3, have been presumed to produce abnormal offspring due to adjacent segregation. In such a situation the chromosome number is normal (46) but the affected child is found to have one structurally abnormal chromosome in the complement. Edwards, Fraccaro, Davies and Young (1962)

were the first to report such a situation in 2 unrelated families. They suggested that in order to establish the existence of structural heterozygosis in man certain requirements be met: "First, at least one chromosome arm of clearly abnormal length must be demonstrated; secondly, the chromosomal aberration and the supposedly related abnormality should segregate in a compatible manner," and thirdly, the phenotypical abnormalities should be generalized, congenital and uncommon. If the carrier of the translocation is a male, direct meiotic confirmation of a multivalent figure at metaphase and a total chromosome count of only 22 completes the investigation (Lindsten, Fraccaro, Klinger, and Zetterquist, 1965). The first 3 criteria have been fulfilled in many large-family studies (Lejeune, Lefourcade, Salmon and Turpin, 1963; Lee, Bowen, Rosenblum and Linsao, 1964; Gustavson, Finley, Finley and Jalling, 1964; Uchida, Wang, Laxdal, Zaleski and Duncan, 1964; Rohde and Catz, 1964; Migeon and Young, 1964; Lord, Casey and Laurance, 1967; Kaplan, Grumbach, Fischgrund and Ferragu, 1966; Punnett, Pinsky, Di George and Gorlin, 1966; Jensen and Melchior, 1967). A family described by Shaw, Cohen and Hildebrandt (1965) raises a point of special importance. The type of translocation and the origin of the propositus did not differ from the cases already mentioned. However, the chromosomes involved in the reciprocal exchange were members of the same group and could not be definitely separated on morphological grounds. In such a situation it is important to know whether the translocation arose between homologues or not. This is the same problem that arises in separating 21/21 and 21/22 translocations in trisomy-21 syndrome. Homologous interchanges give rise to affected offspring and abortions only. The family under discussion demonstrated transmission through more than one generation, which established the translocation as heterologous (4/5).

Walzer, Favara, Ming and Gerald (1966), traced a reciprocal translocation between a member of the B-group and a number 3 chromosome. They found 6 infants with similar types of anomaly in a very extensive family study. They also concluded that there was differential transmission of abnormal segregants between male and female carriers. There were 3 times as many affected offspring from female than from male carriers. In a survey of the literature, Stalder, Bühler and Bühler (1965) concluded that when translocations involved the largest chromosomes (groups A, B and C) phenotypically abnormal offspring or abortion occurred in over 50 per cent of pregnancies.

As noted previously, there is more than one possible unbalanced product from gametes with reciprocal translocations. Usually only one type of segregant is described, but 2 families have been reported with deficiency and duplication in different offspring. In both families the propositus had the

"cri du chat" syndrome and in each instance B and D group chromosomes were involved in the translocation (Lejeune, Lafourcade, Berger and Turpin, 1964; Capoa, Warburton, Breg, Miller and Miller, 1967). Other families were reported by the last-named authors in which translocation was suspected in the parents of subjects with *maladie du cri du chat,* but the site of the exchanged segment could not be defined.

3) As mentioned already, if the product of a translocation is small, it may fail to disjoin at meiosis, and the resulting product of conception has 47 chromosomes. If this chromosome resembles a member of the normal complement, a mistake in diagnosis may occur. The extra element is an indication for further study and this should always be undertaken before counselling. In all instances of non-disjunction associated with translocation the extra element has been small and often resembles a member of group G (Vislie, Wehn, Brogger and Mohr, 1962; Macintyre, Staples, Lapolla and Hempel, 1964; Eisen and Angle, 1964; Pfeiffer, Keuth, Stupperich and Buchner, 1966; Pitt, Webb, Wong, Robson and Ferguson, 1967). Jacobsen, Mikkelsen, Froland and Dupont (1966) reported a family with an abnormal child showing non-disjunction of a translocation product (47 chromosomes) and in the same sibship an abortus with the reciprocal product of only 45 chromosomes. The greatest variety of abnormal products of segregation from a translocation carrier was reported by Hauschteck, Mürset, Prader and Bühler (1966). In this family there was one child with an unbalanced translocation with 46 chromosomes, a second one with 47 chromosomes due to non-disjunction and finally, balanced translocation, as in the mother, in cells grown from an induced abortion.

The rarest product, mentioned above in the cells of an abortion, has been reported in a living child. In this instance, the mother appeared to have a balanced translocation with normal count while the affected offspring lacked the smaller product of the event and had only 45 chromosomes (Pfeiffer, Bachman and Bartel, 1967).

4) Most carriers of a translocation with loss of the small product from acrocentric chromosomes are phenotypically normal. This has been mentioned in connection with D/G, G/G and D/D translocation carriers. It is believed that in those cases, the loss of chromosome material of the pericentric region does not affect development because the missing segment is inactive genetically. It is of course quite conceivable that loss of genetically active material during translocation would produce effects. Reinwein and Wolf (1965) described a patient with 45 chromosomes and a D/B translocation. The phenotype was that of the "cri-du-chat" syndrome and it was presumed that the short arm of chromosome 5 had been deleted during the translocation.

5) There have been several reports of translocations which appear to be balanced in a defective offspring. On studying the parents, they have been found to have apparently the same chromosome complement. One explanation is that the association between the anomalies in the child and the chromosome disorder is fortuitous. However, Lejeune and Berger (1965) and Grouchy (1965) have shown how apparently similar karyotypes may have a different gene complement, normal in one case, abnormal in the other. This type of anomaly, "aneusomy of recombination," requires the assumption that the exchanged segment is not only translocated but inserted into another chromosome. This requires three breaks, two in one chromosome and one in the other (Fig. 4). By the formation of a loop for pairing at meiosis, and

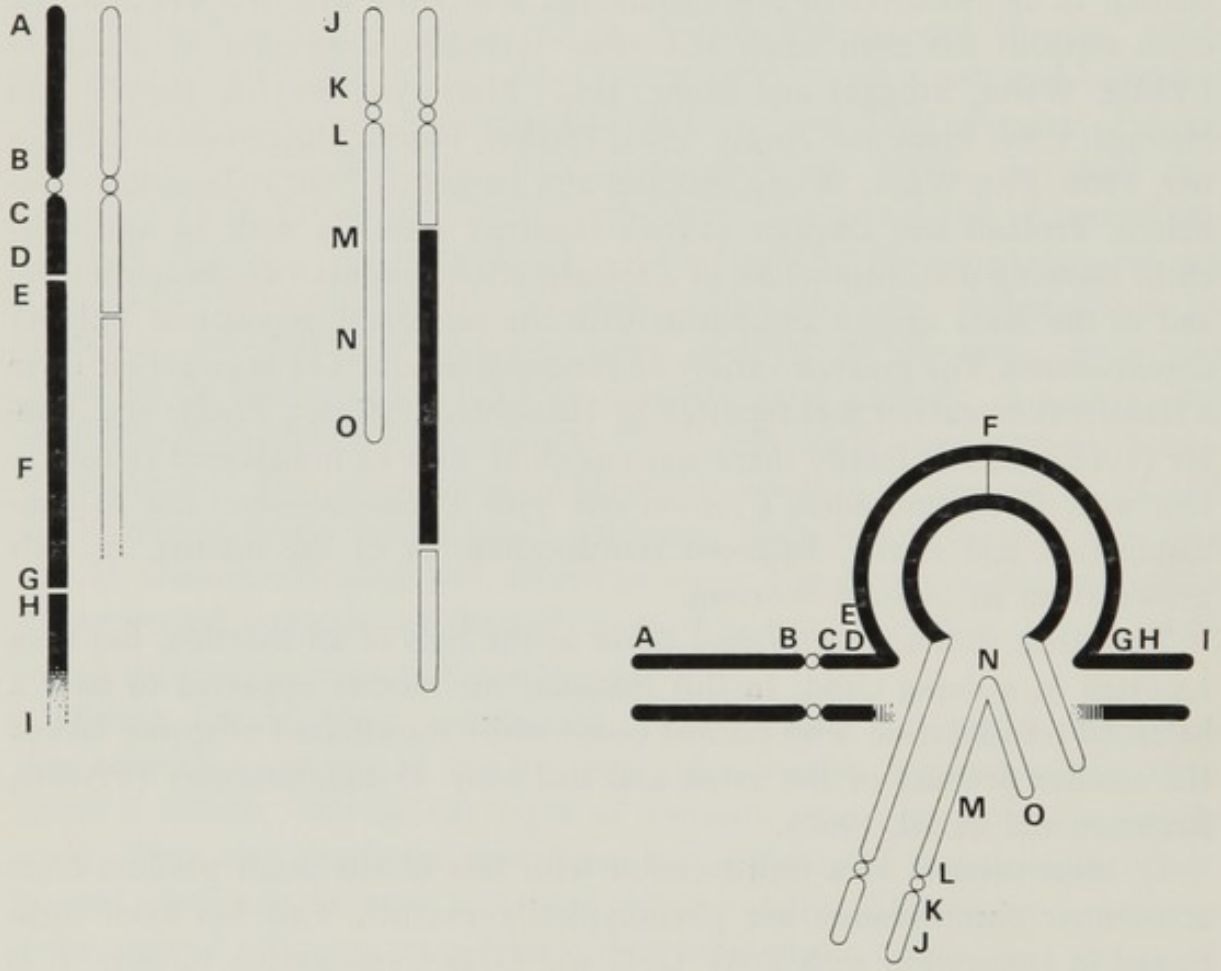



FIG. 4—Illustration of translocation and insertion. The inserted segment pairs with a normal homologue at the loop while the rest of the chromosome pairs with the other normal element. A single cross over within the loop at F produces duplicated and deficient segments but does not change the morphology of the chromosome (modified from Grouchy, 1965).

a single, or any odd number, of cross-overs within this loop, unbalanced but apparently unchanged recombinants are formed (Grouchy, Aussannaire, Brissaud and Lamy, 1966).

## Chromosome Anomalies and Spontaneous Abortions

As already mentioned, one possible indication of a translocation in a balanced heterozygote is the occurrence of repeated abortions. This should be suspected, especially in families with normal offspring, abnormal offspring and recurrent abortion. On the basis of chance, a translocation heterozygote may produce only repeated abortions as a result of unbalanced gametes. This is apparently not a common cause of recurrent abortion. McKay, Hodgkin and Witte (1967) studied 38 couples with repeated abortion without finding translocations. On the other hand, among 4 couples with repeated abortions and one or more children with multiple anomalies, 2 husbands showed consistent balanced translocations. Similarly, Shaw (1966) who studied 30 couples with repeated abortion, found a translocation in only 1 individual.

Schmid (1962), Carr (1963) and Wingate (1965) independently reported structural anomalies of chromosomes in group D or G. They each suggested that these anomalies predisposed to repeated abortion which occurred in these families. It has since been shown that this chromosomal variant, enlargement of the short arm, may be found in subjects with normal family histories (Court Brown, Buckton, Jacobs, Tough, Kuenssberg and Knox, 1966). Whether or not there is an increased risk of abortion in such families has not yet been established.

The management of pregnancy in carriers of balanced translocations has been improved by a new technique. It has now become possible to assess the chromosomes of a fetus by culturing cells aspirated by amniocentesis. There was a low rate of successful culture in the first reports of this procedure (Steele and Breg, 1966; Thiede, Creasman and Metcalfe, 1966). With experience later authors report increasing success with this technique (Turner, Hutchinson and Charles, 1966; Nadler, 1967; Jacobsen and Barter, 1967). In view of the high abortion rate of chromosomally abnormal zygotes before 112 days of gestation (16 weeks after the first day of the last menstrual period) early use of this technique is hardly justified. The method is important but presently limited to handling pregnancies in known carriers of chromosome anomalies in countries with legal abortions for suspected congenital defect.

The problem of translocation heterozygosis as a cause of chromosomally abnormal abortuses was approached from another direction by Singh, Rubinoff and Carr (1966). Contact was made with 47 couples who had produced a chromosomally abnormal abortus. Of these, 19 couples were available for chromosome study and the wife alone in 3 other families. The chromosomes were normal in all subjects studied. This suggests that chromosome anomalies in spontaneous abortions are sporadic events. Further evidence against translocations as a common cause of chromosome anomalies in abortions

comes from obstetrical histories of patients. The histories of women producing chromosomally normal and abnormal abortuses did not differ. The fact that repeated abortion was no more common among women producing abortions with abnormal chromosomes suggests that these defects were chance events (Carr, 1967).

Many workers have now confirmed the common occurrence of chromosome anomalies in spontaneous abortions. In 12 unselected series of spontaneous abortions the incidence of chromosome anomalies varied from 8 to 40 per cent with a mean of 20 per cent (Table 8). The wide variation may reflect a difference in proportion of early to late abortions in different series, though other factors cannot be excluded. The highest rate of chromosome anomalies in abortions is between the beginning of the ninth and the end of the sixteenth week after the first day of the last menstrual period (Carr, 1965). By selecting for factors known to be associated with chromosome anomalies the incidence among abortions has been found to be as low as 2 per cent and as high as 64 per cent (Table 8).

TABLE 8. — *Studies of Chromosome Anomalies in Spontaneous Abortion*

| Reference (unselected) | No. of specimens | No. chromosomally abnormal | % chromosomally abnormal |
|---|---|---|---|
| Boué and Boué, 1966<br>Boué, 1967 | 98 | 36 | 36 |
| Bowen and Lee, 1966 | 22 | 5 | 23 |
| Carr, 1967 | 227 | 50 | 22 |
| Clendenin and Benirschke, 1963 | 10 | 3 | 30 |
| Hall and Källén, 1964 | 8 | 2 | 25 |
| Inhorn, Therman and Patau, 1964 | 88 | 8 | 9 |
| Kadotani and Pergament, 1966 | 77 | 16 | 21 |
| Kerr and Rashad, 1966 | 35 | 6 | 17 |
| Makino et al., 1967 | 37 | 4 | 11 |
| Schlegel et al., 1966 | 30 | 3 | 10 |
| Stenchever, Hempel and Macintyre, 1967 | 101 | 8 | 8 |
| Waxman, Arakaki and Smith, 1967 | 25 | 10 | 40 |
| *Total:* | 758 | 151 | 20 |
| *Reference selected:* | | | |
| Jacobson and Barter, 1967 | 35 | 8 | 23 |
| Szulman, 1965 | 25 | 16 | 64 |
| Thiede and Salm, 1964 | 12 | 7 | 58 |
| Wingate, 1966 | 50 | 1 | 2 |

It is indeed fortunate that the majority of chromosomally abnormal zygotes never go to term but are recognized as defective by the body and rejected. The degree of mortality varies according to the anomaly. There is a striking difference in the age at abortion between trisomic, polyploid and XO specimens which have a mean gestational duration of 74.3, 87.6 and 98.4 days respectively (Carr, 1966).

Those chromosome disorders which are comparable to anomalies found in the living have been discussed under the appropriate heading. In some instances it cannot be established whether the chromosome anomalies found in the liveborn are the same as those demonstrated in abortions. This is true for trisomies in groups D and G. On the other hand, the XO anomaly is known to occur among abortions and liveborn infants.

Most of the lethal chromosome anomalies are of little clinical interest. One must, however, be considered in its relation to the occurrence of an anomaly of the chorionic villi of the placenta. There have been several reports of chromosome study in the condition known as hydatidiform mole (Stolte, Kessel, Seelen and Tijdink, 1960; Atkin and Klinger, 1962; Makino, Sasaki and Fukuschima, 1965; Bourgoin, Baylet, Ballon, and Grattepanche, 1965). Findings varied and normal chromosome complements were usually found. In at least 3 cases, frank hydatidiform mole was associated with triploidy (Atkin and Klinger, 1962; Szulman, 1965; Beischer, Fortune and Fitzgerald, 1967). In 1964, Makino, Sasaki and Fukushima reported 3 triploid abortuses with "abnormal chorionic villi . . . forming small vesicles similar to hydatidiform mole." This has been confirmed by others (Carr, 1965; Schlegel, Neu, Leão, Farias, Lewczak and Gardner, 1966). The observation of this lesion has led to the prediction of tripoidy in 4 specimens (personal observation). The anomaly is illustrated in Fig. 5. It is readily noticeable to the naked eye as long as free villi are visible. This vesiculation of the villi looks exactly like hydatidiform mole in miniature and is more pronounced in fresh than fixed material. The pathalogical appearance is classed as "hydropic degeneration of the villi." A karyotype from cells grown from one of these specimens is shown in Fig. 6. The lesion has been noted with all 3 sex chromosome complexes of triploidy, XXY, XXX and XYY.

During a study over the last 2 years it has become apparent that certain types of chromosome anomaly in abortions are increased in women who become pregnant shortly after discontinuing oral contraceptive medication. The histories and anomalies found are summarized in Table 9. Only 2 types of chromosome disorder have been found, triploidy and 45,X complement. No trisomies have yet appeared in this material. The incidence of triploidy in one series (Carr, 1967), or in the 12 combined unselected series in Table 8, is the same, approximately 4 per cent. Among 11 women who have become

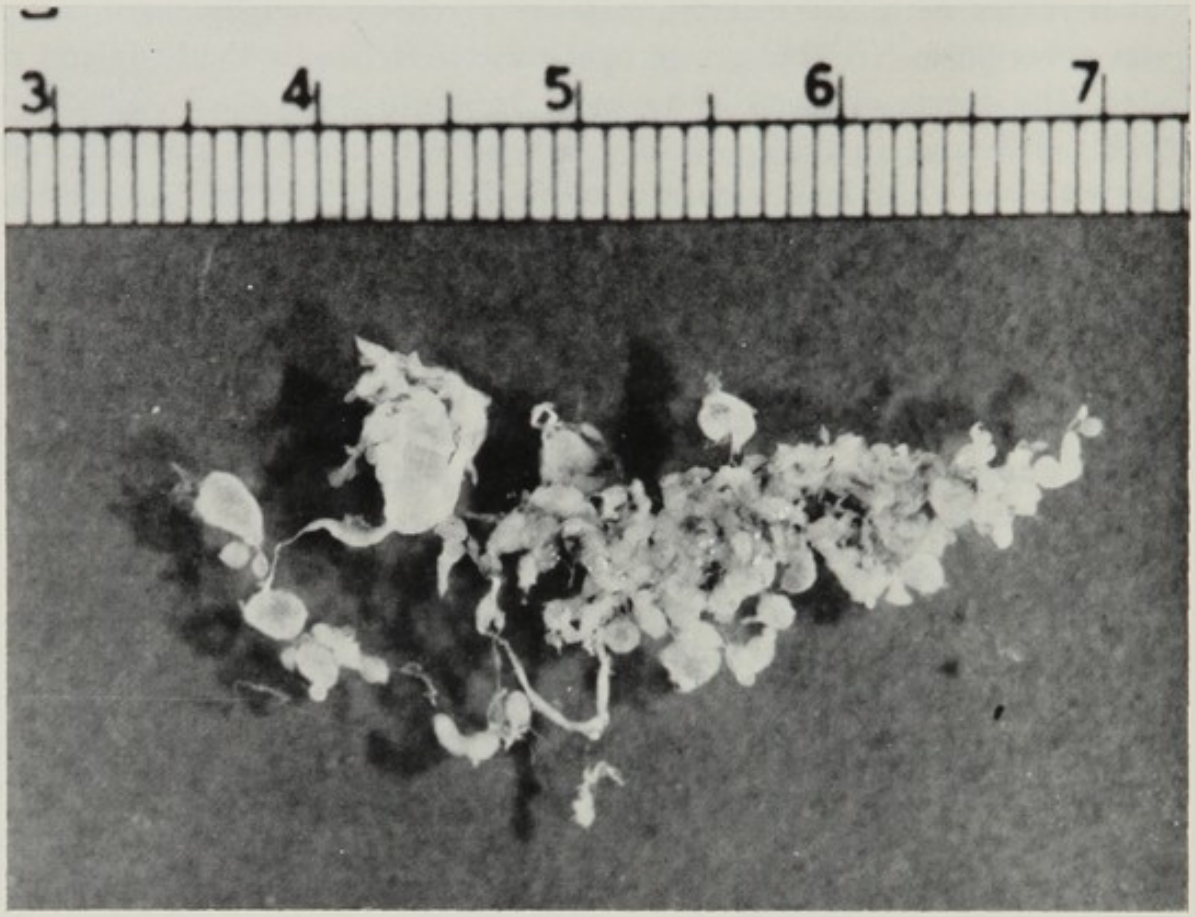



FIG. 5—"Vesiculation of the villi" in a spontaneous abortion (mm. scale).

pregnant after discontinuing oral contraceptives, 45 per cent of available abortuses proved to be triploid or triploid mosaics (Carr, 1967a and additional data). There is a similar but less striking increase in the XO anomaly in abortions under the same circumstances, 27 per cent, compared to about 4 per cent in 12 unselected series.

It seems unlikely that an increase in triploidy will lead to a rise in the incidence of hydatidiform mole. Only a minority of moles are triploid. "Vesicular chorionic villi," which are probably potential moles, are apparently aborted in most instances before they present a typical full-blown picture. The occurrence of triploidy in a percentage of hydatidiform moles does not account for the marked preponderance of chromatin-positive specimens. This observation, noted repeatedly in sex chromatin studies of hydatidiform moles, was recently confirmed and reviewed by Tominaga and Page (1966).

## CHROMOSOMES IN MALIGNANCY

The relationship between chromosome anomalies and malignant change is less clear-cut than in the case of congenital defects. However, there have been important observations of clinical significance.

The only constant chromosome disorder regularly correlated with malig-

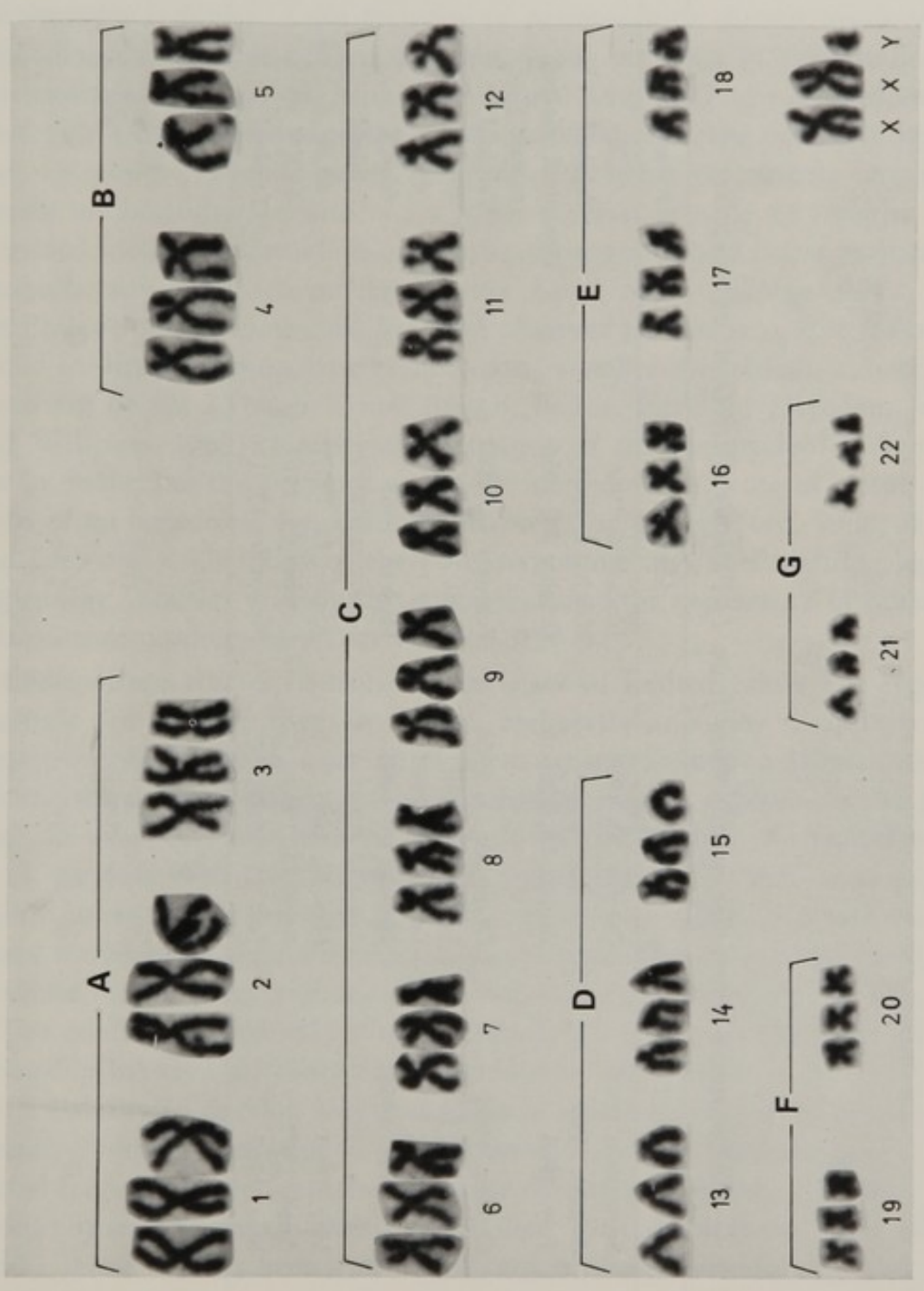



Fig. 6—Triploid chromosome complement of cells grown from material in Fig. 5.

nant disease is the presence of a chromosome in group G with deletion of the long arms. This was first described in a case of chronic myeloid leukemia by Nowell and Hungerford (1960). The finding was soon confirmed, and the deleted chromosome became known as the Philadelphia chromosome or $Ph^1$ (Baikie, Court Brown, Buckton, Harnden, Jacobs and Tough, 1960). With the introduction of the study of direct chromosome preparations in bone

TABLE 9. — *Chromosome Anomalies in Abortuses from 11 Patients After Oral Contraceptives*

| Patient | Age | Ceased medication | L.M.P. | Aborted | Chromosomes |
|---|---|---|---|---|---|
| 1 | 37 | September, 1965 | October 20, 1965 | January 6, 1966 | 45,X |
| 2 | 26 | August, 1965 | ? | March 14, 1966 | Triploid |
| 3 | 25 | July, 1966 | August 15, 1966 | October 28, 1966 | Hexaploid/diploid mosaic |
| 4 | 27 | October, 1966 | December 15, 1966 | February 14, 1967 | Triploid |
| 5 | 24 | December, 1966 | January 28, 1967 | April 26, 1967 | 46,XX |
| 6 | 26 | November, 1966 | February 8, 1967 | May 1, 1967 | 45,X |
| 7 | 20 | December, 1966 | January 17, 1967 | May 18, 1967 | 46,XX |
| 8 | 39 | — | April 10, 1967 | June 7, 1967 | Triploid |
| 9 | 26 | May, 1967 | May 14, 1967 | August 11, 1967 | 45,X |
| 10 | 27 | April, 1967 | April 23, 1967 | October 25, 1967 | 46,XY |
| 11 | 29 | May, 1967 | July 12, 1967 | November 17, 1967 | Triploid |

marrow cells, without prior culture, it became clear that the Philadelphia chromosome is found in the majority of patients with chronic myelogenous (granulocytic) leukemia. Tjio, Carbone, Wang and Frei (1966) studied the chromosomes of 73 people with this disease. Sixty of the 73 showed the deleted ($Ph^1$) chromosome and their mean survival from the time of diagnosis was 45 months. On the other hand the $Ph^1$-negative patients responded poorly to chemotherapy and had a mean survival of only 18 months. This provided further confirmation of improved prognosis and better response to chemotherapy published earlier (Krauss, Sokal and Sandberg, 1964). Once the Philadelphia chromosome has been observed in a patient, it is invariably found in direct bone marrow examination, whether the patient is in clinical remission or not (Tough, Court Brown, Baikie, Buckton, Harnden, Jacobs and Williams, 1962). Usually the diagnosis of chronic myeloid leukemia is not in doubt, but the presence of the $Ph^1$ chromosome is useful in the diagnosis of an occasional atypical case (Nowell and Hungerford, 1962; Bowen and Lee, 1963). Doubling of the Ph chromosome may be a warning sign of impending "blastic" crisis in this disease, as may the appearance of other unrelated chromosome anomalies (Conen, 1967).

Chromosome studies in acute leukemia are of limited value. The types of anomaly are diverse; they are found irregularly and may disappear completely during remission, even on direct marrow examination (Conen, 1967). On the other hand, chromosome examination may be valuable in the prognosis of cases of "preleukemia." Nowell (1965) studied the chromosomes in 23 patients with this diagnosis. He found that in patients who had received no treatment, the finding of chromosome anomalies indicated that patients would soon enter a frank leukemic phase. Negative findings did not preclude the early onset of the disease but the prognosis appeared better.

The etiology of leukemia is still debated. There seems to be a definite relationship between an abnormal chromosome complement in the individual and a liability to develop leukemia. This is especially marked in the case of trisomy-21 syndrome (Conen and Erkman, 1966b). Other known or suspected factors include agents which cause chromosome breaks including radiation, viruses and certain chemicals (Miller, 1964; Nowell and Hungerford, 1966). It has recently been shown that certain diseases associated with a high incidence of chromosome breaks in blood cultures often progress to leukemia (Swift and Hirschhorn, 1966; Sawitsky, Bloom and German, 1966; Hecht, Koler, Rigas, Dahnke, Case, Tisdale and Miller, 1966). If the association between leukemia and chromosome breakage is a direct one, the discovery of agents which have this effect on chromosomes is most significant. Cohen, Marinello and Back (1967) and Irwin and Egozcue (1967) have reported a high incidence of chromosome breaks among users of lysergic acid diethyla-

mide (LSD). The finding has not been confirmed by all workers (Loughman, Sargent and Israelstrom, 1967). More work is in progress in this important subject, relating a commonly-used agent to chromosome damage (Cohen, Hirschhorn and Frosch, 1967).

There is one other type of chromosome anomaly, related in some cases to malignant disease, which is "second only to the Philadelphia chromosome in potential importance" (Baikie, 1966). A chromosome anomaly was first described in association with macroglobulinemia by Bottura, Ferrari and Veiga (1961) and by German, Biro and Bearn (1961). In each instance an extra, large chromosome was found in a proportion of cells cultured from bone marrow or blood. This finding has been confirmed repeatedly in patients with macroglobulinemia, some of whom had malignant disease of the reticulo-endothelial system (Blake, 1966; Ritzmann, Stoufflet, Houston and Levin, 1966).

## CHROMOSOME STUDIES IN POPULATIONS OF NORMAL SUBJECTS

In assessing the significance of chromosome disorders in relation to disease it is important to consider the problem of chromosome disorders in apparently normal individuals. Some progress has been made in this direction, though the problem of selection has not been entirely overcome. In the most detailed study to date, 438 randomly sampled subjects between 15 and 90 years of age had chromosome studies by blood culture technique. In this population there were 2 subjects with reciprocal translocation, 2 with prominent short arms on one member of group D, 2 with marked difference in size between members of the 16 chromosome pair, 5 with a prominent short arm on one member of group G, and one male with pericentric inversion of the Y chromosome (Court Brown, Buckton, Jacobs, Tough, Kuenssberg and Knox, 1966). In addition these authors reported an increase in the proportion of aneuploid cells with advancing age, affecting the sex chromosomes and appearing earlier and to a more marked degree in females than males. The details of a chromosome study of newborn infants is awaited with interest (Valentine and Sergovich, 1967).

## ADDENDUM

Of 26 abortuses from women who became pregnant within six months of discontinuing oral contraceptives, 16 had a chromosome anomaly. Three were trisomic, three were 45,X and 10 were polyploid. The incidence of polyploidy is highly significant when compared with that from an unselected series of patients not receiving oral contraceptives ($p < .001$).

## References

Adams, M. S. 1965. Palm-prints and a ring-D chromosome. Lancet 2: 494-495.

Al-Aish, M. S., F. de la Cruz, L. A. Goldsmith, J. Volpe, G. Mella and J. C. Robinson. 1967. Autosomal monosomy in man. Complete monosomy G (21-22) in a four-and-one-half-year-old mentally retarded girl. New Eng. J. Med. 277: 777-784.

Alexander, D. S. and M. A. Ferguson-Smith. 1961. Chromosomal studies in some variants of male pseudohermaphroditism. Pediatrics 28: 758-763.

Anderson, H. and J. H. Probst. 1963. Renal malformations in gonadal dysgenesis. Acta Paediat. Scand. (Abstr.) Suppl. 140: 142.

Anderson, I. F. 1965. The triplo-X syndrome: Clinical and cytologic features. S. Afr. Med. J. 39: 841-844.

—, E. A. Goeller and C. Wallace. 1964. Sex chromosome abnormalities in a population of 1,662 mental defectives. S. Afr. Med. J. 38: 346-349.

Armendares, S., L. Buentello, J. Sanchez and M. Ortiz. 1967. XO/XX/XXX mosaicism without Turner's syndrome. Lancet 2: 840.

Atkin, N. B. and H. P Klinger 1962. The superfemale mole. Lancet 2: 727-728.

Atkins, L., M. A. O'Sullivan and C. V. Pryles. 1962. Mongolism in three siblings with 46 chromosomes. New Eng. J. Med. 266: 631-635.

Aula, P. and L. Hjelt. 1964. Cytogenetics in mongolism. A review of literature and personal investigation. Ann. Paediat. Fenn. 10: 13-23.

Back, F., P. Dormer, P. Baumann and E. Olbrich. 1967. Trisomy 21 or 22 in Down's syndrome. Lancet 1: 1228.

Bahner, F., G. Schwarz, H. A. Heinz and K. Walter. 1960. Turnersyndrom mit voll Ausebildeten Sekundaren Geschlectsmerkmalen und Fertilitat. Acta Endocr. (Kobehavn) 35: 397-404.

Baikie, A. G. 1966. Chromosomes and leukaemia. Acta Haemat. (Basel) 36: 157-173.

—, W. M. Court Brown, K. E. Buckton, D. G. Harnden, P. A. Jacobs and I. M. Tough. 1960. A possible specific chomosome abnormality in human chronic myeloid leukaemia. Nature 188: 1165-1166.

—,O. M. Garson, S. M. Weste and J. Ferguson. 1966. Numerical abnormalities of the X chromosome. Frequency among inpatients of a general hospital and in a general population. Lancet 1: 398-400.

Bain, A. D. and I. K. Gauld. 1963. Multiple congenital anomalies associated with ring chromosome. Lancet 2: 304-305.

Barr, M. L. 1966. The natural history of Klinefelter's syndrome. Fertil. Steril. 17: 429-441.

— and D. H. Carr. 1960. Sex chromatin, sex chromosomes and sex anomalies. Canad. Med. Ass. J. 83: 979-986.

—, —, A. Morishima and M. M. Grumbach. 1962. An XY/XXXY sex chromosome mosaicism in a mentally defective male patient. J. Ment. Defic. Res. 6: 65-74.

—, —, E. R. Plunkett, H. C. Soltan and R. G. Wiens. 1967. Male pseudohermaphroditism and pure gonadal dysgenesis in sisters. Amer. J. Obstet. Gynec. 99: 1047-1055, 1967.

—, —, H. C. Soltan, R. G. Wiens and E. R. Plunkett. 1964. The XXYY variant of Klinefelter's syndrome. Canad. Med Ass. J. 90: 575-580.

—, E. L. Shaver, D. H. Carr and E. R. Plunkett. 1960. The chromatin-positive Klinefelter syndrome among patients in mental deficiency hospitals. J. Ment. Defic. Res. 4: 89-107.

Bateman, A. J. 1964. Partial aneuploidy. A third mode of inheritance of human disease. Lancet 1: 310-311.

Beçak, W., M. L. Beçak and J. D. Andrade. 1964. A genetical investigation of congenital anesthesia. 1. Cytogenetic studies. Acta Genet. (Basel) 14: 133-142.

—, —, — and A. Manissadjian. 1963. Chromosomal translocation in a mongoloid male child and his normal mother. Arq. Neuropsychiat 21: 161-167.

Beischer, N. A., D. W. Fortune and M. G. Fitzgerald. 1967. Hydatidiform mole and coexistent foetus both with triploid chromosome constitution. Brit. Med. J. 3: 476-478.

Benson, P. F., A. I. Taylor and M. H. Gough. 1967. Chromosome anomalies in primary lymphoedema. Lancet 1: 461-462.

Bergada, C., W. W. Cleveland, H. W. Jones and L. Wilkins. 1962. Gonadal histology in patients with male pseudohermaphroditism and atypical gonadal dysgenesis: Relation to theories of sex differentiation. Acta Endocr. (Kobenhavn). 40: 493-520.

Bergemann, E. 1961. Geschlectschromatinbestimmungen am Neugeborenen. Schweiz. Med. Wschr. 91: 292-294.

—. 1962. Manifestation familiale du karyotype triplo-X. Communication preliminaire. J. Génét Hum. 10: 370-371.

Bias, W. B. and B. R. Migeon. 1967. Haptoglobin: A locus on the $D_1$ chromosome ? Amer. J. Hum. Genet. 19: 393-398.

Biesele, J. J., W. Schmid, C. H. Lee and P. M. Smith. 1962. Translocation between acrocentric chromosomes in a 46-chromosome mongoloid and his 45-chromosome mother. Amer. J. Hum. Genet. 14: 125-134.

Blake, M. N. 1966. A chromosome abnormality associated with macroglobulinaemia. Aust. Ann. Med. 15: 162-168.

Blau, J. N. and D. E. Mutton. 1967. Chromosome studies in the 'sensory syndrome'. Acta Genet. (Basel) 17: 226-233.

Bloom, G. E., P. S. Gerald and L. E. Reisman. 1967. Ring D chromosome: A second case associated with anomalous haptoglobin inheritance. Science 156: 1746-1748.

Bochkov, N. P. 1965. Frequency of sex-chromosome abnormalities in newborn and died children. Proceedings of Symposium on Mutational Process. Prague.

Botella, Llusia J., J. A. Clavero-Nunez, and F. Nogales. 1964. Un caso de feminizacion testicular con cariotypo. Acta Ginec. (Madrid) 15. 389-396.

Bottura, C., I. Ferrari and A. A. Veiga. 1961. Chromosome abnormalities in Waldenstrom's macroglobulinaemia. Lancet 1: 1170.

Boué, J. G. 1967. Personal communication.

— and A. Boué. 1966. Les aberrations chromosomiques dan les avortements spontanés humains. C. R. Acad. Sci. [D] (Paris) 263: 2054-2058.

Bourgoin, P., R. Baylet, C. Ballon and H. Grattepanche. 1965. Exploration d'une hypothèse sur l'étiopathogénie des môles hydatiformes: Étude chromosomique. Rev. Franc. Gynec. Obstet. 60: 673-684.

Bowen, P. and C. S. N. Lee. 1963. $Ph^1$ chromosome in the diagnosis of chronic myeloid leukemia. Report of a case with features simulating myelofibrosis. Bull. Hopkins Hosp. 113: 1-12.

— and —. 1966. Karyotypes of cell strains derived from aborted human fetal tissues. Proc. 3rd Int. Cong. Human Genet., Chicago.

Brandt, N. U., A. Froland, M. Mikkelsen, A. Nielsen and N. Tolstrup. 1963. Galactosaemia locus and the Down's syndrome chromosome. Lancet 2: 700-703.

Breg, W. R., E. E. Castilla, O. J. Miller and J. C. Cornwell. 1963. Sex chromatin and chromosome studies in 1,562 institutionalized mental defectives. J. Pediat. 63: 738-739.

—, O. J. Miller and R. D. Schmickel. 1962. Chromosomal translocations in patients with mongolism and in their normal relatives. New Eng. J. Med. 266: 845-852.

—, —, M. W. Steel and D. Warburton. 1966. Clinical findings in older patients with deletions of a chromosome 4-5 (cri du chat syndrome). Proc. 3rd Int. Cong. Hum. Genet., Chicago.

Brodie, H. R. and L. Dallaire. 1962. The E. syndrome (trisomy 17-18) resulting from a maternal chromosomal translocation. Canad. Med. Ass. J. 87: 558-561.

Brody, J., M. G. Fitzgerald and A. S. D. Spiers. 1967. A female child with five X chromosomes. J. Pediat. 70: 105-109.

Brogger, A., and A. Strand. 1965. Contribution to the study of the so-called pure gonadal dysgenesis. Acta Endocr. (Kobenhavn). 48: 490-505.

Buckton, K. E., D. G. Harnden, A. G. Baikie and G. E. Woods. 1961. Mongolism and leukaemia in the same sibship. Lancet 1: 171-172.

Capoa, A. de., D. Warburton, W. R. Breg, D. A. Miller and O. J. Miller. 1967. Translocation heterozygosis: A cause of five cases of the cri du chat syndrome and two cases with a duplication chromosome number five in three families. Amer. J. Hum. Genet. 19: 586-603.

Carr, D. H. 1963. Chromosome abnormalities and their relation to disease. Canad. Med. Ass. J. 88: 456-461.

—. 1965. Chromosome studies in spontaneous abortions. Obstet. Gynec. 26: 308-326.

—. 1966. Cytogenetics of abortions. *In* Benirschke, K. (Ed.): Comparative aspects of reproductive failure. New York, Springer-Verlag.

—. 1967. Chromosome anomalies as a cause of spontaneous abortion. Amer. J. Obstet. Gynec. 97: 283-293.

—. 1967a. Chromosomes after oral contraceptives. Lancet 2: 830-831.

—, M. L. Barr and E. R. Plunkett. 1961. An XXXX sex chromosome complex in two mentally defective females. Canad. Med. Ass. J. 84: 131-137.

—, R. A. Haggar and A. G. Hart. 1967. Germ cells in the ovaries of XO female infants. Amer. J. Clin. Path. In press.

Carter, C. O., J. L. Hamerton, P. E. Polani, A. Gunlap and S. D. V. Weller. 1960. Chromosome translocations as a cause of familial mongolism. Lancet 2: 678-680.

Casey, M. D., C. E. Blank, D. R. K. Street, L. J. Segal, J. H. McDougall, P. J. McGrath and J. L. Skinner. 1966. YY chromosomes and antisocial behavior. Lancet 2: 859-860.

—, L. J. Segall, D. R. K. Street and C. E. Blank. 1966. Sex chromosome abnormalities in two state hospitals for patients requiring special security. Nature 209: 641-642.

de la Chapelle, A. 1962. Cytogenetical and clinical observations in female gonadal dysgenesis. Acta Endocr. (Kobenhavn) Suppl. 65.

—. 1963. Sex chromosome abnormalities among the mentally defective in Finland. J. Ment. Defic. Res. 7: 129-146.

—, H. Hortling, M. Niemi and J. Wennström. 1964. XX sex chromosomes in a human male. Acta Med. Scand. Suppl. 412: 25-38.

—, —, J. Wennström, M. Niemi and C. J. Johansson. 1965. Two males with female chromosomes. Acta Endocr. (Kobenhavn). Suppl. 100: 90.

Clarke, C. M., J. H. Edwards and V. Smallpiece. 1961. 21 trisomy/normal mosaicism in an intelligent child with some monogoloid characters. Lancet 2: 1028-1030.

Clendenin, T. M. and K. Benirschke. 1963. Chromosome studies on spontaneous abortions. Lab. Invest. 12: 1281-1292.

Close, H G. 1963. Two apparently normal triple-X females. Lancet 2: 1358-1359.

Cohen, M. M. 1966. Chromosomal mosaicism associated with a case of cyclopia. J. Pediat. 69: 793-798.

—, K. Hirschhorn and W. A. Frosch. 1967. In vivo and in vitro chromosomal damage induced by LSD–25. New Eng. J. Med. 277: 1043-1049.

—, M. J. Marinello and N. Back. 1967. Chromosomal damage in human leukocytes induced by lysergic acid diethylamide. Science 155: 1417-1419.

—, A. A. Sandberg, N. Takagi and M. H. MacGillivray. 1967. Autoradiographic investigations of centric fragments and rings in patients with stigmata of gonadal dysgenesis. Cytogenetics (Basel) 6: 254-267.

— and M. W. Shaw. 1965. Two XY siblings with gonadal dysgenesis and a female phenotype. New Eng. J. Med. 272: 1083-1088.

Conen, P. E. 1967. Chromosome studies in leukemia. Canad. Med. Ass. J. 96: 1599-1605.

—. 1967. Personal communication.

— and B. Erkman. 1966. Frequency and occurrence of chromosomal syndromes. I. D-trisomy. Amer. J. Hum. Genet 18: 374-386.

— and —. 1966a. Frequency and occurrence of chromosomal syndromes. II. E-trisomy. Amer. J. Hum. Genet. 18: 387-398.

— and —. 1966b. Combined mongolism and leukemia. Amer. J. Dis. Child. 112: 429-443.

—, —, and R. D. Jeffs. 1964. 47/XXY Klinefelter's syndrome in an infant with abnormal genitalia. J. Urol. 91: 595-599.

— and I. H. Glass. 1963. 45/XO Turner's syndrome in the newborn: Report of two cases. J. Clin. Endocr. 23: 1-10.

Court Brown, W. M., K. E. Buckton, P. A. Jacobs, I. M. Tough, E. V. Kuenssbergs and J. D. E. Knox. 1966. Chromosome studies in adults. London, Cambridge University Press.

—, D. G. Harnden, P. A. Jacobs, N. Maclean and D. J. Mantle. 1964. Abnormalities of the sex chromosome complement in man. Medical Research Council Special Report. London, Her Majesty's Stationary Office.

Dallaire, L. and F. C. Fraser. 1964. Two unusual cases of familial mongolism. Canad. J. Genet. Cytol. 6: 540-547.

Day, R. W., W. Larson and S. W. Wright. 1964. Clinical and cytogenetic studies on a group of females with XXX sex chromosome complements. J. Pediat. 64: 24-33.

J. Pediat. 67: 399-409.

— and C. P. Miles. 1965. Familial Down's syndrome with undetected translocation.

— and S. W. Wright. 1965. Down's syndrome at young maternal ages: Chromosomal and family studies. J. Pediat. 66: 764-771.

Decourt, L., W. Sasso, E. Chiorboli and J. M. Fernandes. 1954. Sobre o sexo genetico nas parientes com sindrome de Turner. Rev. Ass. Med. Brasil 1: 203.

Destiné, M. L., H. H. Punnett, S. Thovicht, A. M. Di George and L. Weiss. 1967. La deletion partielle du bras long du chromosome 18 (syndrome 18q–). Rapport de deux cas. Ann. Génét. (Paris) 10: 65-69.

Dewhurst, J. 1967. The XY female. J. Obstet. Gynaec. Brit. Comm. 74: 353-366.

Edwards, J. H., M. A. Ferguson-Smith, A. Froland, J. Lindsten, P. E. Polani, R. R. Race and R. Sanger. 1966. The contribution of the Xg blood groups to X chromosome aneuploidy: an interim report. Proc. 3rd Int. Cong. Hum. Genet., Chicago.

—, M. Fraccaro, P. Davies and R. B. Young. 1962. Structural heterozygosis in man: analysis of two families. Ann. Hum. Genet. 26: 163-178.

—, D. G. Harnden, A. H. Cameron, V. M. Cross and O. H. Wolff. 1960. A new trisomic syndrome. Lancet 1: 787-790.

Eisen, J. D. and C. R. Angle. 1964. Apparent trisomy E syndrome—a case with partial trisomy C. International Copenhagen Congress on the Scientific Study of Mental Retardation. 139-145.

Elmore, S. M., W. E. Nance, B. J. McGee, M. E. de Montmollin and E. Engel. 1966. Pycnodysostosis, with a familial chromosome anomaly. Amer. J. Med. 40: 273-282.

Engel, E. and A. P. Forbes. 1965. Cytogenetical and clinical findings in 48 patients with congenitally defective or absent ovaries. Medicine 44: 135-164.

Faint, S. and F. J. W. Lewis. 1965. Personal communication.

Fathalla, M. F., M. N. Pashad and M. G. Kerr. 1966. The relationship between ovarian tumours and intersex states. J. Obstet. Gynaec. Brit. Comm. 73: 812-820.

Ferguson-Smith, M. A. 1959. The prepubertal testicular lesion in chromatin-positive Klinefelter's syndrome (primary micro-orchidism) as seen in mentally handicapped children. Lancet 1: 219-222.

—. 1962. Sex chromatin anomalies in mentally defective individuals. Acta Cytol. (Balt.) 6: 73-83.

—. 1965. Karyotype-phenotype correlations in gonadal dysgenesis and their bearing on the pathogenesis of malformations. J. Med. Genet. 2: 142-155.

—. 1967. X-Y chromosomal interchange in the aetiology of true hermaphroditism and of XX Klinefelter's syndrome. Lancet 2: 475-476.

—, D. S. Alexander, P. Bowen, R. M. Goodman, B. N. Kaufman, H. W. Jones and R. H. Heller. 1964. Clinical and cytogenetical studies in female gonadal dysgenesis and their bearing on the cause of Turner's syndrome. Cytogenetics (Basel) 3: 355-383.

Finley, W. H., S. C. Finley, C. J. Rosecrans and C. C. Tucker. 1966. Normal/21-trisomy mosaicism. Amer. J. Dis. Child. 112: 444-447.

Ford, C. E., K. W. Jones, P. E. Polani, J. C. de Almeida and J. H. Briggs. 1959. A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). Lancet 1: 711-713.

Forssman, H. and O. Lehmann. 1962. Chromosome studies in eleven families with mongolism in more than one member. Acta Paediat. Scand. 51: 180-188.

Fraccaro, M., K. Kaijser and J. Lindsten. 1960. Chromosomal abnormalities in father and mongol child. Lancet 1: 724-727.

—, —, and —. 1960a. A child with 49 chromosomes. Lancet 2: 899-902.

Fraccaro, M., J. Lindsten and L. Tiepolo. 1967. Trisomy 21 or 22 in Down's syndrome. Lancet 1: 389.

Fraser, J. H., J. Campbell, R. C. MacGillivray, E. Boyd and B. Lennox. 1960. The XXX syndrome. Frequency among mental defectives and fertility. Lancet 2: 626-627.

Frasier, S. D., R. A. Bashore and H. D. Mosier. 1964. Gonadoblastoma associated with pure gonadal dysgenesis in monozygous twins. J. Pediat. 64: 740-745.

Froland, A. 1967. Seasonal dependence in birth of patients with Klinefelter's syndrome. Lancet 2: 771.

—, A. Lykke and B. Zachu-Christiansen. 1963. Ovarian dysgenesis (Turner's syndrome) in the newborn. Acta Path. Micobiol. Scand. 57: 21-29.

Futterweit, W. 1967. Spermatozoa in seminal fluid of a patient with Klinefelter's syndrome. Fertil. Steril. 18: 492-496.

Gautier, M. 1966. Chromosomes in congenital heart-disease. Lancet 2: 641.

Gedda, L., F. Calabresi, P. Del Porto, A. Del Porto-Mercuri, A. Alfieri, G. Torrioli-Riggio and L. Romei. 1967. On a case of rare chromosomal disorder. Acta Genet. Med. (Roma) 16: 1-7.

Gerald, P. S., S. Warner, J. D. Singer, P. A. Corcoran and I. Umansky. 1967. A ring D chromosome and anomalous inheritance of haptoglobin type. J. Pediat. 70: 172-179.

German, J. L., C. E. Biro and A. G. Bearn. 1961. Chromosomal abnormalities in Waldenstrom's macroglobulinaemia. Lancet 2: 48.

German, J., J. Lejeune, M. N. Macintyre and J. de Grouchy. 1964. Chromosomal autoradiography in the cri du chat syndrome. Cytogenetics (Basel) 3: 347-352.

German, J. L., A. P. de Mayo and A. G. Bearn. 1962. Inheritance of an abnormal chromosome in Down's syndrome (mongolism) with leukemia. Amer. J. Hum. Genet. 14: 31-43.

German, J. and M. Vesell. 1966. Testicular feminization in monozygotic twins with 47 chromosomes (XXY). Ann. Génét. (Paris) 9: 5-8.

Giannelli, F. 1965. Autoradiographic identification of the D(13-15) chromosome responsible for $D_1$ trisomic Patau's syndrome. Nature 208: 669-672.

—, J. L. Hamerton and C. O. Carter. 1965. Cytogenetics of Down's syndrome (mongolism). II. The frequency of interchange trisomy in patients born at a maternal age of less than 30 years. Cytogenetics (Basel) 4: 186-192.

Gilgenkrantz, S., J. M. Gilgenkrantz, J. P. Faucher and M. Pierson. 1966. Un cas de syndrome triplo-X avec malformations multiples. Ann Génét. (Paris) 9: 123-126.

—, J. Sapelier, M. Thiriet, Cl. Kahn and M. Pierson. 1967. A propos de la trisomie 18–. Etude de 4 observations. Ann. Génét. (Paris) 10: 32-38

Goldberg, M. N. and A. L. Scully. 1967. Gonadal malignancy in gonadal dysgenesis. Papillary pseudomucinous cystadenocarcinoma in a patient with Turner's syndrome. J. Clin. Endocr. 27: 341-347.

Goodman, R. M. and B. W. Kaufman. 1962. A 13-15 trisomy with clinical features of the 16-18 trisomy. J. Pediat. (Abstr.) Amer. J. Dis. Child 104: 570.

Greenblatt, R. B., J R. Byrd, P. G. McDonough and V. B. Mahesh. 1967. The spectrum of gonadal dysgenesis. Amer. J. Obstet. Gynec. 98: 151-172.

Grouchy, J. de. 1965a. Chromosome 18: A topologic approach. J. Pediat. 66: 414-431.

—. 1965. A complex familial chromosome translocation. Amer. J. Hum. Genet. 17: 501-509.

—. 1966. Aneusomie de Recombinaison: Three further examples. Amer. J. Hum. Genet. 18: 467-484.

—, J. Bonnette and Ch. Salmon. 1966. Délétion du bras court du chromosome 18. Ann. Génét. (Paris) 9: 19-26.

— and M. Roubin. 1965. Cinq cas de translocations dans un échantillon de 57 trisomiques 21. Ann. Génét. (Paris) 8: 65-66.

—, P. Royer, Ch. Salmon and M. Lamy. 1967. Délétion partielle des bras longs du chromosome 18. Path. Biol. (Paris) 12: 579-582.

Gustavson, K-H. 1964. Down's syndrome. A clinical and cytogenetical investigation. Uppsala, Almquist and Wiksells.

—, S. C. Finley, W. H. Finley and B. Jalling. 1964. A 4-5/21-22 chromosomal translocation associated with multiple congenital anomalies. Acta Paediat. (Scand.) 53: 172-181.

Haddad, H. M. and L. Wilkins. 1959. Congenital anomalies associated with gonadal aplasia. Review of 55 cases. Pediatrics 23: 885-902.

Hall, B. 1962. Down's syndrome (mongolism) with normal chromosmes. Lancet 2: 1026-1027.

—. 1964. Mongolism in newborns. Acta Paediat. Scand. Suppl. 154.

— and B. Källén. 1964. Chromosome studies in abortuses and stillborn infants. Lancet 1: 110-111.

Hambert, G. 1966. Males with positive sex chromatin. Scandinavian University Books. Göteberg, Sweden, Akademiförlaget-Gumperts.

Hamerton, J. L. 1966. Chromosome segregation in three human interchanges. Chromosomes Today 1: 237-252.

—, V. A. Cowie, F. Gianelli, S. M. Briggs and P. E. Polani. 1961. Differential transmission of Down's syndrome (Mongolism) through male and female translocation carriers. Lancet 2: 956-958.

—, G. M. Jagiello and B. H. Kirman. 1962. Sex-chromosome abnormalities in a population of mentally defective children. Brit. Med. J. 1: 220-223.

Hard, W. L., J. Eisen and W. F. Stanage. 1965. The familial transmission of a G/G chromosome in a case of Down's syndrome. S. Dakota J. Med. 18: 15-17.

Harnden, D. G. and J. S. S. Stewart. 1959. The chromosomes in a case of pure gonadal dysgenesis. Lancet 2: 1285-1287.

Hauschka, T. S., J. E. Hasson, M. N. Goldstein, G. F. Koepf and A. A. Sandberg. 1962. An XYY man with progeny indicating familial tendency to non-disjunction. Amer. J. Hum. Genet. 14: 22-30.

Hauschteck, E., G. Mürset, A. Prader and E. Bühler. 1966. Siblings with different types of chromosomal aberrations due to D/E translocation of the mother. Cytogenetics (Basel) 5: 281-294.

Hayashi, T. 1963. Karyotypic analysis of 83 cases of Down's syndrome in Harris County, Texas. Texas Rep. Biol. Med. 21: 28-36.

Hecht, F., J. I. Antonius, P. McGuire and C. G. Hale. 1966. XXY cells in a predominantly XX human male: Evidence for cell selection. Pediatrics 38: 982-985.

—, R. D. Koler, D. A. Rigas, G. S. Dahnke, M. P. Case, V. Tisdale and R. W. Miller. 1966. Leukaemia and lymphocytes in ataxia telangiectasia. Lancet 2: 1193.

— and R. G. Weleber. 1967. Trisomy 21 or 22 in Down's syndrome. Lancet 2: 467.

Heinz, H. A. 1965. Zur Cytogenetik des Mongolismus unter beseonderer Berücksichtigung der familiären. Form. Verh. Deutsch. Ges. Path. 49: 333-338.

—, C. M. Michael, F. W. Schul and K. Walter. 1963. Zur Trisomie des X-Chromosoms. Klin. Wschr. 41: 996-1001.

Hoffenberg, R. and W. P. U. Jackson. 1956. Gonadal dysgenesis in normal looking females. Brit. Med. J. 1: 1281-1284.

Hook, E. B. and J. J. Yunis. 1965. Trisomy-18 in a patient with normal karyotype. J. A. M A. 193: 840-842.

Huang, S-W., I. Emanuel, S-K. Liao and C-C. Hsu. 1967. A cytogenetic study of 77 Chinese children with Down's syndrome. J. Ment. Defic. Res. 11: 147-152.

Hustinx, W. J. 1966. Cytogenetisch onderzoek by enige Families. Drukkerij-Uitgeverij Brakkenstein. Nijmegen.

Inhorn, S. L., E. Therman and K. Patau. 1964. Cytogenetic studies in spontaneous human abortion (Abstr.) Amer. J. Clin. Path. 42: 528.

Insley, J. 1967. Syndrome associated with a deficiency of part of the long arm of chromosome no. 18. Arch. Dis. Child. 42: 140-146.

Irwin, S. and J. Ecozcue. 1967. Chromosomal abnormalities in leukocytes from LSD-25 users. Science 157: 313-314.

Jackson, J. F. and P. W. Ashford. 1967. Familial mongolism due to 21/22 chromosome translocation. J.A.M.A. 200: 722-724.

Jackson, W. P. U., M. Hoffman and H. Makda. 1966. The 45XO/46XY mosaic intersex syndrome. J. Med. Genet. 3: 23-32.

Jacobs, P. A., A. G. Baikie, W. M. Court Brown, H. Forrest, J. R. Roy, J. S. S. Stewart and B. Lennox. 1959. Chromosomal sex in the syndrome of testicular feminisation. Lancet 2: 591-592.

—, —, —, T. N. MacGregor, N. Maclean and D. G. Harnden. 1959. Evidence for the existence of the human "super female." Lancet 2: 423-425.

—, M. Brunton, M. M. Melville, R. P. Brittain and W. F. McClemont. 1965. Aggressive behavior, mental sub-normality and the XYY male. Nature 208: 1351-1352.

—, D. G. Harnden, K. E. Buckton, W. M. Court Brown, M. J. King, J. A. McBride, T. M. MacGregor and N. Maclean. 1961. Cytogenetic studies in primary amenorrhea. Lancet 1: 1183-1189.

— and J. A. Strong. 1959. A case of human intersexuality having a possible XXY sex-determining mechanism. Nature 183: 302-303.

Jacobsen, P. 1966. A ring chromosome in the 13-15 group associated with microcephalic dwarfism, mental retardation and emotional immaturity. Hereditas (Lund) 55: 188-191.

—, M. Mikkelsen, A. Froland and A. Dupont. 1966. Familial transmission of a translocation between two non-homologous large acrocentric chromosomes. Clinical cytogentic and autoradiographic studies. Ann. Hum. Genet. 29: 391-402.

Jacobson, C. B. and R. H. Barter. 1967. Some cytogenetic aspects of habitual abortion. Amer. J. Obstet. Gynec. 97: 666-680.

Jagiello, G. and C. Faiman. 1967. Unilateral fixation of the ossicular chain associated with G-group chromosome deletion. Canad. Med. Ass. J. 96: 1405-1407.

Jagiello, G. M., H. A. Kaminetsky, P. Ricks and R. J. Ryan. 1966. Primary amenorrhea. A cytogenetic and endocrinologic study of 18 cases. J.A.M.A. 198: 30-38.

Jensen, M. K. and J. Chr. Melchior. 1967. Possible partial trisomy of a chromosome of group 6-X-12 and familial translocation heterozygosity in a child with congenital abnormalities. Develop. Med. Child. Neurol. 9: 313-318.

Jeune, M., J. Bertrand, R. Defrenne and M. Forget. 1963. Fréquence des anomalies rénales dans le syndrome de Turner chez l'enfant. Ann. Endocr. (Paris) 24: 856-858.

Jones, H. W., M. A. Ferguson-Smith and R. H. Haller. 1963. The pathology and cytogenetics of gonadal dysgenesis. Amer. J. Obstet. Gynec. 87: 578-600.

Kadotani, T. and E. Pergament. 1966. The nature and implications of chromosome aberrations observed in an abortion population. Proc. 3rd Int. Cong. Hum. Genet., Chicago.

Kaplan, M., R. Grumbach, A. Fischground and O. Ferragu. 1966. Trisomie partielle pour les bras court du chromosome 12. Étude clinique d'une observation. Ann. Pédiat. Paris. 42: 597-606.

Kerr, M. and M. N. Rashad. 1966. Chromosome studies on spontaneous abortions. Amer. J. Obstet. Gynec. 94: 322-329.

Kesaree, N. and P. V. Wooley. 1963. A phenotypic female with 49 chromosomes, presumably XXXXX. J. Pediat. 63: 1099-1103.

Khorsandi, M., D. S. Alexander, A. M. Bryans and M. D. Haust. 1964. Failure to thrive and short stature associated with XXY-chromosomal pattern. Pediatrics 33: 904-911.

Kirch, R. A., E. R. Plunkett, M. S. Smout and D. H. Carr. 1965. Primary ovarian failure. Amer. J. Obstet. Gynec. 91: 630-644.

Klinefelter, H. F. Jr., E. C. Reifenstein, Jr. and F. Albright. 1942. Syndrome characterized by gynecomastia, aspermatogenesis without a-Leydigism and increased excretion of follicle-stimulating hormone. J. Clin. Endocr. 2: 615-627.

Kosenow, W. and R.-A. Pfeiffer. 1967. "YY-Syndrome". Mschr. Kinderheilk 115: 24-31.

Kosowicz, J. 1959. Changes in the medial tibial condyle—a common finding in Turner's syndrome. Acta Endocr. (Kobenhavn) 31: 321-323.

Krauss, S., J. E. Sokal and A. A. Sandberg. 1964. Comparison of Philadelphia chromosome-positive and –negative patients with chronic myelocytic leukemia. Ann. Intern. Med. 61: 625-635.

Lamy, M., N. Josso, J. de Grouchy and A. Bitan. 1965. Anomalies des gonosomes. 41-97. 20e Congres des Pédiatres de Lange Francaise. Ed. L'Expansion Scientifique Francaise. Paris.

Laurent, C., J.–B. Cotton, A. Nivelon and M.–Th. Freycon. 1967. Délétion partielle du bras long d'un chromosome du group D (13-15): Dq–. Ann. Génét. (Paris) 10: 25-31.

Lawrence, R. and A. M. Yuceoglu. 1961. Seminiferous tubule dysgenesis. Amer. J. Dis. Child. 101: 635-638.

Lee, C. S. N., P. Bowen, H. Rosenblum and L. Linsao. 1964. Familial chromosome–2, 3 translocation ascertained through an infant with multiple malformations. New Eng. J. Med. 271: 12-16.

Lejeune, J. 1964. The 21 trisomy– current stage of chromosome research, Vol. 3. In Progress in Medical Genetics, Steinberg A. G. and A. G. Bearn (Eds.): New York, Grune and Stratton Inc., pp. 144-177.

— and R. Berger. 1965. Sur deux observations familiales de translocations complexes. Ann. Génét. (Paris). 8: 21-30.

—, R. Berger, M. O. Réthoré, J. Lafourcade, B. Dutrillaux, P. Canlorbe and B. Labrune. 1967. Deux cas de syndrome 18q–en mosaique (46,XX/46,XX,18q–). Ann. Génét. (Paris) 10: 18-24.

—, M. Gautier and R. Turpin. 1959. Etude des chromosomes somatiques de neuf enfants mongolien. C. R. Acad. Sci. 248: 1721-1722.

—, J. Lafourcade, R. Berger and M. O. Réthoré. 1966. "Cri du chat" syndrome, current stage of investigation. Proc. 3rd Int. Cong. Hum. Genet., Chicago.

—, —, — and R. Turpin. 1964. Ségrégation familiale d'une translocation 5-13 determinant une monosomie et une trisomie partielles du bras courts du chromosome 5: Maladie du "Cri du chat" et sa "réciproque." C. R. Acad. Sci. 258: 5767-5770.

—, —, —, J. Vialette, M. Boeswillwald, P. Seringe and R. Turpin. 1963. Trois cas de délétion partielle du bras court d'un chromosome 5. C. R. Acad. Sci. 257: 3098-3102.

—, —, C. Salmon, and R. Turpin. 1963. Translocation familiale 2-22. Association a un syndrome de Turner haplo-X. Ann. Genet. (Paris) 6: 3-8.

Lemli, L. and D. W. Smith. 1963. The XO syndrome: A study of the differentiated phenotype in 25 patients. J. Pediat. 63: 577-588.

Lévêque, B. and P. Guérineau. 1965. Le syndrome de triplo X. A propos d'une observation. Ann. Pédiat. (Paris) 41: 2622-2626.

Lindsley, D. L., C. W. Edington and E. S. Von Halle. 1960. Sex-linked recessive lethals in Drosophila whose expression is suppressed by the Y chromosome. Genetics. 45: 1649-1670.

Lindsten, J. 1963. The nature and origin of X chromosome aberrations in Turner's syndrome. 1st ed. Almquist and Wiksell. Uppsala.

—, M. Fraccaro, H. P. Klinger and P. Zetterquist. 1965. Meiotic and mitotic studies of a familial reciprocal translocation between two autosomes of group 6-12. Cytogenetics (Basel) 4: 45-64.

Lord, P. M., M. D. Casey and B. M. Lawrence. 1967. A new translocation between chromosomes in the 6-12 and 21-22 groups. J. Med. Genet. 4: 169-176.

Loughman, W. D., T. W. Sargent and D. M. Israelstrom. 1967. Leukocytes of humans exposed to LSD – lack of chromosomal damage. Science 158: 508-510.

Macintyre, M. N., J. M. Hempel, W. I. Staples and M. A. Stenchever. 1964. A ring chromosome in a human male with congenital anomalies. Anat. Rec. 148: 386.

—, W. I. Staples, J. J. Lapolla and J. M. Hempel. 1964. Partial D trisomy in a child whose mother and maternal grandmother demonstrate a D/F translocation. Abstr. Ann. Mtg. Amer. Soc. Hum. Genet.

—, W. I. Staples, A. G. Steinberg and J. M. Hempel. 1962. Familial mongolism (trisomy-21 syndrome) resulting from a 15/21 chromosome translocation in more than three generations of a large kindred. Amer. J. Hum. Genet. 14: 335-344.

Mackay, E. V., K. G. Cockburn and J. G. S. Biggs. 1967. Clinical and chromosomal study of 4 patients with XX/XO mosaicism. Aust. New Zeal. J. Obstet. Gynec. 7: 64-70.

Maclean, N., D. G. Harnden, W. M. Court Brown, J. Bond and D. J. Mantle. 1964. Sex chromosome abnormalities in newborn babies. Lancet 1: 286-290.

—, J. M. Mitchell, D. G. Harnden, J. Williams, P. A. Jacobs, K. A. Buckton, A. G. Baikie, W. M. Court Brown, J. A. McBride, J. A. Strong, H. G. Close and D. C. Jones. 1962. A survey of sex-chromosome abnormalities among 4,514 mental defectives. Lancet 1: 293-296.

Magenis, R. E. and F. Hecht. 1966. $D_1$ trisomy syndrome: studies on parental age, sex and survival. Proc. 3rd Int. Cong. Hum. Genet., Chicago.

Makino, S. 1966. Chromosomes in 150 sexually abnormal patients, (a summarized report). Parts I and II. Cytologia (Tokyo) 31: 309-323 and 349-374.

—, T. Ikeuchi, M. Sasaki, J. Mumamoto, H. Shimba, S. Fujimoto and S. Matsuda. 1967. A preliminary study of the chromosomes in spontaneous abortions. Proc. Japan Acad. 43: 552-555.

—, M. S. Sasaki and T. Fukuschima. 1964. Triploid chromosome constitution in human chorionic lesions. Lancet 2: 1273-1275.

—, — and —. 1965. Cytological studies of tumours, 41. Chromosomal instability in human chorionic lesions. Okajima Folia Anat. Jap. 40: 439-465.

Malvaux, P., H. van den Berghe and M. de Visscher. 1967. Caryotype 47XXX chez une jeune fille de 22 ans phénotypiquement normale. Ann. Endocr. (Paris) 28: 146-147.

Marshall, R., R. E. Newnham, J. R. Rawstron, J. R. Ellis and L. J. Stevens. 1964. Features of 13-15 trisomy syndrome with normal karyotype. Lancet 1: 556.

Marquez-Monter, H. and A. Carnevale-Lopez. 1966. Sex chromatin survey in 3,000 newborn babies in Mexico City. Proc. 3rd Int. Cong. Hum. Genet., Chicago.

McGavin, D. D. M., J. S. Cant, M. A. Ferguson-Smith and P. M. Ellis. 1967. The cri-du-chat syndrome with an apparently normal karyotpe. Lancet 2: 326-330.

McIlree, M. E., W. H. Price, W. M. Court Brown, W. Selby Tulloch, J. E. Newsam and N. Maclean. 1966. Chromosome studies on testicular cells from 50 subfertile men. Lancet 2: 69-71.

McKay, R. J., W. E. Hodgkin and E. H. Witte. 1967. Chromosomes of couples with repeated spontaneous abortions. Pediatrics Res. (Abstr). 1: 207-208.

Meadow, S. R. 1967. Mongoloid features with normal chromosomes—dysplasia epiphysialis punctata. Proc. Roy. Soc. Med. 60: 894-895.

Migeon, B. R. 1966. Short arm deletions in group E and chromosomal "deletion syndromes." J. Pediat. 69: 432-438.

— and W. J. Young. 1964. Reciprocal (D E) translocation: Euploid transmission in three generations. Bull. Hopkins Hosp. 115: 379-388.

Mikkelsen, M. 1966. Transmission of a 13-15/21 translocation in six families. Ann. Hum. Genet. 30: 147-161.

—, A. Froland and J. Ellebjerg. 1963. XO/XX mosaicism in a pair of presumably monozygotic twins with different phenotypes. Cytogenetics (Basel) 2: 86-98.

Miller, O. J., W. R. Breg, D. Warburton, D. A. Miller, I. L. Firschein and K. Hirschhorn. 1966. Alternative DNA replication patterns associated with long arm length of chromosome 4 and 5 in the cri du chat syndrome. Cytogenetics (Basel) 5: 137-151.

Miller, R. W. 1964. Radiation, chromosomes and viruses in the etiology of leukemia. Evidence from epidemiologic research. New Eng. J. Med. 271: 30-36.

Monardo, A. 1965. Gonodal dysgenesis in a woman after seventeen years of regular menses. J. Clin. Endocr. 91: 106-109.

Money, J. and D. Alexander. 1966. Turner's syndrome: Further demonstration of the presence of specific cognitional defects. J. Med Genet. 3: 47-48.

Moore, K. L. 1959. Sex reversal in newborn babies. Lancet 1: 217-219.

Morris, J. M. 1953. The syndrome of testicular feminization in male pseudohermaphrodites. Amer. J. Obstet. Gynec. 65: 1192-1211.

Mosier, H. D., L. W. Scott and L. H. Cotter. 1960. The frequency of the positive sex-chromatin pattern in males with mental deficiency. Pediatrics 25: 291-297.

Mukherjee, B. B., O. J. Miller and W. R. Breg. 1962. Chromosomal rearrangement (a 21/21 isochromosome) as a cause of familial mongolism. Abstr. Ann. Mtg. Amer. Soc. Hum. Genet.

Muldal, S. and C. R. Ockey. 1960. The "double male," a new chromosome constitution in Klinefelter's syndrome. Lancet 2: 492-493.

Nadler, H. L. 1967. Cultivated human fetal cells derived from amniotic fluid. Abstr. Ann. Mtg. Amer. Soc. Hum. Genet.

Nakagome, Y., I. Matsui, K. Komiya, K. Momma and M. Arima. 1966. Sex chromosome abnormalities in mentally defective children. Paediat. Univ. Tokyo. 11: 41-46.

Neu, R. L., J. C. Leão and L. I. Gardner. 1966. Short-arm deletions in G-group chromosomes. Lancet 2: 390.

Nitowsky, H. M., N. Sindhvananda, U. R. Konigsberg and T. Weinberg. 1966. Partial monosomy in the cyclops malformation. Pediatrics 37: 260-269.

Nowell, P. C. 1965. Prognostic value of marrow chomosome studies in human "preleukemia." Arch. Path. (Chicago) 80: 205-208.

— and D. A. Hungerford. 1960. Chromosome studies on normal and leukemia human leukocytes. J. Nat. Cancer Inst. 25: 85-109.

— and D. A. Hungerford. 1962. Chromosome studies in human leukemia. IV. Myeloproliferative syndrome and other atypical myeloid disorders. J. Nat. Cancer Inst. 29: 911-931.

— and —. 1966. The etiology of leukemia: Some comments on current studies. Seminars Hemat. 3: 114-121.

Olanders, S. 1967. Double Barr bodies in women in mental hospitals. Brit. J. Psychiat. 113: 1097-1099.

Oster, J. 1953. A clinicogenealogical investigation comprising 526 mongols living on Seeland and neighbouring islands in Denmark. Copenhagen, Danish Science Press, Ltd.

Palmer, C. G., J. Fareed and A. D. Merritt. 1967. Ring chromosome 18 in a patient with multiple anomalies. J. Med. Genet. 4: 117-123.

Patau, K., D. W. Smith, E. Therman, S. L. Inhorn and H. P. Wagner. 1960. Multiple congenital anomaly caused by an extra autosome. Lancet 1: 790-793.

Penrose, L. S. 1961. Mongolism. Brit. Med. Bull. 17: 184-189.

— and J. D. A. Delhanty. 1961. Familial Langdon Down anomaly with chromosomal fusion. Ann. Hum. Genet. 25: 243-252.

—, J. R. Ellis and J. D. A. Delhanty. 1960. Chromosomal translocations in mongolism and in normal relatives. Lancet 2: 409-410.

Peterson, W. C., R. J. Gorlin, F. Peagler and H. Bruhl. 1966. Cutaneous aspects of the XXYY genotype. Arch. Derm. (Chicago) 94: 695-698.

Pettersson, G. and G. Bonnier. 1937. Inherited sex mosaic in man. Hereditas (Lund). 23: 49-69.

Pfeiffer, R. A. 1963. The transmission of G/G translocation. Lancet 1: 1163.

—. 1966. Deletion der kurzen Arme des Chromosomes Nr. 18. Humangenetik 2: 178-185.

—, K. D. Bachmann and K. Bartel. 1967. Misbildungssyndrom bei autosomaler Defizienz (D2/GI) Z. Kinderheilk. 100: 279-290.

—, U. Keuth, E. Stupperich and Th. Büchner. 1966. Klinik und Cytogenetik einer reziproken, Translokation zwischen Chromosomen der Gruppen B (4-5) und D (13-15). Beitr. Path. Anat. 83: 249-277.

—, D. Palm and J. Jochmus. 1967. Das Erscheinungsbild der Trisomie des X-Chromosome bei Jugendlichen (Triple -X- Syndrom). Mschr. Kinderheilk. 115: 9-18.

— and I. H. Pawlowitzki. 1967. Turner's syndrome in the male with chromosomal mosaicism. Humangenetik 4: 136-139.

Pfitzer, P. 1967. Extra minute chromosome in cyclops. Lancet 2: 102-103.

Philip, J., V. Sele and D. Trolle. 1965. Primary amenorrhea. Fertil. Steril. 16: 795-804.

— and J. Teter. 1964. Significance of chromosomal investigation of somatic cells to determine the genetic origin of gonadoblastoma (gonocytoma III). Acta Path. Microbiol. Scand. 61: 543-550.

— and D. Trolle. 1965. Familial male hermaphroditism with delayed and partial masculinization. Amer J. Obstet. Gynec. 93: 1076-1083.

Pitt, D. B., G. C. Webb, J. Wong, M. K. Robson and J. Ferguson. 1967. A case of translocation (c/14) with mental retardation in two offspring. J. Med. Genet. 4: 177-183.

Plunkett, E. R. and M. L. Barr. 1956. Congenital testicular hypoplasia. Anat. Rec. (Abstr.) 124: 348.

Polani, P. E. 1966. Chromosome anomalies and abortions. Develop. Med. Child. Neurol. 8: 67-70.

—, J H. Briggs, C. E. Ford, C. M. Clarke and J. M. Berg. 1960. A mongol girl with 46 chromosomes. Lancet 1: 721-724.

—, W. E. Hunter and B. Lennox. 1954. Chromosomal sex in Turner's syndrome with coarctation of the aorta. Lancet 2: 120-121.

—, M. H. Lessof and P. M. F. Bishop. 1956. Colourblindness in "Ovarian Agenesis" (Gonadal dysphasia). Lancet 2: 118-120.

Porter, I. H., C. D. Brown and D. Mele. 1966. Paternal transmission of a D/G translocation of Down's syndrome. Amer. J. Dis. Child. 112: 260-261.

Punnett, H. H., L. Pinsky, A. M. Di George and R. J. Gorlin. 1966. Familial reciprocal C/18 translocation. Amer. J. Hum. Genet. 18: 572-583.

Raboch, J. 1964. A clinical study of 100 chromatin– positive men. Fertil. Steril. 15: 331-337.

— and I. Sipova. 1961. The mental level in 47 cases of "true Klinefelter's syndrome." Acta Endocr. (Kobenhavn) 36: 404-408.

Reinwein, H. and U. Wolf. 1965. "Cri du chat" with 5/D– translocation. Lancet 2: 797.

Reisman, L. E., A. Darnell and J. W. Murphy. 1965. Abnormalities with ring chromosome. Lancet 2: 445

Ridler, M. A. C., A. Shapiro and W. R. McKibben. 1963. Sex chromatin abnormalities in female subnormal patients. Brit. J. Psychiat. 109: 390-394.

Ritzman, S. E., E. J. Stoufflet, E. W. Houston and W. C. Levin. 1966. Coexistent chronic myelocytic leukemia, monoclonal gammopathy and multiple chromosomal abnormalities. Amer. J. Med. 41: 981-989.

Rivarola, M. A., J. M. Saez, W. J. Meyer, F. M. Kenny and C. J. Migeon. 1967. Studies of androgens in the syndrome of male pseudohermaphroditism with testicular feminization. J. Clin. Endocr. 27: 371-378.

Rohde, R. A. and B. Catz 1964. Maternal transmission of a new group—C (6/9) chromosomal syndrome. Lancet 2: 838-840.

— and R. Tompkins. 1965. "Cri du chat" due to a ring-B (5) chromosome. Lancet 2: 1075-1076.

Rosenkranz, W. 1965. Klinefelter-Syndrom Bei Kindern von Frauen mit Geschlechtschromosomen – Anomalien. Helv. Paediat. Acta. 20: 359-368.

Sawitsky, A., D. Bloom and J. German. 1966. Chromosomal breakage and acute leukemia in congenital telangiectatic erythema and stunted growth. Ann. Intern. Med. 65: 487-495.

Sergovich, F. R., A. Luce and D. H. Carr. 1967. Chromosome aneuploidy in the father of a mongol. Amer. J. Dis. Child. 114: 407-411.

—, H. C. Soltan and D. H. Carr. 1964. Twelve unrelated translocation mongols: Cytogenetic genetic and parental age data. Cytogenetics (Basel) 3: 34-44.

Schlegel, R. J., R. L. Neu, J. C. Leão, E. Farias, M. J. Aspillaga and L. I. Gardner. 1966. Observations on the chromosomal, cytological and anatomical characteristics of 75 human conceptuses. Cytogenetics (Basel) 5: 430-446.

—, —, —, —, P. Lewczak and L. I. Gardner. 1966. Arborizing amniotic polyps in triploid conceptuses: A diagnostic anatomic lesion? Amer. J. Obstet. Gynec. 96: 357-361.

Schmid. W. 1962. A familial chromosome abnormality associated with repeated abortions. Cytogenetics (Basel) 1: 199-209.

Scott, J. S. 1965. Misfit ovarian tumours. Aust. New Zeal. J. Obstet. Gynec. 5: 156-164.

Shaw, M. W. 1962. Familial mongolism. Cytogenetics (Basel) 1: 141-179.

—. 1966. In discussion of paper by C. B. Jacobson and R. H. Barter. 1967. Some cytogentic aspects of habitual abortion. Amer. J. Obstet. Gynec. 97: 666-680.

—, M. M. Cohen and H. M. Hildebrandt. 1965. A familial 4/5 reciprocal translocation resulting in partial trisomy B. Amer. J. Hum. Genet. 17: 54-70.

Shine, I. B. and G. Corney 1966. Turner's syndrome in monozygotic twins. J. Med. Genet. 3: 124-128.

Singh, R. P. and D. H. Carr. 1966. The anatomy and histology of XO human embryos and fetuses. Anat. Rec. 155: 369-384.

—, A. Rubinoff and D. H. Carr. 1966. Chromosomes and abortions. Lancet 2: 445.

Smith, D. W., K. Patau, E. Therman and S. L. Inhorn. 1962 The No. 18 trisomy syndrome. J. Pediat. 60: 513-527.

Sohval, A. R. (1964). Hermaphroditism with "atypical" or "mixed" gonadal dysgenesis. Amer. J. Med. 36: 281-292.

Sparkes, R. S., R. E. Carrell and S. W. Wright. 1967. Absent thumbs with a Ring $D_2$ chromosome: A new deletion syndrome. Amer. J. Hum. Genet. 19: 644-659.

Stalder, G. R., E. M. Bühler and U. K. Bühler. Nachkommen von balancierten fusions- und translokationsheterzygoten Menschen. Helv. Pediat. Acta 20: 169-184.

Starkman, M. N. and M. W. Shaw. 1967. Atypical acrocentric chromosomes in Negro and Caucasian mongols. Amer. J. Hum. Genet. 19: 162-173.

Steele, M. W. and W. R. Breg. 1966. Chromosome analysis of human amniotic-fluid cells. Lancet 1: 383-385.

—, —, A. I. Eidelman, D. T. Lion and T. A. Terzakis. 1966. A B– group ring chromosome with mosaicism in a newborn with cri du chat syndrome. Cytogenetics (Basel) 5: 419-429.

Stenchever, M. A., J. A. Hempel and M. N. Macintyre. 1967. Cytogenetics of spontaneously aborted human fetuses. Obstet. Gynec. 30: 683-691.

Sternberg, W. H. and D. L. Barclay. 1967. Familial XY gonadal dysgenesis. Lancet 2: 946.

Stolte, L. A. M., H. I. A. M. v. Kessel, J. C. Seelen and G. A. J. Tijdink. 1960. Chromosomes in hydatidiform moles. Lancet 2: 1144-1145.

Subray, N. and S. Prabhaker. 1962. Sex chromatin anomalies in newborn babies in India. Science 131: 1116.

Swift, M. R. and K. Hirschhorn. 1966. Fanconi's anemia. Inherited susceptibility to chromosome breakage in various tissues. Ann. Intern. Med. 65: 496-503.

Swyer, G. I. M. 1955. Male pseudohermaphroditism: A hitherto undescribed form. Brit. Med. J. 2: 709-712.

Szulman, A. E. 1965. Chromosomal aberrations in spontaneous human abortions. New Eng. J. Med. 272: 811-818.

Tagher, P. and L. E. Reisman. 1966. Reproduction in Down's syndrome (mongolism): Chromosomal study of mother and normal child. Obstet. Gynec. 27: 182-184.

Taylor, A. I. 1967. Patau's, Edward's and cri du chat syndromes: A tabulated summary of current findings. Develop. Med. Child. Neurol. 9: 78-86.

Teplitz, R. L., D. Miller, K. M. Harsson and T. S. Rundall. 1967. A human ring D chromosome associated with multiple congenital anomalies. J. Pediat. 70: 936-941.

Teter, J. and K. Boczkowski. 1966. Testicular feminization with and without clitoral enlargement. Amer. J. Obstet. Gynec. 94: 813-819.

— and —. 1967. Occurrence of tumours in dysgenetic gonads. Cancer. 20: 1301-1310.

Therkelsen, A. J. 1964. Sterile male with the chromosome constitution 46XX. Cytogenetics (Basel) 3: 207-218.

Thiede, H. A., W. T. Creasman and S. Metcalfe. 1966. Antenatal analysis of the human chromosomes. Amer. J. Obstet. Gynec. 94: 589-590.

— and S. B. Salm. 1964. Chromosome studies of human spontaneous abortions. Amer. J. Obstet. Gynec. 90: 205-215.

Tjio, J. H., P. P. Carbone, J. Whang and E. Frei III. 1966. The Philadelphia chromosome and chronic myelogenous leukemia. J. Nat. Cancer Inst. 36: 567-584.

Tominaga, T. and E. W. Page. 1966. Sex chromatin of trophoblastic tumors. Amer. J. Obstet. Gynec. 96: 305-309.

Tough, I. M., W. M. Court Brown, A. G. Baikie, K. E. Buckton, D. G. Harnden, P. A. Jacobs and J. A. Williams. 1962. Chronic myeloid leukaemia: Cytogenetic studies before and after splenic irradiation. Lancet 2: 115-120.

Townes, P. L., N. A. Zeigler and L. W. Lenhard. 1965. A patient with 48 chromosomes (XYYY). Lancet 2: 1041-1043.

Turner, B. 1962. Human ring chromosomes. Conference on Genetics and Mental Retardation. Sydney, Australia, Government Printer.

Turner, J. H., D. L. Hutchinson and D. Charles. 1966. Chromosomal studies of the intrauterine human fetus Proc. 3rd Int. Cong. Hum. Genet., Chicago.

Uchida, I. A. 1967. Personal communication.

—, M. Ray and M. Ursel. 1967. Cytogenetic investigations of a random series of mongoloid families. Abstr. Ann. Mtg. Amer. Soc. Hum. Genet.

— and H. C. Soltan. 1963. Evaluation of dermatoglyphics in medical genetics. Pediat. Clin. N. Amer. 10: 409-422.

—, H. C. Wang, O. E. Laxdal, W. A. Zaleski and B. P. Duncan. 1964. Partial trisomy-deficiency syndrome resulting from a reciprocal translocation in a large kindred. Cytogentics (Basel) 3: 81-96.

Uozumi, T., R. Nozaki, K. Matsumoto, T. Seki and J-i Furuyama. 1967. Endocrinological studies of a case of mosaicism (XO/XY/XXY) with clinical appearance of testicular feminization syndrome. Acta Endocr. (Kobenhavn) 54: 193-207.

Valentine, G. H. and F. R. Sergovich. 1967. A new dilemma. Lancet 2: 151.

van der Hagen, C. B. 1966. The frequency of translocations and other chromosomal aberrations in 50 mongoloids and their parents. Proc. 3rd Int. Cong. Hum. Genet., Chicago.

van der Werff ten Bosch, J. J. 1959. Comment on paper by P. M. F. Bishop and P. E. Polani. 1959. Turner's syndrome and allied conditions. Memoirs Soc. Endocr. 7: 171-172.

Verger, P., J.-J. Battin, J. Kermarec and C. Vital. 1962. Syndrome de Bonnevie-Ullrich-Turner-chez un nouveau-né. Arch. Franc. Pediat. 19: 1233-1246.

Vislie, H., M. Wehn, A. Brogger and J. Mohr. 1962. Chromosome abnormalities in a mother and two mentally retarded children. Lancet 2: 76-78.

Walzer, S., B. Favara, P. L. Ming and P. S. Gerald. 1966. A new translocation syndrome (3/B). New Eng. J. Med. 275: 290-298.

Wang, H-C, J. Melnyk, L. T. McDonald, I. A. Uchida, D. H. Carr and B. Goldberg. 1962. Ring chromosomes in human beings. Nature 195: 733-734.

Warburg, E. 1963. A fertile patient with Klinefelter's syndrome. Acta Endocr. (Kobenhavn). 43: 12-26.

Warburg, M. 1960. Anophthalmos complicated by mental retardation and cleft palate. Acta Ophthal. (Kobenhavn). 38: 394-404.

Warkany, J., E. D. Weinstein, S. W. Soukup, J. H. Rubinstein and M. C. Curless. 1964. Chromosome analyses in a children's hospital: Selection of patients and results of studies. Pediatrics 33: 290-305 and 454-465.

Waxman, S. H., D. T. Arakaki and J. B. Smith. 1967. Cytogenetics of fetal abortions. Pediatrics 39: 425-432.

Weber, W. W. 1967. Survival and the sex ratio in trisomy 17-18. Amer. J. Hum. Genet. 19: 369-377.

Welch, J. P., D. S. Borgaonkar and H. M. Herr. 1967. Psychopathy, mental deficiency, aggressiveness and XYY syndrome. Nature 214: 500-501.

Wertelecki, W., A. M. Schindler and P. S. Gerald. 1966. Partial deletion of chromosome 18. Lancet 2: 641.

Wiesli, B. 1962. Vergleich des phânotypischen und zellkernmorphologischen Geschlechtes bei 3029 Neugeborenen. Acta Anat. (Basel) 51: 377-383.

Wilkins, L., M. M. Grumbach and J. J. van Wyk. 1954. Chromosomal sex in ovarian agenesis. J. Clin. Endocr. 14: 1270.

Wingate, L. 1965. Chromosome abrnormality as a possible cause of habitual abortion. Bristol med.-chir. J. 80: 5-7.

Wingate, M. B. 1966. The sex ratio of mid-trimester abortions. J. Obstet. Gynaec. Brit. Comm. 73: 296-298.

Wright, S. W., R. W. Day, H. Muller and R. Weinhouse. 1967. The frequency of trisomy and translocation in Down's syndrome. J. Pediat. 70: 420-424.

Yunis, J. J., E. B. Hook and M. Mayer. 1964a. Deoxyribose-nucleic-acid replication pattern of trisomy 18. Lancet 2: 286-287.

—, — and —. 1964b. Deoxyribonucleic-acid replication pattern of trisomy $D_1$. Lancet 2: 935-937.

—, — and —. 1965. DNA replication analysis in identifying the cytogenetic defect in Down's syndrome (mongolism). Lancet 1: 465-466.

—, —, and —. 1965a. Identification of the mongolism chromosome by DNA replication analysis. Amer. J. Hum. Genet. 17: 191-201.

Zaleski, W. A., C. S. Houston, J. Pozsonyi and K. L. Ying. 1966. The XXXXY chromosome anomaly: Report of three new cases and review of 30 cases from the literature. Canad. Med. Ass. J. 94: 1143-1154.

Zourlas, P. A. and H. W. Jones. 1965. Clinical histologic and cytogenetic findings in male hermaphroditism. III. Male hermaphrodites with asymmetrical gonadal differentiation (mixed gonadal dysgenesis). Obstet. Gynec. 26: 48-58.

— and —. 1965a. Clinical, histologic and cytogenetic findings in male hermaphroditis. II. Male hermaphrodites with feminine external genitalia (testicular feminization). Obstet. Gynec. 25: 768-778.

Zuppinger, K., E. Engel, A. P. Forbes, L. Mantooth and J. Claffey. 1967. Klinefelter's syndrome, a clinical and cytogenetic study in twenty-four cases. Acta Endocr. (Kobenhavn). 54: Suppl. 113.

CHAPTER 2

# Genetics and the Gastrointestinal System

**Richard B. McConnell**

Nuffield Unit of Medical Genetics,
Department of Medicine,
University of Liverpool.
Consultant Physician and Research Fellow

SOME DISORDERS of the gastrointestinal tract have been discussed in detail in recent volumes of *Progress in Medical Genetics*. For instance a chapter in Volume 4 was devoted to the genetics of the glycogen storage diseases (Sidbury, 1965). In the same volume Carter (1965) dealt with harelip, cleft palate and hypertrophic pyloric stenosis. Unlike other systems of the body, the gastrointestinal tract has few disorders inherited in a simple Mendelian manner. In most of the conditions which have been studied in some detail, the evidence suggests that the etiology is partly environmental and partly genetic or that there may be little if any genetic component. In many instances, however, the evidence is conflicting. Much of this confusion may be attributed to heterogeneity within the clinical material; and in choosing which disorders to discuss in this review some preference has been given to those in which genetic studies are helping to clarify clinical diagnoses.

## BLOOD GROUPS AND GASTROENTEROLOGY

Aird et al. in 1953 reawakened interest in the relationship of the ABO blood groups to disease, and since then a very large number of papers on the subject have been published. The blood groups of sufferers from nearly every common disease have been reported. It is striking that rarely has any significantly abnormal distribution been found in a condition outside the gastrointestinal tract. With disorders of the upper alimentary tract, on the

other hand, many large series have been found to differ from adequate controls. It is perhaps not surprising that the ABO blood groups are concerned in the pathology of this region, since the remarkably high concentration of blood group substances in saliva and gastric juice imply that they are of considerable importance in alimentary physiology.

Gastric and duodenal ulcer, carcinoma of the stomach, pernicious anemia, gallstones, cirrhosis of the liver and diabetes have all been reported as associated with one or another of the ABO blood groups. In 1961, C. A. Clarke reviewed this subject in the first volume of *Progress in Medical Genetics,* and since then the associations of group O with duodenal ulcer and group A with carcinoma of the stomach have been confirmed again and again. The association of non-secretor with duodenal ulcer, first demonstrated by Clarke et al. (1956), has also been amply confirmed (see the section on secretor character below).

With gastric ulcer it seems probable that there is no relationship with blood groups, except in the case of antral ulcers, which behave like duodenal ulcers (Johnson et al., 1964). Also contributing to a high group O frequency in some series is the inclusion of gastric ulcers which are concomitant with and probably secondary to duodenal ulcers. The relationship between group A and pernicious anemia is on a less firm basis and more large series are required. With gallstones, cirrhosis and diabetes, there has been conflicting evidence, the explanation of which may be heterogeneity of the clinical material. Perhaps within each of these conditions there are types which are related to a blood group while others are not.

*Clinical Types of Duodenal Ulcer*

*Hematemesis and melena:* Langman and Doll (1965) re-examined their duodenal ulcer data and found that the association with Group O applied par-

TABLE 1. — *Blood Groups of Various Clinical Categories and a Control Population, Showing an Abnormally High Group O Frequency in Hematemesis and/or Melena even when the Barium Meal was Normal*

| Blood group | Control population | Non-bleeding duodenal ulcer | Bleeding and normal barium meal | Bleeding duodenal ulcer |
|---|---|---|---|---|
| O | 3146 | 351 | 268 | 329 |
| A | 2648 | 244 | 174 | 157 |
| Total O + A: | 5794 | 595 | 442 | 486 |
| O as % of O + A | 54.3 | 59.0 | 60.6 | 67.7 |

From Horwich et al, 1966.

ticularly to the patients whose ulcers had bled. This finding has been confirmed in Greece (Merikas et al., 1966) and England (Horwich et al., 1966). The latter workers made the additional observation that there was also a high incidence of group O in people who had had a hematemesis and/or melena and in whom no duodenal ulcer or other lesion could be demonstrated radiologically. In Table 1 patients of groups B and AB have been excluded for ease of analysis and it can be seen that the bleeders without ulcer had a significantly higher group O frequency than the general population control.

However, there is a good deal of evidence against a relationship between a bleeding tendency and group O being the whole explanation of the association between group O and duodenal ulcer. Evans et al. (1967) have found a normal group O frequency in 466 menorrhagic patients, and recent data on perforated duodenal ulcers have shown as high a group O frequency as in bleeding duodenal ulcer.

*Perforation.* There was a suggestion in the data of Horwich et al. (1966) that patients with duodenal ulcers that have perforated have a higher group O frequency than those with uncomplicated ulcers. The same authors (Evans et al, 1967) have studied a further 100 patients who have perforated; and the 2 series together indicate that perforation is as closely association with group O as is hematemesis and melena (Table 2). Langman et al. (1967) also found the group O frequency in perforations to be higher than in patients whose ulcer had neither bled nor perforated. Of 271 perforations 159 (58.7 per cent) were group O, compared with 493 (52.9 per cent) of 932 ulcers causing only pain or obstruction. These London data may not be directly comparable with the Liverpool series. The London data are made up of 192 patients having definitive operations for duodenal ulcer who gave a history of previous perforation and 79 people with stomal ulcer who had had a perforated duodenal ulcer before their partial gastrectomy. Most of the Liver-

TABLE 2. — *Blood Groups of Patients with Duodenal Ulcer which have Perforated, Compared with Ulcers Causing Pain or Obstruction Only*

| | Evans et al., 1967. | | | | Langman et al., 1967. | | | |
|---|---|---|---|---|---|---|---|---|
| Blood | Perforations | | Uncomplicated | | Perforations | | Uncomplicated | |
| group | No. | % | No. | % | No. | % | No. | % |
| O | 128 | 56.9 | 278 | 50.6 | 159 | 58.7 | 493 | 52.9 |
| A | 65 | 28.9 | 209 | 38.1 | 88 | 32.5 | 349 | 37.4 |
| B | 25 | 11.1 | 48 | 8.7 | 17 | 6.3 | 68 | 7.3 |
| AB | 7 | 3.1 | 14 | 2.6 | 7 | 2.6 | 22 | 2.4 |
| *Total:* | 225 | 100.0 | 549 | 100.0 | 271 | 100.1 | 932 | 100.0 |

pool patients, on the other hand, had perforated and been treated with simple suture.

These new data on perforation might be interpreted as indicating that group O is particularly associated with severe or complicated duodenal ulcer. Some series, however, have not shown a significantly higher incidence of group O in patients treated surgically compared with those not submitted to operation. For instance, Horwich et al. (1966) had 271 patients who had no operation and 133 (49.1 per cent) of them were group O compared to 145 (52.2 per cent) of the 278 patients who were operated upon for pain or obstruction. On the other hand, the 143 medically treated ulcer patients of Langman et al. (1967) had only a 45.5 per cent group O frequency compared with 53.8 per cent in 637 operated upon for pain or obstruction only.

*Stomal Ulcer*

It might be considered that further evidence in favor of there being a relationship between group O and severity of ulcer is provided by the very high incidence of group O in stomal ulcer (Doll et al., 1960). Langman et al. (1967) have reviewed the world literature on this aspect of the problem and presented detailed analysis of 305 stomal ulcer patients from London hospitals. The overall group O frequency of 59.8 per cent was indeed higher than in their 1300 duodenal ulcer patients who had not developed a stomal ulcer, and of whom 55.8 per cent were group O. The data in Table 3, however, show that this high frequency was largely due to many of the stomal ulcers having developed in patients who had had a partial gastrectomy following bleeding or perforation, particularly the latter (Jones and Langman, 1967). Thus the high group O frequency in stomal ulcer could be merely a reflection of the high group O frequency in bleeding and perforating ulcers.

It remains to be seen if the high non-secretor rate among stomal ulcer patients also is merely due to a high non-secretor rate in duodenal ulcer patients coming to operation (see below).

TABLE 3. — *Data Extracted from Langman et al. (1967) Showing the Percentage of Group O in a Series of 305 Stomal Ulcer Patients, Subdivided by the Main Symptom Before the Operation for their Duodenal Ulcer, and the Group O Percentages in Surgically Treated Duodenal Ulcer Patients*

| Clinical history | Stomal ulcer | | Duodenal ulcer | |
|---|---|---|---|---|
| | No. | % Group O | No. | % Group O |
| *Pain or obstruction only:* | 152 | 55.9 | 637 | 53.8 |
| *Hematemesis or melena:* | 63 | 60.3 | 202 | 59.9 |
| *Perforation:* | 79 | 63.3 | 192 | 56.8 |

*Secretor Character*

A high non-secretor frequency has been found in several series of duodenal ulcer patients (Ball, 1962; Clarke et al., 1956; Doll et al., 1961; Fodor and Urcan, 1966; Newman et al., 1961 and Pringle et al., 1964). The only other condition found to be associated with the secretor character is rheumatic fever. A normal non-secretor frequency has been found in carcinoma of the stomach, gastric ulcer, diabetes mellitus and ulcerative colitis (see Table 4), as well as in many other non-gastrointestinal conditions (McConnell, 1966).

TABLE 4.—*The Secretor Character in Various Diseases*

| Condition | Number tested | Number non-secretor | Percentage non-secretor |
|---|---|---|---|
| *Duodenal ulcer* | 1540 | 543 | 35.3 |
| *Gastric ulcer* | 404 | 116 | 28.7 |
| *Gastric carcinoma* | 288 | 69 | 24.0 |
| *Diabetes mellitus* | 318 | 83 | 26.1 |
| *Rheumatic carditis* | 712 | 220 | 31.0 |
| *Tonsillectomy* | 885 | 233 | 26.3 |
| *Healthy controls* | 2435 | 590 | 24.2 |

A very high non-secretor rate of 43.8 per cent has been found in 176 patients with stomal ulcer (Langman et al., 1967). Patients with bleeding or perforated ulcers, on the other hand, have not been found by Horwich et al. (1966) or Evans et al. (1967) to have any higher non-secretor frequency than patients with uncomplicated duodenal ulcers (Table 5). This might indicate that there is a real predisposition of non-secretors to develop stomal ulcer, but Langman et al. (1967) found in 334 ulcer patients treated surgically that there were 136 (40.7 per cent) non-secretors, compared with 90 (32.5 per cent) of 277 ulcer patients who did not have surgery. Comparing the distribution of secretor in their stomal ulcer patients with their surgically treated patients, they find the difference to lie well within the limits of random variations. They, therefore, conclude that there is no reason to suppose that non-secretion specifically predisposes to the development of stomal ulcer.

This difference between surgically and medically treated duodenal ulcer has, however, not been found (see Table 5) by other investigators (Newman et al., 1961; Horwich et al., 1965). It would, therefore, be wise to keep an open mind on the nature of the relationship between stomal ulcer and non-secretion until there is more agreement on whether or not non-secretors are more liable to have surgical treatment.

*The Lewis System*

There have been few studies of the relationship of the Lewis blood type system to gastrointestinal disease, probably because of the difficulty of finding strong enough anti-sera for inhibition studies of saliva. The qualitative and quantitative presence of the Lewis substances $Le^a$ and $Le^b$ in saliva and gastric juice (McConnell, 1962) is so closely bound up with the genes at the ABO, H and secretor loci (Race and Sanger, 1962) that one might expect the Lewis genes to be concerned at least in duodenal ulcer. Ball (1962) studied the saliva of Nigerian ulcer patients and controls and found an excess of non-secretors made up of Lewis positive patients. Ball's interpretation of his data has been criticized by the writer (McConnell, 1966) whose own series of 1,000 duodenal ulcer patients did not show any significant difference in Lewis distribution from controls.

With carcinoma of the stomach the writer's series of 320 tested for Lewis was made up entirely of Lewis positive patients — that is, patients whose saliva inhibits anti-$Le^a$ or anti-$Le^b$ or both antisera (McConnell, 1967). From the frequency of Lewis negative (patients whose saliva inhibits neither anti-$Le^a$ nor anti-$Le^b$ sera) in the Liverpool control series, only about 10 Lewis negatives would have been expected. The observation therefore needs to be checked in another population, preferably one with a higher Lewis negative frequency.

*Jejunal Alkaline Phosphatase*

One of the most exciting of the recent discoveries about gastrointestinal physiology has been the demonstration that the alkaline phosphatase pro-

TABLE 5. — *The Secretor Character in Various Subdivisions of Duodenal Ulcer Data According to Various Authors*

| Condition | Iowa data | | | Liverpool data | | | London data | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. | non-sec. | % non-sec. | No. | non-sec. | % non-sec. | No. | non-sec. | % non-sec. |
| *Duodenal ulcers (no operation):* | 242 | 77 | 31.8 | 271 | 102 | 37.6 | 277 | 90 | 32.7 |
| *Duodenal ulcer (operation):* | 228 | 74 | 32.5 | 278 | 97 | 34.9 | 334 | 136 | 40.7 |
| *Bleeding duodenal ulcer:* | — | — | — | 109 | 37 | 33.9 | 120 | 42 | 35.0 |
| *Perforated duodenal ulcer:* | — | — | — | 185 | 65 | 35.1 | — | — | — |
| *Stomach ulcer:* | — | — | — | — | — | — | 176 | 77 | 43.8 |

*Note:* Iowa data from Newman et al. (1961).
Liverpool data from Horwich et al. (1966), and Evans et al. (1967).
London data from Langman et al. (1965), and Langman et al. (1967).

duced by the jejunal mucosa is present in the serum of people of certain ABO blood groups and secretor status but not in others.

The different types of alkaline phosphatases in serum are distinguishable by starch gel electrophoresis (Robinson and Goldsmith, 1967). All human sera show one alkaline phosphatase band of liver or bone origin, but serum from some people shows in addition a second slower-moving band probably derived from small intestine (Hodson et al., 1962). The slower-moving zone is usually only detected after a fatty meal and it is of variable intensity (Beckman et al., 1962). Three phenotypes can be distinguished, those with no second band ($p^0$), those with a weak one ($p^+$) and those with a strong one ($p^{++}$).

Twin and family studies (Arfors et al., 1963, and Beckman, 1964) showed that the different phosphatases are under genetic control even though the mode of inheritance was not clear. Part of the genetic control was due to the relationship with the ABO and secretor genes, but the data suggested that in addition other factors were operative. With only very rare exceptions, all non-secretors belong to the $p^\circ$ phenotype (Beckman, 1964; Bamford et al., 1965; Evans, 1965; Shreffler, 1965). Among secretors the $p^{++}$ phenotype is found only in those of groups O, B and AB (see Table 6). Group A secretors are usually $p^\circ$ 4.5 hours after a fatty meal, and it was only 3 hours later that 6 out of 14 showed the faint slow band of the $p^+$ phenotype (Langman et al., 1966).

Shreffler (1966) studied jejunal mucosa obtained at autopsy and found that the mucosa from 3 non-secretors had much the same alkaline phosphatase content as secretors. Since the ABO and secretor genes did not affect significantly the mucosal levels of the enzyme, he concluded that these genes do not control the capacity to synthesize it, but rather must they play a role in determining the rate of secretion into the serum or in destroying or inhibiting the activity of the enzyme after it has been secreted into the serum. This work needs to be repeated using fresh material, but if, in fact, the blood group and secretor genes affect cell permeability to the extent of preventing the enzyme from passing from the cell into the lymph they may well have a wider influence on absorption from the gut. Whatever the mechanism, it seems likely that secretors of groups O and B have an enzyme system related to the epithelial cells of the small bowel different from that of secretors of group A and non-secretors of all groups. These cells are concerned with the absorption of fat from the gut and with its movement into the lymph of the thoracic duct.

It is of interest that a similar relationship between alkaline phosphatase and blood groups has been reported in sheep (Rendel et al., 1964; Hope, 1966). The level of the alkaline phosphatase activity in the serum of sheep

of group O, all of which have an extra phosphatase band (B phosphatase), was found to be higher than in sheep of group R, which usually only have one band. It has been found possible to induce B phosphatase activity in the blood of B-negative animals by intravenous injection of saliva containing large amounts of O blood group substance. In the sheep, O substance may take part in the production of the second phosphatase or may act as an essential mediator in the release of this phosphatase into the serum.

*The Mechanism of the Associations*

The alkaline phosphatase difference between blood group A and groups O and B may give a clue to the nature of the association between group A and carcinoma of the stomach, but it is unlikely to underlie the group O association with duodenal ulcer. The similarity of people of group O and B as regards serum alkaline phosphatase content is in contrast to their differing liability to duodenal ulcer.

The mechanism of the association between the ABO blood groups and alimentary disease is still uncertain. The high concentration of various blood group substances in gastric juice had indicated that genes at the secretor and Lewis loci, as well as those at the ABO blood group locus, are concerned in gastric physiology, and the confirmation of the relationships of these loci with duodenal ulcer and carcinoma of the stomach stimulated a great deal of research which has not yet succeeded in discovering the nature of the relationships. A simple direct protective action of the mucoid substances has seemed unlikely, first because the total content of blood group substance in gastric juice is the same in secretors as in non-secretors, due to the high titer of $Le^a$ substance in the non-secretors who have no A, B or H substance (Evans et al, 1963); and second, because the increased liability of group O people to develop duodenal ulcer is found in non-secretors, all of whose gastric juices are similar. An alternative possible mechanism of an immunologic basis to the relationship has had no supporting evidence.

TABLE 6. — *The Results of Testing for the Slow Jejunal Alkaline Phosphatase the Serum of Subjects of Different ABO Blood Group and Secretor Status**

| Blood group and secretor type | Jejunal alkaline phosphatase in serum | | |
|---|---|---|---|
| | $p^0$ | $p^+$ | $p^{++}$ |
| *O and B Secretors:* | — | 6 | 18 |
| *A Secretors:* | 8 | 6 | — |
| *All non-secretors:* | 16 | 1 | — |

* The Serum was Collected 7.5 Hours after a Fatty Meal and the Phenotypes $p^0$, $p^+$ and $p^{++}$ Indicate Differing Amounts of Enzyme (From Langman et al., 1966).

On the other hand there has been a good deal of circumstantial evidence to support the theory of a pleiotropic effect of the ABO and secretor genes on the type of gastric mucosa, quite separate from their action of determining the serological properties and distribution of the group-specific mucopolysaccharides. Such a pleotropic effect would not however take into account the high concentration of blood group substances in gastric juice, which is unlikely to be fortuitous. The relationship with jejunal alkaline phosphatase is however almost certainly due to a pleiotropic effect of the ABO and secretor genes.

A picture is slowly building up of a large number of genes which not only affect the expression of each other but also determine the type of gastric mucosa, its ability to produce acid, and its liability to bleed, to atrophy and to become malignant. It seems likely that the genes concerned with intestinal alkaline phosphatase can join those at the ABO blood group, secretor, Lewis and Hh loci whose interrelationships have been detailed (McConnell, 1966). Many more loci will have to be added before the picture is complete, and the effects of these genes may turn out to be much more extensive than merely on the gastric mucosa.

## HIRSCHSPRUNG'S DISEASE

There are few clinical conditions in which genetic research has been more rewarding than Hirschsprung's disease (also known as congenital megacolon). One of the commoner congenital abnormalities, it is due to absence of the myenteric plexus from part of the gut. In the majority of cases only the rectum and sigmoid colon are aganglionic but in about 20 per cent a larger part of the colon or even the ileum is involved. A division clinically into short and long segment cases is possible.

Boys are affected much more frequently than girls. In one small series only 1 in 18 cases was a girl (Richardson and Brown, 1962). In other series there were 45 girls in 207 (Bodian and Carter, 1963) and 8 in 63 (Passarge, 1967). Furthermore the sex ratio varies with the length of bowel affected,

TABLE 7. — *Sex Ratio in Hirschsprung's Disease According To the Length of Aganglionic Bowel**

| Upper limit of aganglionic bowel | Number of cases | Number of | | Sex ratio |
|---|---|---|---|---|
| | | Boys | Girls | |
| *Descending colon or above* | 35 | 21 | 14 | 1.5 |
| *Sigmoid colon* | 129 | 104 | 25 | 4.2 |
| *Rectum only* | 41 | 36 | 5 | 7.2 |

the proportion of girls increasing with the length of the aganglionic segment (Table 7). Yet another interesting point is that nearly all reported series of patients with the condition have contained mongols (trisomy-21 syndrome) (Graivier and Sieber, 1966), usually only 1 or 2 per cent, but Passarge (1967) found that 6 of his 63 index patients had the trisomy-21 syndrome.

*Incidence in Families*

Several systematic family studies have been made (Althoff, 1962; Bodian and Carter, 1963; Gordon et al., 1966 and Passarge, 1967). The striking result in these studies is that the proportion of affected sibs is significantly higher with long-segment cases than with short-segment ones. For instance Bodian and Carter (1963) found 8 affected in 63 sibs of long segment cases and only 10 in 318 sibs of short segment cases. Another interesting result concerned sex ratios (Table 8), the proportion of affected brothers being higher than affected sisters (9 in 173 boys and 1 in 145 girls) of short segment patients, while in the sibs of long segment patients the sex ratio was more nearly unity (5 of 35 brothers and 3 of 28 sisters). Passarge (1967) found that his 55 male index patients had 121 sibs, 3 of whom were probably affected, while the 8 female index patients had 2 affected among 23 sibs. Finally, in most of the investigations there has been found a remarkable consistency in the length of aganglionic bowel within families, though Emanuel et al., (1965) did not find this.

*Inheritance*

These data have many features in common with those concerning hypertrophic pyloric stenosis (Carter, 1965) in which there is a similar sex ratio. They are consistent with a quantitative genetic effect. A polygenically determined susceptibility to environmental factors is the most likely explanation.

TABLE 8. — *Numbers of Affected Brothers and Sisters of Cases of Hirschsprung's Disease**

| Categories | Number of index cases | *Brothers* | | *Sisters* | |
|---|---|---|---|---|---|
| | | Total | Affected | Total | Affected |
| *Long-segment cases* | 22 M | 24 | 3 | 17 | 2 |
| | 15 F | 11 | 2 | 11 | 1 |
| *Total:* | 37 | 35 | 5 | 28 | 3 |
| *Short-segment cases* | 140 M | 143 | 8 | 121 | 1 |
| | 30 F | 30 | 1 | 24 | 0 |
| *Total:* | 170 | 173 | 9 | 145 | 1 |

* From Bodian and Carter, 1963.

The association with mongolism may indicate a chromosomal factor.

The sex ratio implies that the female baby is more resistant to the initiating environmental factors. When she does develop it she would either have to be under exceptional environmental stress or have a very high concentration of the genes determining susceptibility, as has been found with hypertrophic pyloric stenosis (Carter, 1965).

From the data available it is not possible to be sure which explanation is correct. The sex ratios in long and short segment cases and the quantitative nature of their genetic components suggests that the second is correct. In addition the data of Passarge (1967) show a greater proportion of affected sibs of female patients (2 of 23) than of male patients (only 3 of 121 sibs). On the other hand the more extensive data of Bodian and Carter (1963) in Table 8 provide no support for this second explanation, either in the sibs of long or of short segment cases. Clearly there is a need for the collection of more data to investigate this aspect of the inheritance of Hirschsprung's disease.

This demonstration of the quantitative nature of genetic susceptibility to the environment, and of how the resistance conferred by female sex is overcome by a greater genetic component, can probably serve as a model for other conditions commoner in males than in females and not due to major X or Y linked genes. At times the sex difference may be due to the greater exposure of the male to the environmental factors, as perhaps in lung cancer, but it is likely that often, as in Hirschsprung's disease, the environment operates equally on males and females and the comparative immunity of women may in some indirect way be due to her two X chromosomes. When the female does develop the disease it implies an exceptional concentration of the genetic factors underlying susceptibility.

TABLE 9.—*The Number of Reports of Familial Occurrence of Ulcerative Colitis and Crohn's Disease**

| Relationship | Number of families | | |
|---|---|---|---|
| | Ulcerative Colitis | Crohn's Disease | Mixed Crohn's and ulc. colitis |
| *Parent—one child* | 39 | 14 | 3 |
| *Parent—multiple children* | 1 | 2 | 2 |
| *Two sibs* | 43 | 19 | 3 |
| *Three or more sibs* | 5 | 5 | 3 |
| *Twins* | 4 | 6 | 0 |
| *Collateral relatives* | 13 | 10 | 6 |
| *Husband and wife* | 2 | 0 | 0 |

* From Almy and Sherlock, 1966.

## CROHN'S DISEASE AND ULCERATIVE COLITIS

### *Crohn's Disease (Regional Ileitis)*

There has been a large number of reports of 2 or more people in one family having Crohn's disease. Table 9 is from Almy and Sherlock (1966). It can be seen that the combination most frequently reported was 2 siblings but that the disease occurred almost as often in a parent and child.

No systematic study has been carried out in which the incidence of the disease in the relatives of patients with the disease was compared with the incidence in the relatives of controls, but several workers have reported the number of their patients who had affected relatives. From these results it is clear that the familial incidence of Crohn's disease is low, only 1 in 20 to 1 in 50 having an affected first degree relative. This means that the incidence in first degree relatives is between 1 in 100 and 1 in 250, which is almost certainly a higher incidence than is found in the general population, but how much higher is uncertain. There is an obvious need for a study of the incidence in the relatives of healthy controls who have been carefully matched with a series of patients with the disease.

There has never been a report of both husband and wife affected with Crohn's disease. If the disease were wholly environmental this would imply that the causative factors only operate during childhood or adolescence. It is much more likely that both genetic and environmental factors are concerned. It is clear that there is no simple pattern of inheritance of Crohn's disease such as would imply the operation of a single gene. It is likely that the increased liability of relatives of patients is due to polygenic inheritance.

Further support for there being a genetic influence in Crohn's disease comes from reports of its association with ankylosing spondylitis (Acheson, 1960; McBride et al., 1963) a condition in which genetic influences have been well established (Hersh et al., 1950).

### *Ulcerative Colitis*

There have been many reports of familial incidence in series of patients with ulcerative colitis. Rarely has there been any control, and one can only guess whether or not the incidence found in relatives is more than that in the general population (Lennard-Jones et al., 1962; Barker, 1962). Binder et al., (1966) had a good control and found 5.3 per cent of patients had an affected relative compared with 0.7 per cent of the controls.

There now seems little doubt that this familial aggregation is partly due to genetic factors and is not entirely due to the family having a common environment. All the evidence is in favor of there being quite an important hereditary basis to the etiology of the disease. The data suggest that there is an inherited liability to develop the disease and that this liability is deter-

mined by several different genes. Environmental factors are also obviously of importance but the data do not allow any estimate to be made of the relative importance of heredity and environment.

*The Association of Crohn's Disease and Ulcerative Colitis*

For many years there have been reports of both Crohn's disease and ulcerative colitis occurring in the same family. At first it may have been possible to attribute these reports to misdiagnosis or coincidence, but it must now be admitted that these 2 relatively uncommon diseases, diagnosed according to accepted criteria, occur together in the same family too often to be due to chance. Almy and Sherlock (1966) have collected reports of 17 families in which both diseases are known to have occurred (see Table 9). They reported a family with 4 cases of Crohn's disease in one sibship, and another in a cousin. In the same family ulcerative colitis occurred in a cousin and a nephew (Sherlock et al., 1963).

It is curious that the index patients in the families with both diseases have nearly always been suffering from Crohn's disease. In small series of patients with Crohn's disease it is often found that relatives have had ulcerative colitis, while in reports of some large series of patients with ulcerative colitis there has been no mention of Crohn's disease in their relatives. Table 10 shows what are probably the best available data on this aspect of the problem.

Most authorities consider Crohn's disease and ulcerative colitis to be different diseases, and in their typical forms they certainly behave as 2 distinct entities. Edwards and Truelove (1964) had among their 624 patients with ulcerative colitis, 14 of whom they knew also to have Crohn's disease of the small intestine. These patients definitely did not have Crohn's disease of the colon. Crohn and Yarnis (1958) reported 60 cases of the combined disease in 676 cases of Crohn's disease. Thus, in addition, to the familial aggregation of the 2 diseases there are clinical grounds for suspecting that they may not be distinct entities.

TABLE 10. — *The Numbers of Affected Relatives of Patients with Ulcerative Colitis and Crohn's Disease**

| Disease | Disease in relatives | | |
|---|---|---|---|
| | Ulcerative Colitis | Reg. enteritis | Ileocolitis |
| 1084 Ulc. colitis | 75 | 13 | 1 |
| 185 Reg. enteritis | 8 | 13 | 1 |
| 70 Ileocolitis | 4 | 1 | 0 |

* From Kirsner and Spencer, 1963.

Further, both diseases are associated with ankylosing spondylitis, both in individuals and in families (McBride et al., 1963). The spondylitis cannot be regarded as a complication of the intestinal diseases, since symptoms of arthropathy come first in the majority of cases. All 3 diseases may be manifestations of one general disorder. If this were so, one might expect to find occasional patients with all 3 conditions, but in fact only one such case has been recorded (Steinberg and Storey, 1957). Common factors in the genotypes may underlie the inter-relationships between the 3 diseases and determine liability to each of them.

It is probable that too little is known at present of the etiology of Crohn's disease and of ulcerative colitis for a precise analysis of these relationships to one another to be made. However, it has been established that at least there is a strong relationship between them. There are several possible bases to this relationship:

1) They may be the same disease with the same genetic basis and the same etiological environmental factors. This is very unlikely in view of the very different macroscopic and microscopic picture in most cases of the two diseases.

2) They may both be due to the same environmental factors and therefore tend to occur in the same family, but these factors produce different pathology in people of different genotypes. Thus relatives would tend to be affected with the same disease. Against this explanation is the probability that Crohn's disease is not so evenly distributed geographically as is ulcerative colitis (Levrat, Peycelon and Lamber, 1962). If in fact Crohn's disease does not occur in certain districts where ulcerative colitis is found, it is unlikely that they share the same external etiological factor.

3) They may be due to different environmental factors which result in different pathology, but the 2 polygenic systems which determine liability to the 2 diseases may have a number of genes in common. This could result in the relatives of patients with one disease having an increased liability to develop the other compared with people in the general population. In favor of this possibility is the relationship which both diseases have with ankylosing spondylitis. On the whole this explanation requiring overlapping genotypes is most likely to be correct.

## POLYPOSIS

Although there is much that is not clear about polyps of the gastrointestinal tract, there is fairly general agreement that there exist at least 3 genetically distinct varieties of intestinal polyposis. These are:

1. Familial polyposis of the colon

2. The Gardner syndrome — polyposis of small and large intestines with osteomata, fibromata and sebaceous cysts
3. The Peutz-Jeghers syndrome — generalized gastrointestinal polyposis with melanotic spots of the buccal mucosa, lips and digits

In addition there are probably other inherited varieties of polyposis; some of the more distinct entities are as follows:

4. Discrete polyps of the rectum and colon
5. Colonic polyposis and tumors of the nervous system
6. Colonic polyposis with sebaceous cysts
7. Multiple polyps of the colon and stomach
8. Gastric polyposis
9. Colonic polyposis with development of a duodenal polyp.

In addition there are 3 varieties of gastrointestinal polyposis in which no evidence of a hereditary tendency yet exists. They are:

1. Juvenile polyps (Knox et al, 1960)
2. Metaplastic polyps (Morson, 1962)
3. Solitary polyps of the small intestine (Gannon et al., 1962).

*Familial Polyposis of the Colon*

In classical polyposis coli the polypoid lesion is a benign adenoma, indistinguishable from the common, discrete polypoid adenoma. In the typical case the whole colon from anus to ileocaecal valve is thickly carpeted by polyps. There is, however, a wide variation in the number of polyps, and an occasional family member whose offspring are affected is found to have only widely scattered polyps. Thus the differential diagnosis in a single isolated patient with no family history is not always a simple straightforward matter.

Even though familial polyposis of the colon is probably the commonest of the hereditary polyposis, it is still a rare condition, and it was estimated that in the state of Michigan it had a minimum frequency of only 1 in 8,300 births (Reed and Neel, 1955). The most important recent contribution to the study of the genetics of the condition is the monograph of Veale (1965) analyzing the material at St. Mark's Hospital, London.

The published pedigrees of familial polyposis clearly indicate that the usual manner in which this trait is inherited is as a dominant character, transmitted by both males and females. The gene is therefore autosomal. Very occasionally the condition may appear to skip a generation, suggesting that the penetrance of the gene is incomplete, but this may be due to late onset in an individual who dies early of some other cause and is consequently not scored as affected.

A remarkable number of the patients diagnosed as having polyposis coli lack a family history of the disease. Thus only the propositus was affected in

11 of the 41 families described by Dukes (1952). Nine out of 24 (Reed and Neel, 1955) and 24 out of 71 (Veale, 1965) are other figures which have been reported. Although Dukes considered that these might represent non-familial phenocopies, Veale ascribed such isolated cases to fresh mutations. Reed and Neel calculated that the relative fitness of individuals with the gene was 0.78, which is in close agreement with Veale's figure of 0.82. Assuming that a state of genetic equilibrium exists, and that the loss of genes in each generation is balanced by mutation, Reed and Neel calculated a mutation rate of 1.3 per 100,000 loci per generation. Veale's rate was nearly 3 times higher at 3.8 per 100,000. Only when one of these isolated cases has been shown to transmit his polyposis to an offspring will it be absolutely definite that he has true familial polyposis and is not a phenocopy. Among Veale's 47 families in which polyposis was plainly inherited, there were 3 propositi whose parents and sibs were apparently unaffected but who had passed the condition on to their offspring.

The frequently inconsistent genetic pattern suggests that a single abnormal gene may not be the complete explanation of the inheritance of familial polyposis. Taking into account the variability of both the age at which symptoms first occur and the number of polyps, it seems probable that additional factors modify the expression of the gene. Even though there is almost certainly one major gene for the condition, in some families its expression may be so modified that the inheritance is virtually polygenic. In addition it is quite possible that environmental factors may influence expressions of the gene.

*The Gardner Syndrome*

It is now appreciated that polyposis of the colon occasionally is associated with neoplasms of both hard and soft tissues elsewhere in the body. This syndrome was first described by Gardner (1951) and families with several cases have now been described in different parts of the world. It is considerably rarer than familial polyposis coli. The lesions which occur outside the gut are epidermoid cysts and fibromas, and osteomas particularly of the mandible and maxilla.

In contrast to familial polyposis of the colon, polyps may not develop in Gardner's syndrome until after the age of 30 or even 40 years, but the bone and soft tissue lesions develop earlier than the polyps. Gardner's syndrome also differs from familial polyposis in that the colon polyps are more scattered and a polyp occasionally occurs in the small intestine (Gumpel and Carballo, 1956; Gorlin and Chaudhry, 1960). Like familial polyposis, however, there is a high incidence of carcinoma; there is a high likelihood of malignancy developing by the age of 60 years.

Mesenteric fibromatosis, which often occurs after intestinal surgery (Shepherd, 1958), is another manifestation of Gardner's syndrome. It was the only extra-colonic sign of Gardner's syndrome in 60 per cent of 22 cases recently reviewed (Simpson et al., 1964). Fibromas (desmoids) tend to develop in abdominal incisional scars (Smith, 1959), suggesting that these patients have unusual mesenchymal tissue properties.

Examination of the published pedigrees leaves no doubt that the Gardner syndrome is determined by a single pleiotropic gene and that the trait is transmitted as an autosomal dominant. This gene is certainly distinct from that determining familial polyposis, because no family has been described containing both types of polyposis. The genes for the 2 types could, however, be allelic, and McKusick (1962) discusses how linkage studies could be used to test this possibility. More families will have to be studied before it will be possible to assess the variability of the Gardner gene. Isolated cases are reported whose parents show no signs of the syndrome. In one such case a son of the propositus had an osteoma of the forehead (Savage, 1964). There is the possibility of a fresh mutation having occurred.

*The Peutz-Jeghers Syndrome*

A major review of the genetics of the Peutz-Jeghers syndrome was made by Dormandy (1957).

The most important development of recent years has been the realization that the polyps of Peutz-Jeghers syndrome are not adenomas but are congenital malformations or hamartomas, and that they have no particular propensity to undergo malignant change (Morson, 1962; Gannon et al., 1962). In spite of the view of most authorities that the polyps are not premalignant there have been a few reports of gastrointestinal malignancy in patients with the syndrome (Po et al., 1963; Achord and Proctor, 1963; Bailey, 1957; and Williams and Knudsen, 1965).

The incidence is probably about the same or perhaps rather higher than that of familial polyposis coli (Kutscher et al., 1960).

Examination of many of the published pedigrees leaves no doubt that the syndrome is determined by a single pleiotropic gene and that a dominant mode of inheritance is usual. The likelihood of the pigmentation and the polyposis being caused by 2 closely linked genes is very slight, as no instance of crossing over has ever been reported. The single gene may not have complete penetrance, however, as pedigrees with skipped generations have been published, and the number of isolated cases diagnosed has been far too large for them all to be due to fresh mutations. For instance, in one series of 9 fully developed examples of the syndrome only 3 had histories of relatives being affected (Gannon et al., 1962). Alternatively, the apparently unaffected

carriers of the gene may have had only a few polyps which never caused trouble.

Expression of the gene is very variable and only a proportion of the affected members of a family have the fully developed syndrome. Sometimes mucocutaneous pigmentation is the only manifestation of the gene, and very occasionally the polyposis is found without any pigmentary changes. Farmer, Hawk and Turnbull (1963) reported 3 cases in the same family, each representing a different manifestation of the syndrome. The propositus had the full syndrome, her brother had typical pigmentation alone and her father only the polyposis. The propositus had 1 gastric polyp, 4 in the small intestine and 4 in the colon, while her father in addition to jejunal polyps had had so many colonic polyps that total colectomy had been carried out for what was considered to be familial polyposis coli. This diagnostic mistake is understandable, since the patient has no abnormal pigmentation and had a large number of polyps in the colon and rectum.

Bartholomew et al., (1962) say that of the 182 patients with the syndrome who had been reported in the literature up to 1961 only 88 had a family history of both abnormal pigmentation and of polyposis. An additional 17 patients had relatives with the pigmentation alone.

Dormandy (1957) in his review of the syndrome referred to 2 isolated case reports of associated granulosa-celled tumor. A third case of Peutz-Jeghers syndrome with this rare tumor has now been reported (Christian et al., 1964) and it seems possible that it is part of the syndrome.

*Discrete Polyps of the Colon and Rectum*

Sigmoidoscopy and barium studies of the colon in apparently healthy people occasionally reveal a solitary adenomatous polyp. Sometimes there are several but when this occurs they are well scattered. The proportion of the population in whom these discrete adenomatous polyps is found increases with advancing years. Woolf, Richards and Gardner (1955) were the first to produce evidence of heredity being involved in producing these solitary polyps. In their large family there was not only a very high incidence of small adenomatous polyps of the rectum and rectosigmoid, reaching 50 per cent of the children in the third generation, but also a high incidence of death from carcinoma of the colon. A third of the adults of the second generation had died of gastrointestinal cancer (Richards and Woolf, 1956). The polypoid lesions seen were similar, pathologically and in age and sex distribution, to those found in other reported studies of solitary polyps of the colon. The authors considered that the findings strongly suggested a definite familial trait in the occurrence of solitary polyps and that there was a dominant form of inheritance in this family. It is uncertain whether this heredi-

tary influence on solitary polyps applies outside this one family, but there have been reports suggesting that this may be so (McKusick, 1962).

*Colonic Polyposis and Tumors of the Nervous System*

Turcot, Després and St. Pierre (1959) reported a young man with a spinal medulloblastoma and his sister with a frontal glioblastoma both of whom had polyposis of the colon. There was no history of any other relatives being affected by either polyposis or tumors of the central nervous system, but it may be significant that the parents of the patients were third cousins. This raises the possibility that in this family polyposis of the colon and tumor of the nervous system was inherited as a recessive condition, and that it is an entity distinct from classical familial polyposis of the colon. More data are required before any more definite conclusion can be reached. Neurological tumors are not rare in young people and it may be only a coincidence that this brother and sister were affected.

*Colonic Polyps with Sebaceous Cysts*

Soon after Gardner (1954) described intestinal polyposis associated with osteomata and soft tissue tumors, Oldfield (1954) published a detailed report of a family in which numerous sebaceous cysts occurred in association with a diffuse polyposis of the colon which resembled that found in Gardner's syndrome. Two of the members of this family had died of carcinoma of the colon. It has been noted earlier that many patients with Gardner's syndrome have sebaceous cysts. It is therefore possible that the condition in Oldfield's family is genetically identical with Gardner's syndrome, but there are several features which suggest that this polyposis with associated sebaceous cysts is determined by a different gene. The family had been under observation for many years because of the high incidence of multiple sebaceous cysts which were large, liable to infection, and unsightly, and were sufficiently striking for the family to have been reported in 1937, even though at that time no case of polyposis had been recognized. The 3 individuals in whom polyposis was recognized all had sebaceous cysts and careful examination had failed to reveal any of the soft tissue and bony tumors which are usually part of Gardner's syndrome. It seems likely that the polyposis and sebaceous cysts are determined by a single pleiotropic gene distinct from that which determines Gardner's syndrome, and also distinct from another gene which determines the dominant inheritance of sebaceous cysts associated with various tumors but not with polyposis (Munro, 1938).

*Multiple Polyposis of the Colon and Stomach*

There have been several reports of patients with multiple polyposis of the colon and stomach, without associated soft tissue or bony tumors. This condition appears to be distinct from both familial polyposis coli and Gardner's

syndrome. As it has been described in one family in 2 brothers, an uncle and a son, it is almost certainly inherited as a dominent character (Halsted et al., 1950). There were also 2 sisters, one with polyposis coli who was not gastroscoped and the other with a negative barium enema but in whom 3 small polyps were seen on gastroscopy. Since the uncle had carcinoma of the rectum and one of the brothers had carcinoma of the colon, the colonic polyposis had similar malignant propensities to classical familial polyposis of the colon. Polyposis of colon and stomach has been described in another family in which 2 sisters, aged 11 and 16 years, were affected (Boley et al., 1961).

Another family has been reported (Murphy et al., 1962) in which the propositus was a boy who had a total colectomy at the age of 15 years for colonic polyposis and who died at the age of 16 years of carcinoma of the stomach. Two male first cousins were known to have polyposis of the colon, and the maternal grandfather had died of carcinoma of stomach. Both parents, however, were reported to be without relevant symptoms or signs, though they were not investigated. It is possible that this family is a third example of a genetically distinct variety of gastrointestinal polyposis, in which both the colon and stomach are involved and in which the inheritance is dominant with incomplete penetrance and variable expression.

*Gastric Polyposis*

Polyposis of the stomach occurring without small or large intestine polyposis has been regarded as a rare condition and in a review (Roxburgh, 1962) it was stated that it is hardly ever familial. It has, however, been reported in 2 brothers (Rø, 1962). Because of marked symptoms one had a total gastrectomy in 1934 at 45 years of age and the other in 1947 at 46 years of age. At the operations it was found that the stomachs were carpeted from the cardia to the pylorus. No extragastric polyps were noticed. The first brother died of a cerebral catastrophe at the age of 70 and the other was still alive, aged 61. This familial occurrence strongly suggests that heredity is concerned in gastric polyposis, but it obviously is insufficient to indicate the mode of inheritance. Nor is the possibility ruled out that these 2 brothers had the gene for multiple polyposis of the colon and stomach, but that its expression was incomplete in them and only their stomachs were involved.

*Colonic Polyposis with Development of a Duodenal Polyp*

Chiat et al., (1962) have reported the case of a young woman with multiple polyposis of the colon who, 7 years after colectomy, presented with bleeding from a large sessile benign adenoma of the second part of the duodenum. There is no mention of the patient's family history, and on the available evidence it is not possible to decide whether this duodenal polyp was an incidental development in a case of familial polyposis coli or whether the

patient was an example of yet another distinct type of gastro-intestinal polyposis.

## CONCLUSION

Study of the genetical aspects of gastroenterological disorders leaves one with two main impressions: first, the great importance of environmental factors and their interaction with genotype; and second, the inexactitude of most clinical diagnoses. Compared with other systems there are few traits with simple Mendelian inheritance and the inherited component of many of the disorders seems to be polygenic. On the other hand, the study of the physiological traits with simple inheritance, such as the blood groups and secretor character and various digestive enzymes, gives hope of being able to identify the genotypes determining more complete physiological processes and liability to some of the common disorders.

## REFERENCES

Acheseon, E. D. 1960. Associations between ulcerative colitis, regional enteritis and ankylosing spondylitis. Quart. J. Med. 29: 489-499.

Achord, J. C. and H. D. Proctor. 1963. Malignant degeneration and metastasis in Peutz-Jeghers syndrome. Arch Intern. Med. III; 498-502.

Aird, I., H. H. Bentall, and J. A. F. Roberts. 1953. A relationship between cancer of stomach and the ABO blood groups. Brit. Med. J. 1: 799-801.

Almy, T. P. and P. Sherlock. 1966. Genetic aspects of ulcerative colitis and regional enteritis. Gastroenterology 51: 757-761.

Althoff, W. 1962. Zur Genetik der Hirschsprungschen Krankheit. Z. Menschl. Vererb. Konstitiutionsl. 36: 314-340.

Arfors, K. E., L. Beckman and L. G. Lunden. 1963. Genetic variations of human serum phosphatases. Acta Genet. (Basel). 13: 89-94.

Bailey, D. 1957. Polyposis of gastro-intestinal tract: the Peutz syndrome. Brit. Med. J., ii: 433-438.

Ball, P. A. J. 1962. Influence of the secretor and Lewis genes on susceptibility to duodenal ulcer. Brit. Med. J. 2: 945-950.

Bamford, K. F., H. Harris, J. E. Luffman, E. B. Robson and T. E. Cleghorn. 1965. Serum-alkaline-phosphatase and the ABO blood-groups. Lancet I: 530-531.

Barker, W. F. 1962. Familial history of patients with ulcerative colitis. Amer. J. Surg. 103: 25-26.

Bartholomew, L. G., C. E. Moore, D. C. Dahlin and J. M. Waugh. 1962. Intestinal polyposis associated with mucocutaneous pigmentation. Surg. Gynec. Obstet., 115: 1-11.

Beckman, L. 1964. Associations between human serum alkaline phosphatase and blood groups. Acta Genet. (Basel). 14: 286-297.

—, G. B. Björling and A. Heiken. 1966. Human alkaline phosphatases and the factors controlling their appearance in serum. Acta Genet. (Basel). 16: 305-312.

Binder, V., E. Weeke, J. H. Olsen, P. Anthonisen and P. Riis. 1966. A genetic study of ulcerative colitis. Scand. J. Gastroent. 1: 49-56.

Bodian, M. and C. O. Carter. 1963. A family study of Hirschsprung's disease. Ann. Hum. Genet. (Lond.) 26: 261-277.

Boley, S. J., W. M. P. McKinnon and V. F. Marzulli. 1961. The management of familial gastrointestinal polyposis involving stomach and colon. Surgery 50: 691-696.

Carter, C. O. 1965. The inheritance of common congenital malformations. *In:* Steinberg, A. G. and Bearn, A. G. (Eds.): Progress in Medical Genetics, Vol. 4. New York and London, Grune and Stratton, chapter 3.

Chiat, H., S. T. Ross, D. E. Janelli and P. R. Mandel. 1962. Familial polyposis of the colon with subsequent development of a duodenal polyp: A report of a case. Dis. Colon Rectum 5: 444-445.

Christian, C. D., Th. G. McLoughlin, E. R. Cathcart, and M. M. Eisenberg. 1964. Peutz-Jeghers syndrome associated with functioning ovarian tumor. J. A. Med. A. 190: 935-938.

Clarke, C. A. 1961. Blood Groups and Disease. *In* Steinberg, A. G. (Ed.): Progress in Medical Genetics, Vol. 1. New York and London, Grune and Stratton, chapter 4.

—, J. W. Edwards, D. R. W. Haddock, A. W. Howel-Evans, R. B. McConnell and P. M. Sheppard. 1956. ABO blood groups and secretor character in duodenal ulcer. Population and sibship studies. Brit. Med. J. 2: 725-731.

Crohn, B. B. and H. Yarnis. 1958. Regional Ileitis. Second Edition. New York, Grune and Stratton Inc.

Doll, R., H. Drane and A. C. Newall. 1961. Secretion of blood group substances in duodenal, gastric and stomal ulcer, gastric carcinoma and diabetes mellitus. Gut 2: 352-359.

Doll, R., B. F. A. Swynnerton, and A. C. Newall. 1960. Observations on blood group distribution in peptic ulcer and gastric cancer. Gut 1: 31-35.

Dormandy, T. L. 1957. Gastrointestinal polyposis with mucocutaneous pigmentation (Peutz-Jeghers syndrome). New Eng. J. Med. 256: 1186-1190.

Dukes, C. E. 1952. Familial intestinal polyposis. Ann. Eugen. 17: 1-29.

Edwards, F. C. and S. C. Truelove. 1964. The course and prognosis of ulcerative colitis.. Part 4. Carcinoma of the colon. Gut 5: 15-22.

Emanuel B., M. P. Padorr and O. Swenson. 1965. Familial absence of myenteric plexus (congenital megacolon). J. Pediat. 67: 381-386.

Evans, D. A. P. 1965. Confirmation of association between ABO blood groups and salivary ABH secretor phenotypes and electrophoretic patterns of serum alkaline phosphatases. J. Med. Genet. 2: 126-127.

—, L. Horwich, R. B. McConnell and M. F. Bullen. 1967. The influence of the ABO blood groups and secretor status on bleeding and on perforation of duodenal ulcers. Gut 9: 319-322.

Farmer, R. G., W. A. Hawk, and R. B. Turnbull. 1963. The spectrum of the Peutz-Jeghers syndrome. Report of 3 cases. Amer. J. Dig. Dis. 8: 953-961.

Fodor, O. and S. Urcan. 1966. Hereditary aspects of duodenal ulcer in relation to blood group and secretor status. Rev. Roum. Méd. Int. 3: 301-303.

Gannon, P. G., D. C. Dahlin, L. C. Bartholomew, and O. H. Beahrs. 1962. Polypoid glandular tumors of the small intestine. Surg. Gynec. Obstet. 114: 666-672.

Gardner, E. J. (1951). A genetic and clinical study of intestinal polyposis, a predisposing factor for carcinoma of the colon and rectum. Amer. J. Hum. Genet. 3: 167-176.

Gordon, H., M. Torrington, J. H. Louw, and S. Cywes. 1966. A genetical study of Hirschsprung's disease. Congenital intestinal aganglionosis. S. Afr. Med. J. 40: 720-721.

Gorlin, R. J. and A. P. Chaudhry. 1960. Multiple osteomatosis, fibromas, lipomas and fibrosarcomas of the skin and mesentery, epidermoid inclusion cysts of the skin, leiomyomas and multiple intestinal polyposis: a heritable disorder of connective tissue. New Eng. J. Med. 263: 1151-1158.

Graiver, L. and W. K. Sieber. Hirschsprung's disease and mongolism. Surgery 60: 458-461.

Gumpel, R. C. and J. D. Carballo. 1956. A new concept of familial adenomatosis. Ann. Intern. Med. 45: 1045-1058.

Halsted, J. A., E. J. Harris, and M. K. Bartlett. 1950. Involvment of the stomach in familial polyposis of gastro-intestinal tract. Report of a family. Gastroenterology 15: 763-770.

Hersh, A. H., R. M. Stecher, W. M. Solomon, R. Wolpaw and H. Hauser. 1950. Heredity in ankylosing spondylitis; a study of 50 families. Amer. J. Hum. Genet. 2: 391-408.

Hodson A. W., A. L. Latner, and L. Raine. 1962. Isoenzymes of alkaline phosphatase. Clin. Chim. Acta. 7: 255-261.

Hope, R. M. 1966. Association between serum alkaline phosphatase variants and the R-O-i blood group system in the Australian Merino. Aust. J. Biol. Sci. 19: 1171-1174.

Horwich, L., D. A. P. Evans, R. B. McConnell and W. T. A. Donohue. 1966. ABO blood groups in gastric bleeding. Gut 7: 680-685.

Johnson, H. D., A. H. G. Love, N. C. Rogers and A. P. Wyatt. 1964. Gastric ulcers, blood groups and acid secretion. Gut 5: 402-411.

Jones, J. H. and M. J. S. Langman. 1967. Pre- and post-operative symptoms of patients with stomal ulcers. Gut 8: 133-136.

Kirsner, J. B. and J. A. Spencer. 1963. Familial occurrences of ulcerative colitis, regional enteritis, and ileocolitis. Ann. Intern. Med. 59: 133-144.

Knox, W. G., R. E. Miller, C. F. Begg, and H. A. Zintel. 1960. Juvenile polyps of the colon: a clinicopathologic analysis of 75 polyps in 42 patients. Surgery 48: 201-210.

Kutscher, A. H., E. V. Zegarelli, R. M. Rankow, and T. W. Slaughter. 1960. Incidence of Peutz-Jaghers syndrome: Data derived from a survey of hospitals. Amer. J. Dig. Dis. 5: 576-577.

Langman, M. J. S. and R. Doll. 1965. ABO blood group and secretor status in relation to clinical characteristics of peptic ulcers. Gut 6: 270-273.

—, —, and R. Saracci. 1967. ABO blood group and secretor status in stomal ulcer. Gut 8: 128-132.

—, E. Leuthold, E. B. Robson, J. Harris, J. E. Luffman, and H. Harris. 1966. Influence of diet on the "intestinal" component of serum alkaline phosphatase in people of different ABO blood groups and secretor status. Nature. 212: 41-43.

Lennard-Jones, J. E., G. W. Cooper, A. C. Newell, C. W. E. Wilson, and A. F. Jones. 1962. Observations on idiopathic proctitis. Gut 3: 201-206.

Levrat, M., R. Peycelon, and R. Lamber. 1962. Une observation d'iléite de Crohn. Terrain prédiposant constitutionnel. Arch. Mal. Appar. Dig. 51: 1201-1203.

McBride, J. A., M. J. King, A. G. Baikie, G. P. Crean, and W. Sircus. 1963. Ankylosing spondylitis and chronic inflammatory diseases of the intestines. Brit. Med. J. 2: 483-486.

McConnell, R. B. 1962. The blood group substances in gastric juice. *In* Smith, A. N. (Ed.): Surgical Physiology of the Gastro-intestinal Tract. Royal College of Surgeons of Edinburgh, pp. 98-105.

—. 1966. The Genetics of Gastro-intestinal Disorders. London. Oxford University Press.

—. 1967. The genetics of carcinoma of the stomach. *In* Shivas, A. A. (Ed.): Racial and Geographical Factors in Tumour Incidence. Edinburgh University Press. pp. 107-113.

McKusick, V. A. 1962. Genetic factors of intestinal polyposis. J. A. M. A. 182: 271-277.

Merikas, G., P. Christakopoulos, and E. Petropoulos. 1966. Distribution of ABO blood groups in patients with ulcer disease: its relationship to gastro-duodenal bleeding Amer. J. Dig. Dis. 11: 790-795.

Morson, B. C. 1962. Some peculiarities in the histology of intestinal polyps. Dis. Colon Rect. 5: 337-344.

Munro, T. A. 1938. Hereditary sebaceous cysts. J. Genet. 35: 61-72.

Murphy, E. S., M. Mireles, and A. Beltran. 1962. Familial polyposis of the colon and gastric carcinoma. Concurrent conditions in a 16-year-old boy. J. A. M. A. 179: 1026-1028.

Newman, E., G. S. Naifeh, J. E. Auer, and J. A. Buckwalter. 1961: Secretion of ABH antigens in peptic ulceration and gastric carcinoma. Brit. Med. J. 1: 92-94.

Oldfield, M. C. 1954. The association of familial polyposis of the colon with multiple sebaceous cysts. Brit. J. Surg. 41: 534-541.

Passarge, E. 1967. The genetics of Hirschsprung's disease; evidence of heterogeneous etiology and a study of sixty-three families. New Eng. J. Med. 276: 138-143.

Po, B. T., C. M. McMurray, and O. F. Noel. 1963. Congenital familial polyposis of the gastro-intestinal tract (Peutz-Jeghers syndrome). Amer. Surg. 29: 639-642.

Pringle, R., A. J. Wost, and C. A. Green. 1964. The significance of ABO groups and secretor status in duodenal ulcer. Brit. J. Surg. 51: 341-343.

Race, R. R. and R. Sanger. 1962. Blood groups in man. 4th Edition. Oxford, Blackwell Scientific Publications.

Reed, T. E. and J. V. Neel. 1955. A genetic study of multiple polyposis of the colon (with an appendix deriving a method of estimating relative fitness). Amer. J. Hum. Genet. 7: 236-263.

Rendel, J., O. Aalund, R. A. Freedhand, and F. Møller. 1964 The relationship between the alkaline phosphatase polymorphism and blood group O in sheep. Genetics 50: 973-986.

Richards, R. C. and C. Woolf. 1956. Solitary polyps of the colon and rectum; a study of inherited tendency. Amer. Surg. 22: 287-294.

Richardson, W. R. and I. H. Brown. 1962. Hirschsprung's disease in infants and children. Amer. Surg. 28: 149-164.

Rø, J. 1962. Polyposis ventriculi hos to brødre. Nord. Med. 68: 1065-1067.

Robinson, J. C. and L. A. Goldsmith. 1967. Genetically determined variants of serum alkaline phosphatase: a review. Vox Sang. 13: 289-307.

Roxburgh, R. A. 1962. The case for total gastrectomy in multiple polyposis of the stomach. Gut 3: 224-231.

Savage, P. T. 1964. Polyposis coli associated with multiple tumours in other parts of the body (Gardner's syndrome). Proc. Roy. Soc. Med. 57: 402-403.

Shepherd, J. A. 1958. Familial polyposis of the colon with associated connective tissue tumours. J. Roy. Col. Surg. Edin. 4: 31-38.

Sherlock, P., B. M. Bell, H. Steinberg, and T. P. Almy. 1963. Familial occurrence of regional enteritis and ulcerative colitis. Gastroenterology 45: 413-420.

Shreffler, D. C. 1965. Genetic studies of blood group–associated variations in a human serum alkaline phosphatase. Amer. J. Hum. Genet. 17: 71-86.

—. 1966. Relationship of alkaline phosphatase levels in intestinal mucosa to ABO and secretor blood groups. Proc. Soc. Exp. Biol. Med. 123: 423-427.

Sidbury, J. B. 1965. The genetics of the glycogen storage diseases. *In* Steinberg, A. G. and Bearn, A. G. (Eds.): Progress in Medical Genetics, Volume 4. London and New York, Heinemann and Grune and Stratton. Chapter 2.

Simpson, R. D., E. G. Harrison, and C. W. Mayo. 1964. Mesenteric fibromatosis in familial polyposis. Cancer (Philad.), 17: 526-534.

Smith, W. G. 1959. Desmoid tumors in familial multiple polyposis. Proc. Mayo Clin. 34: 31-38.

Steinberg, V. L. and G. Storey. 1957. Ankylosing spondylitis and chronic inflammatory lesions of the intestines. Brit. Med. J. 2: 1157-1159.

Turcot, J., J-P. Després, and F. St. Pierre. 1959. Malignant tumors of the central nervous system associated with familial polyposis of the colon: Report of two cases. Dis. Colon Rectum. 2: 465-468.

Veale, A. M. O. 1965. Intestinal Polyposis. London, Cambridge University Press.

Williams, J. P. and A. Knudsen. 1965. Peutz-Jeghers syndrome with metastasizing duodenal carcinoma. Gut 6: 179-184.

Woolf, C. M., R. C. Richards, and E. J. Gardner. 1955. Occasional discrete polyps of colon and rectum showing inherited tendency in kindred. Cancer (Philad.), 8: 403-408.

CHAPTER 3

# The Genetics of Thyroid Disease

**G. R. Fraser**

Division of Medical Genetics, Department of Medicine
University of Washington, School of Medicine,
Seattle, Washington

THE SPECTRUM OF DISEASES affecting the thyroid gland may be taken as a model of the systems of interactions between genetic and environmental factors which play a role in the causation of human disease in general. It is an exceptionally well studied spectrum because of the recent introduction of many elegant investigative procedures using radioactive isotopes of iodine, and of techniques to detect autoantibodies to constituents of the gland. An attempt will be made in this review to define the role of genetic factors in etiology within the framework of the conventional classification of clinical types of thyroid disease.

## INFANTILE HYPOTHYROIDISM WITH GOITER (SPORADIC GOITROUS CRETINISM)

Intensive studies of the pathway of synthesis of thyroid hormones have led to the tentative definition of at least 5 genetically determined defects which may properly be regarded as inborn errors of metabolism (Stanbury, 1966). Family data are consistent with the hypothesis that these defects in hormone synthesis are inherited in an autosomal recessive manner. They lead to compensatory hyperplasia of the thyroid gland on account of pituitary stimulation. The degree to which this compensatory thyro-pituitary feedback mechanism is successful in overcoming partial blocks in synthesis and insuring an adequate supply of thyroid hormones determines the age of onset and severity of the retardation of mental and physical development (cretinism) attributable to hypothyroidism. Although this is the only form

of thyroid disease in which a marked preponderance of affected females does not occur, females tend to be more severely involved (Fraser, 1965a).

These defects implicate the following steps in the intra-thyroidal metabolism of iodine.

1) Trapping and concentration of inorganic iodide from the blood.
2) Oxidation in inorganic iodide to elemental iodine followed by incorporation into tyrosyl radicals of thyroglobulin in the form of mono- and diiodotyrosine (MIT and DIT).*
3) Coupling of MIT and DIT which takes place within the thyroglobulin molecules to form the thyroid hormones — triiodothyronine ($T_3$) and tetraiodothyronine or thyroxine ($T_4$).
4) Deiodination of surplus MIT and DIT.

In addition to cases due to defects in these steps, a further sub-group of goitrous hypothyroidism which is marked by the presence of iodoproteins in the serum can be defined. The mechanism whereby such abnormal constituents are liberated into the serum is not understood, and this sub-group may be heterogeneous. Thus, possible mechanisms include genetically determined defects of the synthesis or breakdown of thyroglobulin so that a precursor protein or degradation product is present in excess and diffuses out of the thyroid gland; alternatively, the serum iodoprotein may represent a normal constituent of the gland which only takes part in iodination when thyroglobulin synthesis is defective.

Present knowledge of the biochemical and physiological basis of these defects has been recently discussed by Stanbury (1966). Here an attempt will be made to consider outstanding problems that are specifically of genetic interest concerning this group of conditions.

One weakness of the scheme proposed is that only in the case of one of these defects (deiodination) has the actual enzyme involved (iodotyrosine deiodinase) been defined. This defect is not confined to the thyroid and may best be demonstrated by intravenous injections of labelled MIT and DIT. In affected persons, these substances are not deiodinated peripherally but are excreted unchanged in the urine. This is not always true, however, since Kusakabe and Miyake (1964) have described 3 goitrous sisters born of a first cousin marriage in whom deiodination was defective in the thyroid but not peripherally.

In the case of the other defects, the evidence on which reduced activity of

* This group contains 2 distinct categories which may be distinguished by the fact that in one of them the thyroid defect is accompanied by congenital perceptive deafness (Pendred's (1896) syndome).

an enzyme is based is largely inferential, since the enzymes concerned have not been isolated and no tests are available directly to measure their activity. Thus, more complex genetically determined abnormalities may be involved. These may affect the structure of the cell membrane or the synthesis of thyroglobulin; alternatively, aberrant metabolic pathways determined by genes not primarily controlling the synthesis of intra-thyroidal enzymes may lead to the formation of substances which inhibit various steps in hormone production. In view of this uncertainty, it is not surprising to find many cases of goitrous hypothyroidism which do not fall unequivocally into these categories. Such cases have been discussed by Wiener and Linderboom (1964) who have suggested that many may represent abnormalities of thyroglobulin synthesis. Again, Michel, Rall, Roche and Tubiana (1964) found abnormal iodoproteins in the thyroid glands of 3 patients with goitrous hypothyroidism; they indicated that the presence of these abnormal constituents might be primarily responsible for the disease, or might be secondary, due to generalized disorganization of intra-thyroidal metabolism. Among other explanations which are possible in such "mixed" cases are:

1) The same enzyme may catalyse 2 steps in the synthesis of thyroid hormones.
2) A defect in the iodination of tyrosyl radicals may lead to a spatial configuration of MIT and DIT molecules in thyroglobulin which is unfavorable for coupling.
3) A mutation of control rather than structural genes may affect two or more enzymes.

Thus, the nosography of this group of diseases is far from being definitively resolved. Nevertheless some salient features stand out as being well established. From the genetic point of view, for example, it is certain that several distinct autosomal recessive defects of thyroxine synthesis exist.

The importance of these rare defects is out of all proportion to their frequency because of the light they throw on the modes of gene action in the causation of thyroid disease in general. For example, in all of these defects, heterozygotes have been described as having a propensity to goiter formation much in excess of the normal population, and in the case of the deiodinase defect they have been shown to have an activity of this enzyme which is intermediate between that of normal persons and of affected homozygotes (Stanbury, 1966). Such heterozygotes, of course, may be expected to be relatively common, and it is certain that these and other genetically determined blocks in thyroxine synthesis play some part in the causation of non-toxic goiter, which is often inherited in a dominant manner largely limited to the female sex (Fraser, 1962, 1963a).

*Pendred's Syndrome (Deafness with Goiter)*

It would seem possible that elucidation of the nature of those defects in thyroxine synthesis which are not as yet well defined will come from the clarification of the biological relationship between goiter due to a block in organic binding of iodine and deafness in Pendred's syndrome (Figure 1). Although most investigators hold, in common with Stanbury (1966), that this condition is entirely distinct from the difficulty in organic binding without deafness, some link between the two must exist. A very revealing finding in this context is shown in Figure 2; in this family (discussed by Fraser

Clinical Notes:

MEDICAL, SURGICAL, OBSTETRICAL, AND THERAPEUTICAL.

DEAF-MUTISM AND GOITRE.

By Vaughan Pendred, M.R.C.S. Eng., L.R.C.P. Lond.,
late house surgeon to guy's hospital.

THE curious association of deaf-mutism and goitre occuring in two members of a large family has induced me to record these cases. Why this association? Perhaps some readers of THE LANCET may be able to throw some light on the cause of this combination of diseases: Absence of thyroid—oretinism; overgrowth of thyroid—deaf-mutism. I append the family history as recounted to me by the mother. The family is an Irish one, and the parents have been upwards of forty years resident in Durham. The father, aged sixty-six years, and the mother, aged sixty-seven years, are alive and healthy. They have had ten children, five sons and five daughters. In an epidemic of small-pox twenty-five years ago the whole family was attacked with the exception of the younger of the deaf-mutes, and four males and one female died, although all had been vaccinated, and that recently, as they were children. The remaining son and two of the daughters are healthy and vigorous. The first goitre case is the first-born of the family—a spare woman now aged thirty-eight years. She is deaf and can only mumble indistinctly; little care has been taken to educate her and so she is imbecile. The goitre is a large multilobular hard tumour, the greater part on the right side of the neck; from time to time she suffers from dyspnœic attacks. The growth was first observed after the small-pox—i.e., at thirteen years of age. The second surviving girl is now aged twenty-eight years, and is the fifth of the family; she is a small, spare, intelligent woman, her expression being in marked contrast to her sister's. She is not absolutely deaf and can mumble incoherently; her education has been attended to with so much success that she has been "in service." The tumour is larger than in the other case, but is of the same character; it has been growing for about fifteen years, and during the last year has caused both dyspnœa and dysphagia, which have become so urgent that I have sent her to-day to Newcastle Infirmary for operation.

Durham.

FIG. 1—Dr. Vaughan Pendred's original description of the syndrome of deafness with goiter from the Lancet (1896), 2, 532. This figure has already been reproduced previously (Fraser, 1964) and the author is grateful to the Editors of the *Lancet* and the *Journal of Medical Genetics* for permission to use it here.

(1965a) the proposita with the block in question but with virtually normal hearing has 2 aunts with Pendred's syndrome.

Nor is the distinction proposed by Stanbury (1966) entirely valid, that persons who are not deaf and who manifest this particular block are grossly hypothyroid (goitrous cretins) while persons with Pendred's syndrome are euthyroid. Fraser (1965a) has shown that hypothyroid and euthyroid persons with Pendred's syndrome may coexist in the same family and, to compound difficulties in differentiation, gross hypothyroidism leading to a diagnosis of goitrous cretinism may be followed in one and the same individual at a later age by the classical symptomatology of Pendred's syndrome (Figure 3). Moreover, many case reports of the type of goitrous cretinism marked by a block in the incorporation of iodide into organic compounds mention speech

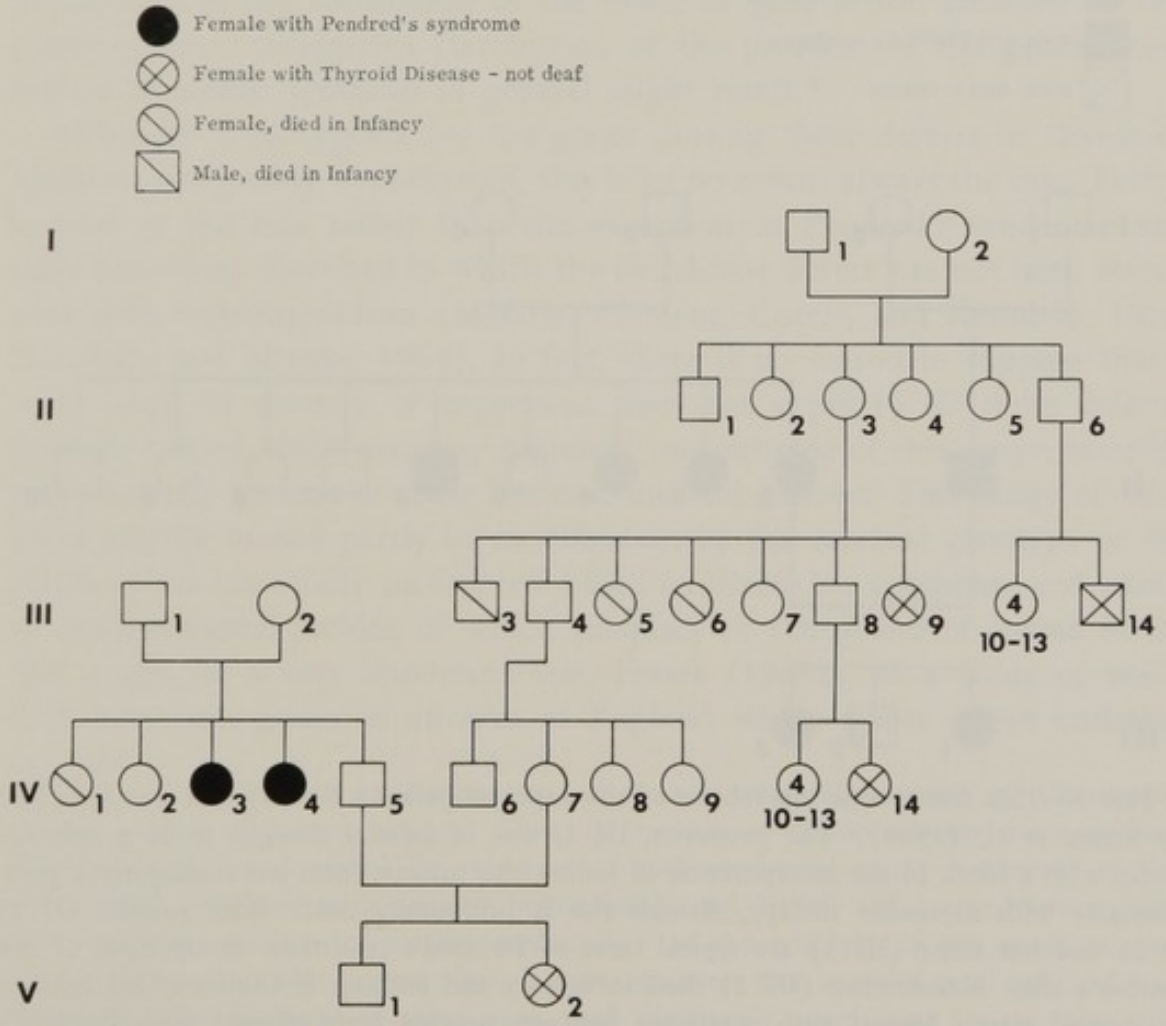



FIG. 2—The proposita, V 2, is a goitrous cretin with a block in the incorporation of iodide into organic form and with normal hearing but has 2 aunts (IV 3; IV 4) with typical Pendred's syndrome. She presumably inherited one mutant allele for Pendred's syndrome from her father and a distinct mutant allele affecting thyroxine synthesis from her mother. The mother's mutant allele seems to be expressed rather frequently in heterozygotes as is evidenced by goiter in herself and thyroid disease in several other members of her family (III 9; III 14; IV 14).

defects or deafness; such associations do not seem to occur with other types of goitrous cretinism (Fraser, 1965a). Nevertheless, it remains probable that there are 2 or more distinct defects of thyroxine synthesis marked by a block at the stage of organic incorporation of iodine.

The situation is further complicated by the fact that the defect in organic binding of iodide is not the only one present in Pendred's syndrome. Fraser, Morgans and Trotter (1960) showed that some difficulty in coupling of the iodotyrosines existed in addition. Also, later studies on the nature of the physiological and biochemical aspects of the derangement of hormone synthesis in Pendred's syndrome have revealed a complex situation, which has been well discussed by Sacrez, Juif, Mandry, Kellershohn, Rivière and Comar (1967) and Rivière, Comar, Kellershohn, Juif, Mandry, Sacrez and Methlin (1967). These authors detected MIT and DIT in the sera of cases of Pendred's syndrome and, on this basis, suggested a difficulty in the intra-thyroidal deiodination of these substances. In addition, they reviewed their own

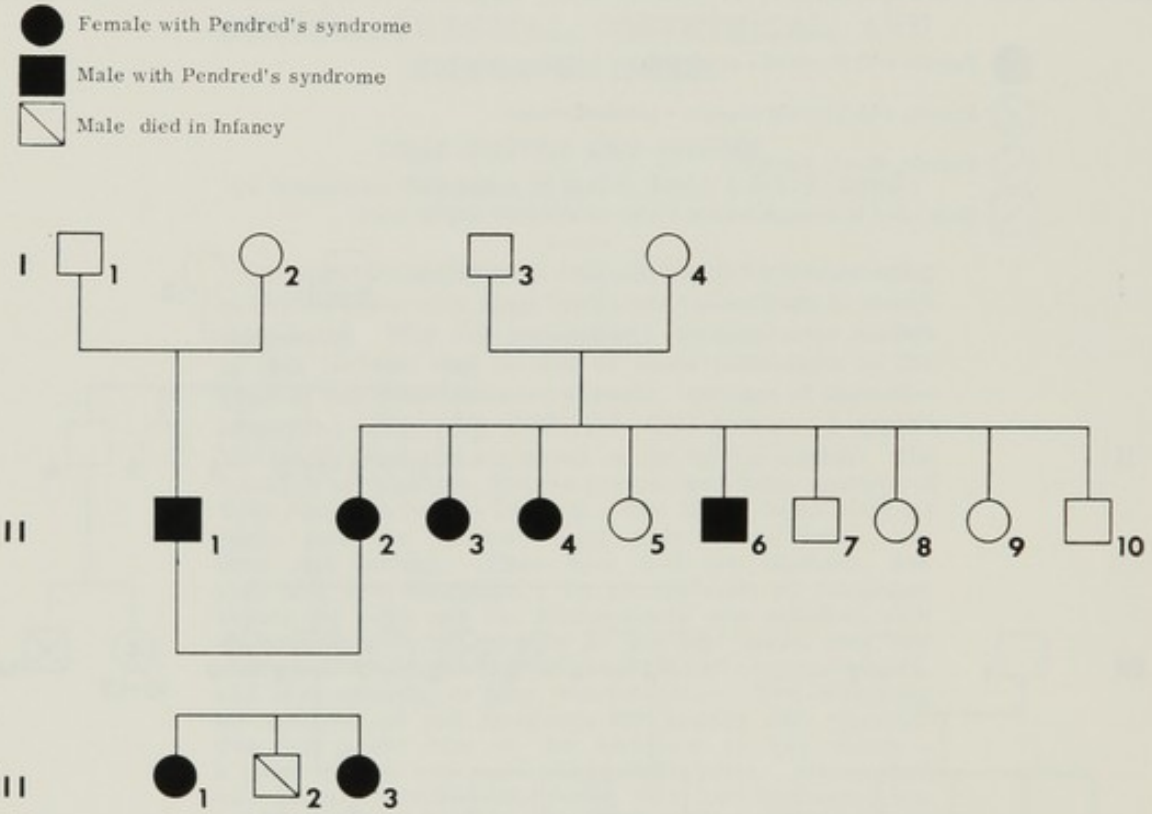



FIG. 3—This family was the subject of the paper of Jackson (1954) and was restudied by Fraser et al. (1969). The proposita, III 1, was in infancy thought to be a goitrous cretin with a block in the incorporation of iodine into organic form but subsequently grew normally with thyroxine therapy, though she is profoundly deaf. Both parents (II 1; II 2) and her sister (III 3) are typical cases of Pendred's syndrome, as are three of her mother's sibs. Her brother (III 2) died in infancy and nothing is known of his hearing or thyroid state. Several such marriages between persons both affected with Pendred's syndrome with, as expected, only affected offspring have been ascertained by Fraser (1965a) as a consequence of a strong assortative mating tendency among the deaf.

and other cases of the association of childhood deafness with goitrous hypothroidism in which no evidence could be demonstrated of a block in the organic incorporation of iodine and in which suggestions of other defects in thyroxine synthesis were made.

A partial explanation of these anomalies may be afforded by a lack of uniformity among investigators in the use of diagnostic techniques, in particular, the discharge test with $KClO_4$ or KCNS used to demonstrate the block in organic incorporation of iodide. It is of course possible, moreover, that congenital deafness may coexist coincidentally with a goiter due to a defect in thyroxine synthesis. One such case was mentioned by Fraser (1965a). Nevertheless, although it remains probable that Pendred's syndrome is the manifestation of the pleiotropic effects of a gene which include difficulties at the stage of organic incorporation of iodine, it would seem likely that the nature of the block in thyroxine synthesis is somewhat complex. The crucial importance of Pendred's syndrome would seem to lie in the association of childhood perceptive deafness with this block for, if advantage can be taken of the clue to the elucidation of the mode of gene action afforded by this pleiotropism, considerable clarification of the problem of the genetic control of thyroxine synthesis in general might result.*

Although homozygotes for the genes causing these defects in thyroxine synthesis are grossly hypothyroid, this is by no means always the case. Euthyroidism is the rule rather than the exception in Pendred's syndrome and cases have been described in which the deiodinase defect has not been associated with hypothyroidism (McGirr, Clement, Currie, and Kennedy, 1959; Kusakabe and Miyake, 1964). In fact, there is no reason to suppose that a wide range of severity of expression does not apply to all these defects; goitrous cretins, therefore, may represent one extreme of this range, sampled preferentially because of their intrinsic medical interest. This range of variation may be caused partly by modification by the residual genotype of the effects of an essentially incomplete block in thyroxine synthesis, and partly by environmental factors, of which adequacy or abundance of iodine in the diet might be a very important one. Fraser (1963a), in a study of males with non-toxic goiter in an area of England where goiter is not endemic,

* All such pleiotropic associations are, of course, potentially capable of illuminating obscure aspects of human biology. Thus, in the case of childhood perceptive deafness, Fraser (1964) has discussed the relevance of such conditions as the syndromes of Pendred, Usher (deafness with retinitis pigmentosa), and Jervell and Lange-Nielsen (deafness with abnormalities of cardiac conduction) to the problem of the mode of gene action. Although at the present time, it does not seem that knowledge is sufficiently advanced to take advantage of these clues, such pleiotropic associations are no more striking, *a priori,* than that of juvenile hepatic cirrhosis with cataracts, which is now well understood as being the result of an autosomal recessive disorder of galactose metabolism.

found suggestive evidence of the operation of simple genetic factors, both in homozygous and heterozygous form, in a substantial proportion.

## Sporadic Non-toxic Goiter

It is clear that genetic factors play an important role in the pathogenesis of sporadic goiter in general. It has already been stressed that homozygosity for the genes causing more or less well-defined defects in thyroxine synthesis is not necessarily associated with hypothyroidism. Thus, not only patients with sporadic goitrous cretinism but also some with non-toxic euthyroid goiter may be homozygous for such genes. There are many indications that this is the case, apart from the studies to which reference has been made above. For example, many years before the introduction of radioiodine to studies of thyroid disease, Long (1939) described 3 sibs with non-toxic goiter. A characteristic feature, well demonstrated in this family, of goiters due to such a mechanism is their tendency to recur after surgery since the compensatory pituitary thyrotrophic hormone (TSH) stimulation continues. Other such cases of recurrence of goiter after surgery were described by Poate and McIndoe (1940) and Heptinstall and Porritt (1952). Constant TSH stimulation leads to a high uptake of radioiodide by the thyroid gland and such high uptakes were described in 3 cases of non-toxic goiter by Bell and Eisenbeis (1953).

The prevalence of Pendred's syndrome is probably no greater than 5 to 10 in 100,000 in most populations (Fraser, 1965a) and there is no reason to suppose that homozygosis for the genes causing other defects of thyroxine synthesis is substantially greater. Such mechanisms, therefore, are clearly inadequate to explain all cases of non-toxic sporadic goiter, a condition which may affect as many as 4 per cent of individuals in areas where goiter is not endemic (Means, DeGroot and Stanbury, 1963).

It should be noted, however, that heterozygosis for the genes causing defects in thyroxine synthesis is relatively common. Thus, if it is assumed that 6 such defects exist, for each of which the frequency of homozygotes is 1/20,000, then approximately 8 per cent of the population is heterozygous for one of these genes. It is suggested therefore that commonly, though not invariably, such heterozygosis is one of the primary causes of hyperplasia of the thyroid gland leading to non-toxic sporadic goiter which may evolve through a colloid to a nodular state. Such an hypothesis receives support from the very common observation of a strong family history of goiter in cases of sporadic goitrous hypothyroidism.

Heterozygosis of this type — or even double heterozygosis, which would be expected to occur with a not insubstantial frequency — is clearly not sufficient to cause goiter. If it were, the family history of goiter in cases of spor-

adic goitrous hypothyroidism would be far more striking than it is; for example, all parents of such cases are obligatory heterozygotes. It may well be, however, that hyperplasia of the thyroid gland results from a minor difficulty in thyroxine synthesis, due to such heterozygosis, interacting with other factors. Among the most potent of these is sex, and this explains the common pattern noted above (see page 91) of apparently dominant inheritance of sporadic goiter*, largely limited to the female sex. It is generally assumed that the greater lability of the thyroid gland in females, leading to increased propensity to all types of thyroid disease, including non-toxic nodular goiter, is associated with the general hormonal control of their *milieu interieur* and, in particular, with their exposure to the cyclic changes of menstruation and the disturbances characterizing puberty, pregnancy, and the menopause. That this greater lability may, however, have other causes is suggested by the fact that non-toxic goiter is relatively common among males with the XXY chromosomal constitution (Fraser, 1963b). This point is discussed further in the section dealing with adult hypothyroidism.

A genetic polymorphism which may play a role in the determination of susceptibility to hyperplastic changes in the thyroid gland is that governing the ability to taste phenylthiocarbamide (PTC). The findings of Harris, Kalmus and Trotter (1949) and of Kitchin, Howel Evans, Clarke, McConnell and Sheppard (1961), that non-tasters are more prone to develop nodular goiter, serve as a model of genetic modifying mechanisms which interact with other factors to cause goiter. Kitchin et al (1961), in fact, interpret their findings as suggesting an increased susceptibility to the effect of dietary goitrogens of a similar chemical constitution to PTC.

Undoubtedly the most important modifying factor is the supply of iodide in the diet. It is to be expected that, if the diet is abundant in iodine, minor defects in thyroxine synthesis will have little or no significance. If, on the other hand, supplies of iodine do not greatly exceed minimal requirements, such minor difficulties may well tip the balance, and hyperplasia of the thyroid gland, leading eventually to goiter, may be initiated.

Thus, it is suggested that non-toxic sporadic goiter is due to an interaction of minor difficulties of thyroxine synthesis with secondary modifying factors — of which sex and dietary deficiency of iodine or excess of goitrogens are the major ones thus far defined. These minor difficulties of thyroxine syn-

* Ascher (1920) described an association of goiter with blepharochalasis and a double upper lip. While it is probable that the combination of these other traits is inherited in an autosomal dominant manner, the association with goiter is so rare that it is possible that it was coincidental in Ascher's (1920) patients and that, therefore, goiter is not, as has sometimes been claimed, a facultative component of this dominantly inherited malformation syndrome.

thesis need not necessarily be due to heterozygosis for a specific gene but may be of more complex causation. Each step in hormone synthesis is undoubtedly under the control of many genes and consequently considerable variation probably exists in any population; persons with goiter may be predominantly drawn from one extreme, the less efficient, of these distribution curves. Nor need the postulated relative deficiency of iodine necessarily be closely associated with the diet; excessive renal (Cassano, Baschieri and Andreani, 1957) or intestinal (Van Middlesworth, 1957) loss may also be implicated, and this again may represent unfavorable extremes of a normal range of variation which is under polygenic control.

## Endemic Goiter

Endemic goiter is primarily due to environmental and not to genetic causes. Nevertheless, a brief discussion is of some relevance to the preceding section on sporadic goiter. There are, in fact, no unequivocal criteria available to separate the two (de Visscher, Beckers, de Crombrugghe and Herveg, 1964). Rather, a continuous spectrum of variation exists in the prevalence of goiter in populations, from those populations in which it is rare to those in which considerable thyroid hyperplasia may be present in virtually every individual.

Undoubtedly one of the most potent causes of endemic goiter is iodine deficiency. This is, of course, essentially an easily correctable cause, and the United States affords an excellent example of the virtual elimination of endemic goiter by the introduction of additional iodine into the diet. However, such iodine deficiency is never absolute and, although in some areas of endemic goiter it may be so severe that virtually 100 per cent of the population will show thyroid enlargement, even in these areas the degree of such enlargement will vary from individual to individual. In determining the degree of response of the thyroid gland to the iodine deficiency, therefore, secondary factors are important. These factors will be particularly important when the primary iodine deficiency is less marked.

Some of these secondary factors are environmental, such as economic ability to supplement the diet. In addition, epidemics of nodular goiter have been described, especially in Italy (Costa and Cottino, 1967), and it has been suggested that they may represent the initial stages of endemic goiter; though their exact cause has not been defined, it is likely to be environmental. Other secondary factors are genetic and essentially the same as those which play a role in the pathogenesis of sporadic goiter. A particularly instructive series of studies in this regard has recently been performed in Greece (Malamos, Miras, Koutras, Kostamis, Binopoulos, Mantzos, Levis, Rigopoulos, Zerefos and Tassopoulos, 1966; Koutras, Tassopoulos and Mar-

ketos, 1967). These authors have concluded that the differences in gland size among individuals who are taking a diet uniformly deficient in iodine are due to the interplay of many factors of which the rates of iodine loss from the body, the relative efficiency of the iodide-trapping mechanism of the gland and its biosynthetic capacity may be among the more important. As in the case of sporadic goiter, complex genetic control of these continuously varying attributes may be postulated. In some cases this control is essentially polygenic; in others, heterozygosis for genes causing defects in thyroxine synthesis when in homozygous form plays an important role. Recent careful family and twin studies in areas of endemic goiter in Greece (Hadjidakis, Koutras and Daikos, 1964; Malamos, Koutras, Kostamis, Kralios, Rigopoulos and Zerefos, 1966; Malamos, Koutras, Kostamis, Rigopoulos, Zerefos and Yataganas, 1967) have clearly shown that, as far as endemics of moderate intensity are concerned, complex genetic factors do play some part in the determination of susceptibility to the effects of iodine deficiency leading to hyperplasia of the thyroid gland.

## ENDEMIC CRETINISM

One of the main arguments put forward against the hypothesis that iodine deficiency is the universal cause of endemic goiter has been the rather striking dichotomy between areas in which endemic goiter has and has not been accompanied by an increased prevalence of the mental and physical retardation known for many centuries as the cretinoid degeneration.* The protagonists of the unitary iodine deficiency theory of endemic goiter argue that endemic cretinism is a manifestation of fetal involvement due to maternal

* It should be noted that the word *cretin* was originally used to describe such unfortunate degenerates in the Alpine areas of endemic goiter. Platter (1614) gave the following description of such individuals " . . . many infants are wont to be affected: who besides their innate simple-mindedeness, the head is now and then misformed, the tongue immense and tumid, dumb, a struma often at the throat, they show a deformed appearance, and seated in solemn stateliness, staring and a stick resting between their hands, their bodies twisted variously, their eyes wide apart, they show immoderate laughter and wonder at unknown things." One suggested explanation of the etymological derivation of the word *cretin* is that it is a perversion of the word *"chrétien,"* used because of the good-natured friendliness of these unfortunates.

The application of the word to hypothyroid conditions arising in infancy in areas where goiter is not endemic ("sporadic cretinism") has led to some confusion, since the two conditions are very different. Curling (1850) was perhaps responsible for this misnomer, which is based on the resemblances noted between 2 English infants with proved absence of the thyroid gland and some endemic cretins whom he had seen in Switzerland. Endemic cretinism, however, is a heterogeneous condition and only a minority of such cases owe their disability to uncomplicated hypothyroidism. The term rather connotes the geographical aggregation of a widely varying spectrum of associations of idiocy, dwarfism, deafness, goiter, and neurological disturbances.

thyroid deficiency in areas (usually, but not invariably, mountainous) of exceptionally severe endemic goiter. On the other hand, it is not unreasonable to postulate that other factors are operative. One such possibility might be the widespread use in the diet of substances which are goitrogenic to adults and at the same time potentially severely toxic in fetal life and in infancy.

It is a common and paradoxical difficulty of scientific investigations that material advances, while increasing the possibilities of dealing with a particular problem, may also lead to its virtual disappearance so that such studies become extremely difficult. In fact, cretinism has almost vanished in our own century from the European Alps after holding sway for many hundreds of years, and the only family studies of any importance of this condition date from 30 years and more ago (Lang, 1929; Eugster, 1934, 1935, 1936). These studies do not provide evidence that genetic factors are of primary importance in the etiology of endemic cretinism; rather they speak in favor of much the same type of complex determination that has been postulated for endemic goiter.

## Sporadic Non-Goitrous Infantile Hypothyroidism (Sporadic Non-Goitrous Cretinism)

It has already been noted in a previous section that sporadic goitrous cretinism is a simply inherited autosomal recessive disease. In the majority of cases, however, sporadic cretinism is not accompanied by goiter; indeed, the thyroid gland is typically very much reduced in size or absent altogether. In this larger group, which is itself biologically heterogeneous, the role of genetic factors is unclear; a complex interaction of genotype and environment seems to be involved. The position is further complicated by the fact that autosomal recessive inborn errors of thyroxine synthesis may, because of treatment or because of natural involution of goiter associated with aging, lead to a final clinical picture virtually indistinguishable from non-goitrous cretinism.

Non-goitrous sporadic cretinism may be due either to a true aplasia of the thyroid or to a failure of adequate development of the gland caused by an arrest during its descent during embryonic life from its position at the back of the tongue to the neck. Another possible cause is destruction of the thyroid gland by autoantibodies in infancy and childhood. More typically, such a process leads to juvenile Hashimoto's disease (see below) and is accompanied by goiter. This may, however, be followed by involution of the gland and gross hypothyroidism. If this process of involution is exceptionally rapid, the goitrous phase may be very short and remain undetected.

Osler (1893, 1897) attempted to define the relative numerical importance of the various biological categories making up the clinical entity of sporadic

cretinism. He distinguished severe congenital cretinism, usually associated with absence of the thyroid gland, from a milder type in which a goiter was often present and from a third category where the disease does not make its appearance until early childhood (juvenile myxedema). He gave the first careful histological description of the thyroid gland in a case of sporadic cretinism. Although the gland was small (4 grams), the description is similar in many ways to modern descriptions of the thyroid in goitrous cretins. It is interesting to note that this child was a deaf-mute and may well have been a case of Pendred's syndrome. Osler tried to form some estimate of the proportion of sporadic cretins who had thyroid glands, either enlarged or of normal size. Thus, in the 37 cases where information was available, goiter was present in 7 and a palpable thyroid gland in 14 more. This is rather a high proportion when compared to that found in modern series but, as Osler pointed out, at the time his paper was written athyreotic cretins tended to die in early infancy.

Finally, though he came to no specific conclusions on this point, Osler's papers illustrate the familial nature of sporadic cretinism. Four sibships account for 9 of his 60 cases and in one further case the parents were first cousins; data concerning presence or absence of goiters in these familial cases are unfortunately not available. One family where 3 cretinous siblings were afflicted with large goiters (one indeed had died at thyroidectomy) is especially worthy of note in that the 2 survivors were deaf and "spoke but little."*

Osler pointed out clearly that sporadic cretinism was a heterogeneous condition; and it is possible to infer from his data that one sub-group is of simple genetic etiology. Two recent surveys in Denmark (Andersen, 1961) and in Michigan, U.S.A. (Lowrey, Aster, Carr, Ramon, Beierwaltes and Spafford, 1958; Beierwaltes, Carr, Ramon, Stafford, Aster and Lowrey, 1959; Carr, Beierwaltes, Neel, Davidson, Lowrey, Dodson and Tanton, 1961; Neel, Carr, Beierwaltes and Davidson, 1961) have been made of large series of hypothyroid children (cretins) using modern investigative techniques. The first of these surveys is notable for the finding that cretinism due to an ectopic situation of the thyroid gland is more common than was previously thought to be the case, and that true athyreotic cretinism was the exception rather than the rule. These surveys, as well as that of Fraser (1961), show that about a third of all cases of cretinism result from defects of thyroxine synthesis due to an autosomal recessive gene or genes.

Only a beginning has been made in the elucidation of the etiology of the majority of cases which are not due to such autosomal recessive defects; it is

*Pendred described the syndrome of deafness with goiter which now bears his name at much the same time (1896).

not even clear whether aplasia and ectopic situation of the thyroid gland represent variants of the same basic process or not. The findings of Childs and Gardner (1954), most of which have been confirmed in later surveys, may provide some pointers towards elucidation of etiology. These authors found a preponderance of females among cases of non-goitrous cretinism. Clearly this cannot be due to the cyclic hormonal changes that are invoked as the cause of the greater representation of females among adults with thyroid disease.* They also found slight parental age and birth rank effects, in that cretins tended to be offspring of mothers over 30 and products of the third and later pregnancies. High birth weight is also a characteristic of these children; this may be a secondary effect due to the failure of function of the fetal thyroid.

Lastly, Childs and Gardner (1954) as well as Neel et al. (1961) found an increase in the rate of consanguineous marriages among the parents of these children. Neel et al. (1961) interpreted this finding as support for the theory that cases of sporadic cretinism represent phenodeviants who, in the words of Lerner (1954) "fall below the threshold of the obligate proportion of loci needed in the heterozygous state to ensure normal development." While the role of individual genes in such a situation can only rarely be identified, the finding of a very significant excess of non-tasters for PTC in 3 series of non-goitrous cretins (Shepard and Gartler, 1960; Shepard, 1961; Fraser, 1961; Shepard and Andersen, 1964) may be significant in this context.† It is of interest to note also that the selection experiments of Cole (1966) suggested polygenic inheritance of hypothyroidism in the White Leghorn fowl.

The possible deleterious role of maternal thyroid autoantibodies passed across the placenta, a mechanism which has been implicated in rare cases of congenital and apparently athyreotic cretinism,‡ remains to be adequately explored. Such exploration is rendered difficult in retrospective studies, since the presence of the antibodies may be temporary. The birth rank and par-

*Of significance in this connection is the report of Boyle and McGirr (1965) of cretinism in a case of Klinefelter's syndrome with XXY chromosomal constitution.

†These findings have been challenged by Azevedo, Snyder and Krieger (1965) on the ground that falsely negative responses to the test used in determining taste sensitivity to PTC are common among the mentally immature. Nevertheless, it remains probable (Fraser, 1965b) that the gross excess of non-tasters among sporadic non-goitrous cretins is a true one and not due to such a phenomenon, since a substantial excess of non-tasters was found also among their parents.

‡ Many such cases are familial (Blizzard, Chandler, Landing, Pettit and West, 1960; Sutherland, Esselborn, Burket, Skillman and Benson, 1960). Thus they may simulate the autosomal recessive inheritance of the goitrous type of sporadic cretinism as contrasted to the usual isolated occurrence of the non-goitrous type. Another type of non-goitrous cretinism which has been reported as occurring in more than one member of a family is

ental age effects apparent in some surveys of this condition may be of significance in this context, since the prevalence of thyroid autoantibodies increases with age (Hill, 1961); the high incidence of thyroid disease among relatives of propositi, which has often been recorded, may also be relevant to this hypothesis.

## ADULT HYPOTHYROIDISM

The most common cause of adult hypothyroidism (myxedema) is autoimmune damage to the thyroid gland. Such persons may be goitrous or without goiter. It is probable that a goiter may often be present at the beginning of the disease but that involution of the gland subsequently takes place. Thus lymphadenoid goiter (Hashimoto's struma) is thought to represent the active phase of thyroid autoimmunity, and gross myxedema to represent the end phase after complete destruction of the gland. This group of diseases is not controlled by any simple genetic mechanism but, in view of the central position of autoimmune phenomena in investigative medicine at the present time, much interest has been aroused by the fact that the aggregation of thyroid disease which has been noted for many years in relatives of persons with adult hypothyroidism is reflected by familial aggregation of thyroid autoantibodies also (Hall, Owen and Smart, 1960; Doniach and Roitt, 1966).

This familial predisposition to the formation of autoimmune antibodies is likely to be under genetic control. While a single gene theory is untenable, Burch and Rowell (1963) have suggested that dominant germinal mutations at a number of X-chromosomal loci are of importance, combined with a number of autosomal somatic mutations. This hypothesis has the merit of explaining the predominance of females among persons affected with these diseases and is an alternative to the currently accepted view that this predominance is associated with differences in hormonal status affecting pituitary-thyroid-gonad interactions. Such dominant X-chromosomal mutations, moreover, may play some part in the predisposition both of females and of XXY males to thyroid disease, such as sporadic goiter and sporadic non-goitrous cretinism, which are not marked by autoimmunity.

Another mechanism which has been suggested as a possibility, at least for the maternal transmission of Hashimoto's thyroiditis, is the transplacental passage of thyroid autoantibodies (Hjort and Pedersen, 1962; Volpe, Ezrin, Johnston and Steiner, 1963). According to this hypothesis, such passage leads to disturbance of immunological tolerance to thyroglobulin in the fetus who is predisposed, therefore, to form autoantibodies in later life. Such

---

the Kocher-Debré-Sémélaigne syndrome, in which cretinism is associated with muscular pseudohypertrophy (Cross, Mollander, Rimoin and McKusick, 1968). In some cases, furthermore, non-goitrous cretinism may occur in 2 or more sibs because of the general non-specific tendency towards familial aggregation of congenital malformations.

"false genetic" transmission through maternal-fetal interaction could at best only represent an ancillary mechanism, since family studies show that the predisposition to thyroid disease and to autoantibody production occurs among the paternal as well as the maternal relatives of propositi.

### THYROID DISEASE AND CHROMOSOMAL ABERRATIONS

Fialkow (1964) first put forward the hypothesis that autoantibodies predisposed to chromosomal aberrations. This hypothesis has stimulated a great number of studies, and a formidable amount of evidence bearing on this point has now accumulated. Fialkow (1966, 1967) reviewed this evidence in the case of trisomy 21 (mongoloid idiocy). He showed that thyroid autoantibodies and thyroid disease were unusually common in family members of patients with the trisomy-21 syndrome and suggested that such antibodies in the mothers of the patients predisposed to meiotic abnormalities. A dissenting view has recently been put forward by Wren, Evans, Vetters and Chew (1967) who found no such excess of autoantibodies in members of the families of patients with the trisomy-21 syndrome.

The situation in the case of the various chromosomal variants of gonadal dysgenesis or Turner's syndrome has been recently discussed by Fialkow (1967) and Vallotton and Forbes (1967). These authors found an increased prevalence of autoantibodies to a variety of tissues, especially thyroid, and an increased prevalence of thyroid disease among patients and their fathers and mothers.

Several investigators, however, have failed to find such a phenomenon in the case of Klinefelter's syndrome with an XXY chromosomal constitution (Day and Wright, 1964; Ferguson-Smith, Anderson, Frøland and Gray, 1966; Vallotton and Forbes, 1967). This finding contrasts with the observation of an increased predisposition of XXY males to various types of thyroid disease; these are, however, diseases which are not usually accompanied by autoantibody formation, such as sporadic goiter (see page 97) and sporadic non-goitrous cretinism (see page 102). On the other hand, the incidence of diabetes mellitus is increased in XXY individuals and members of their families (Nielsen, 1966a, 1966b) and the same has been found in the case of Turner's syndrome (Forbes and Engel, 1963). Burch and Burwell (1965) are of the opinion that diabetes mellitus, like Hashimoto's disease, is also an autoimmune disorder.

Burch, Rowell and Burwell (1966a, 1966b) have attempted to interpret the corpus of data available on the prevalence of thyroid autoantibodies and of diabetes mellitus in the families of patients with trisomy-21, Turner's and Klinefelter's syndromes in terms of an extension of Fialkow's (1964) hypothesis. They suggest that the meiotic accidents which lead to chromosomal

aberrations are due to autoantibodies directed against the germ cells, and that this is yet another aspect of the generalized disturbance of normal immunological tolerance which is under complex genetic control and, therefore, aggregated in these families. Moreover, they postulate that the various associations of Klinefelter's, Turner's and trisomy-21 syndromes with diabetes mellitus and thyroid autoimmunity reflect differences in the genotypes segregating in these families, each such genotype tending to contain certain combinations of alleles which predispose to the conditions found in association.

## THYROTOXICOSIS

Levitt (1954) clearly foreshadowed the current unitary concept of thyrotoxicosis, Hashimoto's disease and myxedema as representing different phases of the same basic process. This underlying unity is reflected by the presence of circulating autoantibodies in a large proportion of cases of these diseases and in members of their families. Thus the studies of Saxena (1965) and of Howel Evans, Woodrow, McDougall, Chew and Evans (1967) have shown a very high incidence of such antibodies among parents and sibs of patients with thyrotoxicosis. Moreover, in these family studies and in those of Bartels (1941), cases of 2 or more of these diseases were found within the same family, although there is probably greater inter- than intra-familial variation in the evolution of the disease process. The same tendency is seen in studies in which the propositi have Hashimoto's disease (Hall, Saxena and Owen, 1962; Doniach, Nilsson and Roitt, 1965).

It seems that, at least in the active state, thyrotoxicosis is marked by the presence of an abnormal thyrotrophic hormone, the so-called long-acting thyroid stimulator or LATS. This substance seems itself to be an immunoglobulin (Meek, Jones, Lewis and VanderLaan, 1964; Beall and Solomon, 1966) so that some primary disturbance of the control of the immune response seems likely.

In the face of such a complex situation, it is hardly surprising that divergent theories of the mode of inheritance of thyrotoxicosis have been proposed. This variety of hypotheses is reminiscent of the situation regarding the mode of inheritance of diabetes mellitus which has long been a matter of controversy among geneticists. It is perhaps significant to note that Burch and Burwell (1965) believe that the pathogenesis of the 2 groups of disease are similar. Moreover, in both cases, the diseases in question are undoubtedly heterogeneous; and rare juvenile forms exist which are not associated with autoimmune disturbances but with autosomal recessive inheritance presumably reflecting inborn errors of metabolism. In the case of thyroid disease, these correspond to sporadic goitrous cretinism, while in the case of diabetes mellitus, juvenile forms are associated with optic atrophy in diseases inher-

ited in an autosomal recessive fashion (Rose, Fraser, Friedmann and Kohner, 1966).

This parallel may be extended to pernicious anemia. Again, while the main body of cases is associated with autoantibody formation against the gastric parietal mucosa and with familial aggregation of no regular pattern, rarer juvenile autosomal recessive cases are also described (Mohamed, McKay and Galloway, 1966). There seems, moveover, to be much overlap both in families and in individuals among all 3 groups of diseases (Landing, Pettit, Wiens, Knowles and Guest, 1963; Doniach and Roitt, 1966). It has been suggested by Burch and Rowell (1963) that this overlap, which may include other diseases which these authors believe to be due to an autoimmune etiology, is due to the fact that certain of the germinal and somatic mutations which they invoke may be common predisposing causes of several of these disease processes.*

Thyrotoxicosis is the most thoroughly investigated abnormality in thyroid function that has been investigated from the genetic viewpoint. One of the earliest and most comprehensive of such surveys is that of Lewit, Rywkin, Serejsky, Fogelson, Dorfman and Lichzieher (1930). These authors suggested irregular or "conditional" dominant inheritance in the case of primary thyrotoxicosis. On the other hand, Bartels (1941) and Martin and Fisher (1945), suggested recessive inheritance in this disease. It is clear that genetic determination of this group of diseases is complex, though the ideas of Lewit et al. (1930) are probably nearer the truth. In fact, we are not yet near a definitive solution of the problem of genetic determination of this group of thyroid diseases and of the control of autoimmunity in general.

It is possible (Means et al., 1963) that some cases of toxic nodular goiter ("secondary" thyrotoxicosis) represent a disease which is basically different in etiology from "primary" thyrotoxicosis. Thus, Plummer (1928) suggested that in a multi-nodular goiter one of the nodules may of itself, over one or two decades, become hyperactive and produce thyrotoxicosis. This may occur in sporadic goiters, but more often in endemic goiters, usually those arising in lowland areas. If such a distinct entity exists, then it must be regarded as a development of the natural history of sporadic or endemic goiter — as the case may be — and, therefore, to follow the patterns of genetic control outlined in those sections. It may be of significance that Lewit et al (1930), Bar-

* It is of interest to note that Schmidt's syndrome of thyroid and adrenal insufficiency with coexistent diabetes mellitus (Solomon, Carenter, Bennett and Harvey, 1965) is thought to have an autoimmune basis; and there are suggestions (Phair, Bondy and Abelson, 1965; Matz and Zucker, 1967) that the ovary and parathyroids may also be involved in this class of pluriglandular autoimmune disorders. Associations of such pluriglandular disorders with pernicious anemia have also been reported (Doniach and Roitt, 1966).

tels (1941) and Martin and Fisher (1951) all believed that there was a difference in the pattern of inheritance between toxic nodular goiter ("Plummer's disease") and "primary" thyrotoxicosis. Furthermore, the findings of Kitchin et al (1961) of an excess of tasters for PTC among cases of "primary" and a small excess of non-tasters among cases of "secondary" thyrotoxicosis again suggest that the two are distinct diseases.

## MALIGNANT NEOPLASIA

Most malignant tumors of the thyroid gland are not determined in a simple way by genetic factors. The etiology of malignant neoplasia in general is of course even more complex than that of autoimmune disease and, indeed, there may be connections between the two (Burch and Burwell, 1965). We intend here only to discuss those rare forms of thyroid malignancy which are under simple genetic control.

Schimke and Hartmann (1965) have recently presented 2 families showing dominant inheritance of an association of amyloid-producing medullary thyroid carcinoma and pheochromocytoma, and have reviewed the findings in previously reported patients with this association. Since occasionally parathyroid adenomas have also been reported, these authors have suggested that this syndrome may be a variant of another dominantly inherited condition, multiple endocrine adenomatosis. Whether this is so or not, this particular type of thyroid carcinoma undoubtedly should be added to the growing list of malignant neoplasms which are primarily determined by single abnormal genes.

In addition to this syndrome, it is clear that hyperplasia of the thyroid of the type of sporadic or endemic non-toxic goiter predisposes to malignant change. Thus the genetic factors determining such neoplasia are essentially those which are responsible for the goiter. It has been possible to induce malignant neoplasia in the Syrian hamster by giving a diet grossly deficient in iodide (Fortner, George and Sternberg, 1960), and in the rat by prolonged administration of goitrogenic drugs (Purves, Griesbach and Kennedy, 1951). In both cases, the primary cause of thyroid hyperplasia has been prolonged stimulation of the gland by pituitary TSH in a compensatory effort to overcome the block in thyroxine synthesis and this is, of course, precisely the situation which occurs in the case of the autosomal recessive defects responsible for sporadic goitrous cretinism and for some types of sporadic goiter.

It is not surprising, therefore, that a suspicion of malignant change has often been recorded in the case of goiters of this type (Potter and Morris, 1935; Lerman, Jones and Calkins, 1946; Roberts, 1957; Thieme, 1957; Elman, 1958; McGirr et al, 1959; Fraser, 1963a; Nilsson, Borgfors, Gamstorp,

Holst and Lidén, 1964). In no case, however, has a truly malignant and metastatic evolution of the neoplasia been recorded, and it is probable that the diagnosis of such malignancy is mistaken and due to a deceptive histological picture marked by considerable pleomorphic appearances of the gland due to constant and prolonged stimulation by TSH (Smith, 1960). Nevertheless, it remains likely that these changes are potentially malignant and, in such cases therefore, a situation occurs wherein predisposition to malignant change is largely determined by autosomal recessive inheritance involving a single gene locus.

## Inherited Variation in Thyroxine-Binding Proteins

In previous sections, simply inherited variations which play a role in the pathogenesis of thyroid disease have been described which affect the enzyme systems responsible for the trapping of iodide by the thyroid gland and the subsequent synthesis of thyroid hormones. Probably genetically determined defects of the structure of thyroglobulin also exist. However, the intra-thyroidal metabolism of iodine is not the only possible site where defects determined by simple patterns of inheritance may occur. It has been suggested that such defects may influence both the renal and intestinal excretion of iodide and iodine-containing compounds; in addition, peripheral utilization of the thyroid hormones may be defective (Hutchison, Arneil and McGirr, 1957). The evidence for the existence of such simply inherited extra-thyroidal defects is, however, inconclusive. On the other hand, unequivocal evidence exists for simply heritable variation in the proteins responsible for the transport of thyroxine in the serum.

Thyroxine is bound in the serum mainly to 3 proteins — albumin and the thyroxine-binding pre-albumin and alpha-globulin components. It is in the last of these (TBG) that variation has been described. Beierwaltes and Robbins (1959) and Beierwaltes, Carr and Hunter (1961) described a family in members of which the thyroxine-binding capacity of TBG was much increased. The trait was inherited in a dominant manner; although males with this condition were involved more than females, in that the increase in thyroxine-binding by TBG was much greater, probable male to male transmission in this family argues against sex linkage. A further family of this type was described by Florsheim, Dowling, Meister and Bodfish (1962).

The converse situation (decreased binding capacity of TBG) has also been reported. Thus Nicoloff, Dowling and Patton (1964) described this phenomenon in 6 members of a family. Again the trait seemed to be inherited in a dominant manner. Though male to male transmission did not take place, the 3 females seemed to be involved to the same extent as the 3 males, arguing against sex-linkage. On the other hand, sex-linked inheritance of a similar de-

fect was considered probable in 2 families by Nikolai and Seal (1966) and Marshall, Levy and Steinberg (1966). In these 2 families a total of 16 males had no demonstrable binding by TBG, whereas in 16 females values for thyroxine-binding by TBG were lower than the normal range. The pedigree information, moreover, was fully consistent with sex-linked inheritance, with these 16 males representing affected hemizygotes and the 16 females heterozygotes.

Thus it would seem that at least 3 simply inherited defects exist, either increasing or decreasing the thyroxine-binding capacity of TBG. These variant forms are reflected by abnormally high or low levels of serum protein bound iodine (PBI) but do not seem to have any gross clinical effect on thyroid function. It would be surprising, however, if this pronounced variation in such a basic process as serum transport of thyroid hormones did not play a role in the total picture of thyroid homeostasis. Therefore, it is likely that the genes involved are of importance, in association with other factors, in the pathogenesis of thyroid disease. In this connection the apparent sex-linked nature of some of these genes, and the more pronounced expression in the male sex of others which seem to be autosomal, should be noted, since this may be of significance in the context of the female predisposition to thyroid disease. At least one of these abnormal alleles is probably of appreciable frequency since Marshall et al. (1966) have shown that approximately 3 per cent of healthy young males have inexplicably low PBI levels. This is of importance since such persons may well be treated unnecessarily with thyroid hormones because of a suspicion of hypothyroidism. Conversely, high levels of PBI when the TBG binding is increased may lead to a false diagnosis of hyperthyroidism.

At the moment nothing is known of the nature of this variation of TBG. The genes involved could be structural mutants giving rise to an altered TBG molecule, or they could affect the control of the rate of synthesis of TBG; yet other possibilities for their mode of action exist. It should be noted that genetically determined variations exist also in serum albumin (Adams, 1966) and pre-albumin (Fagerhol and Braend, 1965) in man. Such variations, however, do not seem to have been related to any changes in the binding of thyroid hormones or to their transport in the serum.

## SUMMARY

As in the case of all other disease processes affecting the human organism, the development and evolution of a disorder of the thyroid gland is due to a complex interaction of many factors, both genetic and environmental. This interaction is likely to be different in each case, so that each individual with thyroid disease represents to some extent a unique biological phenomenon.

Nevertheless, patterns can be traced in that in a few rare diseases, single gene inheritance seems to be largely responsible, usually by affecting one of the enzymes concerned in thyroxine synthesis. Even in such cases, however, secondary modification, both genetic and environmental, of the results of the primary defect may determine the evolution of the lesion and the degree of clinical disease resulting.

In the vast majority of cases, pathogenesis is considerably more complex. Some of the main etiological factors likely to be involved are deficiency of iodide and presence of excessive amounts of goitrogens in the diet; heterozygosis for genes causing defects in thyroxine in homozygous form; and the predisposition to form antibodies against the body's own tissues. Thyroid function also seems to be influenced by genetic variation at the locus determining taste sensitivity to PTC, in a way which is not currently understood. Finally, sex seems to play a role, in that females are more susceptible than males to all types of thyroid disease except autosomal recessive forms of sporadic goitrous cretinism and, even in this case, they tend to be more severely involved.

We are only at the beginning of understanding how these various factors are implicated in the production of each case of thyroid disease and how they interact with the effects of variant forms of the many other enzymes, proteins and hormones involved in the complex chain of events between the ingestion of iodide and the availability and effect of thyroid hormones at their final sites of action.

## REFERENCES

Adams, M. S. 1966. Genetic diversity in serum albumin. J. Med. Genet. 3: 198-202.

Andersen, H. J. 1961. Studies of hypothyroidism in children. Acta Paediat. (Uppsala) Suppl. 125.

Ascher, K. W. 1920. Blepharochalasis mit Struma und Doppellippe. Klin. Mbl. Augenheilk. 65: 86-96.

Azevedo, E., L. H. Snyder, and H. Krieger. 1965. Phenylthiocarbamide-tasting in the mentally immature. Lancet 1: 1223-1224.

Bartels, E. D. 1941. Heredity in Graves' disease. Op. Dom. Biol. Hered. Hum. (Kbh): 2: 1-384.

Beall, G. N., and D. H. Solomon. 1966. On the immunological nature of the long-acting thyroid stimulator. J. Clin. Endocr. 26: 1382-1388.

Beierwaltes, W. H., E. A. Carr, and R. L. Hunter. 1961. Hereditary increase in the thyroxine-binding sites in the serum alpha-globulin. Trans. Ass. Amer. Physicians 74: 170-179.

—, —, G. Raman, N. R. Stafford, R. A. Aster, and G. U. Lowrey. 1959. Institutionalized cretins in the State of Michigan. J. Mich. Med. Soc. 58: 1077-1095.

—, and J. Robbins. 1959. Familial increase in the thyroxine-binding sites in serum alpha-globulin. J. Clin. Invest. 38: 1683-1688.

Bell, G. O., and C. H. Eisenbeis, C. H. 1955. The non-toxic nodular hyperplastic goiter. New Eng. J. Med. 253: 812-814.

Blizzard, R. M., R. W. Chandler, B. N. Landing, M. D. Pettit, and C. D. West. 1960. Maternal auto-immunization to thyroid as a probable cause of athyreotic cretinism. New Eng. J. Med. 263: 327-336.

Boyle, J. A., and E. M. McGirr. 1965. Coexisting cretinism and Klinefelter's syndrome. Brit. Med. J. 1: 1170.

Burch, P. R. J., and R. G. Burwell. 1965. Self and not-self. A clonal induction approach to immunology. Quart. Rev. Biol. 40: 252-279.

—, and N. R. Rowell. 1963. Aetiological aspects of chronic discoid and systemic lupus erythematosus, systemic sclerosis and Hashimoto's thyroiditis. Some immunological implications. Lancet 2: 507-513.

—, —, and R. G. Burwell. 1966(a). Autoimmunity and chromosomal aberrations. Lancet 2: 170.

—, —, and —. 1966(b). Autoantibodies in Klinefelter's syndrome. Lancet 2: 748-749.

Carr, E. A., W. H. Beierwaltes, J. V. Neel, R. Davidson, G. H. Lowrey, V. N. Dodson, and S. H. Tanton. 1961. The various types of thyroid malfunction in cretinism and their relative frequency. Pediatrics 28: 1-16.

Cassano, C., L. Baschieri, and D. Andreani. 1957. Fattori dismetabolici nella genesi del gozzo endogeno. *In* Le Tireopatie vol. V: 703.

Childs, B., and L. I. Gardner. 1954. Etiologic factors in sporadic cretinism. An analysis of ninety cases. Ann. Hum. Genet. 19: 90-96.

Cole, R. K. 1966. Hereditary hypothryroidism in the domestic fowl. Genetics 53: 1021-1033.

Costa, A., and F. Cottino. 1967. An epidemic of acute nodular goitre. Lancet 2: 50.

Cross, H. E., C. S. Hollander, D. L. Rimoin, and V. A. McKusick. 1968. Familial agoitrous cretinism accompanied by muscular hypertrophy. Pediatrics 41: 413.

Curling, T. B. 1850. Two cases of absence of the thyroid body. Med.-Chir. Trans. 33: 303-306.

Day, R. W., and S. W. Wright. 1964. Thyroid autoantibodies and sex chromosome abnormalties. Lancet 1: 667.

De Visscher, M., C. Beckers, B. de Crombrugghe, and J. P. Herveg. 1964. Is there any fundamental difference between endemic and sporadic non-toxic goitre? Acta Endocr. (Kbh.) 45: 365-369.

Doniach, D., L. R. Nilsson, and I. M. Roitt. 1965. Autoimmune thyroiditis in children and adolescents. II. Immunological correlations and parent study. Acta Paediat. (Uppsala) 54: 260-274.

—, and I. M. Roitt. 1966. Family studies on gastric autoimmunity. Proc. Roy. Soc. Med. 59: 691-694.

Elman, D. S. 1958. Familial association of nerve deafness with nodular goiter and thyroid carcinoma. New Eng. Med. 259: 219-223.

Eugster, J. 1934. Zur Erblichkeitsfrage der Endemischen Struma. I. Arch Klaus-Stift. Vererbungsforsch. 9: 275-363.

—. 1935. Zur Erblichkeitsfrage der Endemischen Struma. II. Arch. Klaus-Stift. Vererbungsforsch. 10: 101-192.

—. 1936. Zur Erblichkeitsfrage der Endemischen Struma. III. Arch. Klaus-Stift. Vererbungsforsch. 11: 369-539.

Fagerhol, M. K., and M. Braend. 1965. Serum prealbumin: polymorphism in man. Science 149: 986-987.

Ferguson-Smith, M. A., J. R. Anderson, A. Frøland, and K. G. Gray. 1966. Frequency of autoantibodies in patients with chromatin positive Klinefelter's syndrome and their parents. Lancet 2: 566-568.

Fialkow, P. 1964. Autoimmunity: a predisposing factor to chromosomal aberrations? Lancet 1: 474-475.

—. 1966. Autoimmunity and chromosomal aberrations. Amer. J. Hum. Genet. 18: 93-108.

—, 1967. Autoantibodies and chromosomal aberrations. Lancet 1: 1106.

Florsheim, W. H., J. T. Dowling, L. Meister, and R. E. Bodfish. 1962. Familial elevation of serum thyroxine-binding capacity. J. Clin. Endocr. 22: 735-740.

Forbes, A. P., and E. Engel. 1963. The high incidence of diabetes mellitus in 41 patients with gonadal dysgenesis and their close relatives. Metabolism 12: 428-439.

Fortner, J. G., P. A. George, and S. S. Sternberg. 1960. Induced and spontaneous thyroid cancer in the Syrian (golden) Hamster. Endocrinology 60: 364-376.

Fraser, G. R. 1961. Cretinism and taste sensitivity to phenylthiocarbamide. Lancet 1: 964-965.

—. 1962. Some genetical determinants in the pathogenesis of goitre. J. Génét. Hum. 11: 240-250.

—. 1963(a). A genetical study of goitre. Ann. Hum. Genet. 26: 335-346.

—. 1963(b). Goitre in Klinefelter's syndrome. Brit. Med. J. 1: 1284.

—. 1964. Profound childhood deafness. J. Med. Genet. 1: 118-125.

—. 1965(a). Association of congenital deafness with goitre (syndrome of Pendred)—a study of 207 families. Ann. Hum. Genet. 28: 201-249.

—. 1965(b). Phenylthiocarbamide-tasting in cretins. Lancet 2: 134.

—, M. E. Morgans, and W. R. Trotter. 1960. The syndrome of sporadic goitre and congenital deafness. Quart. J. Med. 29: 279-295.

Hadjidakis, S. G., D. A. Koutras, and D. K. Daikos. 1964. Endemic goitre in Greece: family studies. J. Med. Genet. 1: 82-87.

Hall, R., S. G. Owen, and G. A. Smart. 1960. Evidence for genetic predisposition to formation of thyroid antibodies. Lancet 2: 187-188.

—, K. M. Saxena, and S. G. Owen. 1962. A study of parents of patients with Hashimoto's disease. Lancet 2: 1291-1292.

Harris, H., H. Kalmus, and W. R. Trotter. 1949. Taste sensitivity to phenylthiourea in goitre and diabetes. Lancet 2: 1038-1039.

Heptinstall, R. H., and A. Porritt. 1952. Recurrent 'nodular goitre' in children. Brit. J. Surg. 39: 433-441.

Hill, O. W. 1961. Tydroglobulin antibodies in 1297 patients with thyroid disease. Brit. Med. J. 1: 1793.

Hjort, T., and G. T. Pedersen. 1962. Thyroid antibodies and "thyroglobulin" in the serum in pregnant and parturient women and in newborn infants. Lancet 2: 259-263.

Howel Evans, A. W., J. C. Woodrow, C. O. M. McDougall, A. R. Chew, and R. W. Evans. 1967. Antibodies in the families of thyrotoxic patients. Lancet 1: 636-641.

Hutchison, J. H., G. C. Arneil, and E. M. McGirr. 1957. Deficiency of an extra-thyroid enzyme in sporadic cretinism. Lancet 2: 644.

Jackson, A. D. M. 1954. Non-endemic goitrous cretinism. Arch. Dis. Child. 29: 571-574.

Kitchin, F. D., W. Howel Evans, C. A. Clarke, R. B. McConnell, and P. M. Sheppard. 1959. P.T.C. taste response and thyroid disease. Brit. Med. J. 1: 1069-1074.

Koutras, D. A., C. N. Tassopoulos, and S. Marketos. 1967. Endemic goiter in Greece. Salivary iodide clearance in goitrous and non-goitrous persons. J. Clin. Endocr. 27: 783-788.

Kusakabe, T., and T. Miyake. 1964. Thyroidal deiodination defect in three sisters with simple goiter. J. Clin. Endocr. 24: 456-459.

Landing, B. H., M. D. Pettit, R. L. Wiens, H. Knowles, and G. M. Guest. 1963. Antithyroid antibody and chronic thyroiditis in diabetes. J. Clin. Endocr. 23: 119-120.

Lang, T. 1929. Sippschaft untersuchungen über Allgäuer Kretinen und Schwachsinnige. Z. Ges. Neurol. Psychiat. 119: 109-152.

Lerman, J., H. W. Jones, and E. Calkins. 1946. Studies on two sporadic cretinous brothers with goiter, together with some remarks on the relation of hyperplasia to neoplasia. Ann. Intern. Med. 25: 677-701.

Lerner, I. M. 1954. Genetic Homeostasis. London and New York: Wiley.

Levitt, T. 1954. The Thyroid. Edinburgh and London: Livingstone.

Lewit, S. G., L. A. Rywkin, M. J. Serejsky, L. J. Fogelson, I. A. Dorfman, and I. B. Lichzieher. 1930. Uber die Genetik des Basedow'schen Krankheit und des ihr nahestehenden pathologischen Formen. Mediko-Biologicheskij Zhurnal 6: 368.

Long, L. D. 1939. Preadolescent recurrent nodular goiter in three children of the same family. Trans. Amer. Goiter Ass. 306-312.

Lowrey, G. H., R. H. Aster, E. A. Carr, G. Ramon, W. H. Beierwaltes, and N. R. Spafford. 1958. Early diagnostic criteria of congenital hypothyrodism. A comprehensive study of forty-nine cretins. Amer. J. Dis. Child. 96: 131-143.

Malamos, B., D. A. Koutras, P. Kostamis, A. C. Kralios, G. Rigopoulos and N. Zerefos. 1966. Endemic goiter in Greece: epidemiologic and genetic studies. J. Clin. Endocr. 26: 688-695.

—, —, —, G. A. Rigopoulos, N. S. Zerefos, and X. A. Yataganas. 1967. Endemic goiter in Greece: a study of 379 twin pairs. J. Med. Genet. 4: 16-18.

—, K. Miras, D. A. Koutras, P. Kostamis, D. Binopoulos, J. Mantzos, G. Levis, G. Rigopoulos, N. Zerefos, and C. N. Tassopoulos. 1966. Endemic goiter in Greece: metabolic studies. J. Clin. Endocr. 26: 696-704.

McGirr, E. M., W. E. Clement, A. R. Currie, and J. S. Kennedy. 1959. Impaired dehalogenase activity as a cause of goitre with malignant changes. Scot. Med. J. 4: 232-241.

Marshall, J. S., R. P. Levy, and A. G. Steinberg. 1966. Human thyroxine-binding globulin deficiency. A genetic study. New Eng. J. Med. 274: 1469-1473.

Martin, L., and R. A. Fisher. 1945. The hereditary and familial aspects of exophthalmic goitre and nodular goitre. Quart. J. Med. 14: 207-219.

—, and —. 1951. The hereditary and familial aspects of toxic nodular goitre (secondary thyrotoxicosis). Quart. J. Med. 20: 293-297.

Matz, R., and S. Zucker. 1967. Adrenal insufficiency, diabetes mellitus and amenorrhea: an incomplete form of pluriglandular insufficiency. Metabolism 16: 522-528.

Means, H., L. J. DeGroot, and J. B. Stanbury. 1963. The Thyroid and its Diseases. 3rd edition. New York: McGraw Hill.

Meek, J. C., A. E. Jones, W. J. Lewis, and W. P. VanderLaan. 1964. Characterization of the long-acting thyroid stimulation of Graves' disease. Proc. Nat. Acad. Sci. USA. 52: 342-349.

Michel, R., J. E. Rall, J. Roche, and M. Tubiana. 1964. Thyroidal iodoproteins in patients with goitrous hypothyroidism. J. Clin. Endocr. 24: 352-358.

Mohamed, S. D., E. McKay, and W. H. Galloway. 1966. Juvenile familial megaloblastic anaemia due to selective malabsorption of vitamin $B_{12}$. Quart. J. Med. 35: 433-453.

Neel, J. V., E. A. Carr, W. H. Beierwaltes, and R. T. Davidson. 1961. Genetic studies on the congenitally hypothyroid. Pediatrics 27: 269-285.

Nicoloff, J. T., J. T. Dowling, and D. D. Patton. 1964. Inheritance of decreased thyroxine-binding by the thyroxine-binding globulin. J. Clin. Endocr. 24: 294-298.

Nielsen, J. 1966(a). Diabetes mellitus in parents of patients with Klinefelter's syndrome. Lancet 1: 1376.

—. 1966(b). Klinefelter's syndrome and diabetes mellitus. Lancet 2: 748.

Nikolai, T. F., and U. S. Seal. 1966. X-chromosome linked familial decrease in thyroxine-binding globulin activity. J. Clin. Endocr. 26: 835-841.

Nilsson, L. R., N. Borgfors, I. Gamstorp, H-E. Holst, and G. Lidén. 1964. Non-endemic goitre and deafness. Acta Paediat. (Uppsala) 53: 117-131.

Osler, W. 1893. On sporadic cretinism in America. Amer. J. Med. Sci. 106: 503-518.

—. 1897. Sporadic cretinism in America. Trans. Congr. Amer. Phys. Surg. 4: 169-206.

Pendred, V. 1896. Deaf-mutism and goitre. Lancet 2: 532.

Phair, J. P., P K. Bondy, and D. M. Abelson. 1965. Diabetes mellitus, Addison's disease and myxedema—report of two cases. J. Clin. Endocr. 260-265.

Platter, F. 1614. Quoted by R. H. Major. 1945. *In* Classic Descriptions of Disease. 3rd edition. Oxford: Blackwell

Plummer, H. S. 1928. The function of the thyroid gland containing adenomatous tissue. Trans. Ass. Amer. Physicians 43: 159-167.

Poate, H. R. G., and P. H. McIndoe. 1940. Recurrent adenomata of thyroid. Med. J. Aust. 1: 187-191.

Potter, E. B., and W. R. Morris. 1935. Carcinoma of the thyroid gland. Report of five cases in young individuals. Amer. J. Surg. 27: 546-550.

Purves, H. D., W. E. Griesbach, and T. H. Kennedy. 1951. Studies in experimental goitre; malignant change in a transplantable rat thyroid tumour. Brit. J. Cancer 5: 301-310.

Rivière, R., D. Comar, C. Kellershohn, J.-G. Juif, J. Mandry, R. Sacrez, and G. Methlin. 1967. Le syndrome de Pendred. II. Les explorations radio-isotopiques. Ann. Pédiat. 43: 10-19.

Roberts, K. D. 1957. Thyroid carcinoma in childhood in Great Britain. Arch. Dis. Child. 32: 58-60.

Rose, F. C., G. R. Fraser, A. I. Friedmann, and E. M. Kohner. 1966. The association of juvenile diabetes mellitus and optic atrophy: clinical and genetical aspects. Quart. J. Med. 35: 385-405

Sacrez, R., J.-G. Juif, J. Mandry, C. Kellershohn, R. Rivière, and D. Comar. 1967. Le syndrome de Pendred. I. Etude critique, clinique et génétique. Ann. Pédiat. 43: 5-9.

Saxena, K. M. 1965. Inheritance of thyroglobulin antibody in thyrotoxic children. Lancet 1: 583.

Schimke, R. N., and W. H. Hartmann. 1965. Familial amyloid-producing medullary thyroid carcinoma and pheochromocytoma. Ann. Intern. Med. 63: 1027-1039.

Shepard, T. H. 1961. Phenylthiocarbamide non-tasting among congenital athyrotic cretins: further studies in an attempt to explain the increased incidence. J. Clin. Invest. 40: 1751-1757.

—, and H. J. Andersen. 1964. Phenylthiocarbamide non-tasting among different types of cretinism and thyroid disorders. Acta Endocr. (Kbh.) Suppl. 89: 32.

—, and S. M. Gartler. 1960. Increased incidence of nontasters of phenylthiocarbamide among congenital athyreotic cretins. Science 131: 929.

Smith, J. F. 1960. The pathology of the thyroid in the syndrome of sporadic goitre and congenital deafness. Quart. J. Med. 29: 297-303.

Solomon, N., C. J. Carpenter, I. L. Bennett, and A. M. Harvey. 1965. Schmidt's syndrome (thyroid and adrenal insufficiency) and coexistent diabetes mellitus. Diabetes 14: 300-304.

Stanbury, J. B. 1966. Familial goiter. *In* J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson (Eds.): The Metabolic Basis of Inherited Disease. 2nd edition. p. 215. New York: McGraw Hill.

Sutherland, J. M., V. M. Esselborn, R. L. Burket, T. B. Skillman, and J. T. Benson. 1960. Familial non-goitrous cretinism apparently due to maternal antithyroid antibody: report of family. New Eng. J. Med. 263: 336-341.

Thieme, E. T. 1957. A report of the occurrence of deaf-mutism and goiter in four of six siblings of a North American family. Ann. Surg. 146: 941-948.

Vallotton, M. B., and A. P. Forbes. 1967. Autoimmunity in gonadal dysgenesis and Klinefelter's syndrome. Lancet 1: 648-651.

Van Middlesworth, L. 1957. Thyroxine excretion, a possible cause of goitre. Endocrinology 61: 570-573.

Volpe, R., C. Ezrin, M. W. Johnston, and J. W. Steiner. 1963. Genetic factors in Hashimoto's struma. Canad. Med. Ass. J. 88: 915-919.

Wiener, J. D., and G. E. Linderbloom. 1964. The possible occurrence of two inborn errors of iodine metabolism in one patient. Acta Endocr. (Kbh.) 47: 385-401.

Wren, P. J., D. A. P. Evans, J. M. Vetters, and A. Chew. 1967. Autoimmune antibodies in Mongol families. Lancet 2: 186-188.

CHAPTER 4

# Genetic Aspects of Autoimmunity

**Philip J. Fialkow**

Departments of Medicine and Genetics
University of Washington
Seattle, Washington

AN ANIMAL with an intact immune system reacts to foreign invaders such as bacteria with the formation of antibodies which bring about either the destruction or inactivation of the intruders. However, in order to maintain its integrity, the animal must not mount an immune attack against its own tissues. Ehrlich and Morgenroth (1900) referred to this refusal to react to self as "horror autotoxicus." More recently, Burnet and Fenner (1949) introduced the term "self-recognition" to refer to the function of the immune system concerned with the ability to distinguish between "self" and "not-self." Once this distinction is made, significant immunologic responses are directed only against material foreign to the normal animal; but if there is a breakdown in immunologic homeostasis, then the response may be directed against self-constituents. If this reaction results in significant tissue destruction, an autoimmune disease* may ensue. Evidence for the pathogenesis of autoimmune reactions is most convincing in the diseases of experimental animals which develop after immunization with autologous antigens. However, immunologic aberrations have recently been described in a variety of human diseases, and the hypothesis that autoimmunity is involved in some of them has gained increasing acceptance.

---

* The term "autoimmune disease" is in general use, although "autosensitive" or "autoallergic" may be more correct etymologically.

Aided by Grant GM 15253 of the National Institutes of Health.

A striking feature of these diseases is the presence of circulating auto-antibodies that have the capacity to react with a specific antigen of the host, such as thyroglobulin. It is generally agreed that in most instances these auto-antibodies are not the agents primarily responsible for the disease. Nevertheless, the not infrequent occurrence of such antibodies in asymptomatic subjects may indicate a latent or subclinical form of the disease. Thus, regardless of their pathogenic significance, the presence of circulating antibodies may be utilized as a kind of "marker" in family studies of patients with putative autoimmune diseases.

The diseases regarded by one or more investigators as "autoimmune" are now so numerous that only some of them can be included in this discussion. For convenience, these diseases can be divided into two groups. One group consists of the so-called organ-specific autoimmune diseases, and is primarily characterized by circulating antibodies directed against antigens specific to a given organ (e.g., the thyroid) which is the major site of tissue destruction. The other group includes diseases like systemic lupus erythematosus, in which the lesions are widespread, and the circulating antibodies are directed against antigens common to many organs. Some of these "autoimmune" diseases occur with increased frequency in patients with immunologic deficiency states (e.g., agammaglobulinemia), and their asymptomatic relatives have an increased frequency of circulating antibodies (Fudenberg, German and Kunkel, 1962). Genetic aspects of the systemic "autoimmune" and immunologic deficiency diseases have been reviewed in past volumes of *Progress in Medical Genetics* (Holman, 1962; Peterson, Cooper and Good, 1965). In this review, only selected examples of diseases associated with organ-specific antibodies are discussed; they are referred to as autoimmune, as opposed to "autoimmune" or "so-called autoimmune." However, it must not be inferred from this terminology that autoimmunity has been conclusively shown to be of direct pathogenic importance in any of the diseases in man discussed herein.

Since the most extensive data on organ-specific lesions in man have been obtained from studies of the autoimmune thyroid diseases, especially chronic lymphocytic thyroiditis (CLT), these conditions are considered in some detail. The observations which are relevant to pathogenic mechanisms in CLT are in broad outline applicable to the other organ-specific autoimmune diseases, such as pernicious anemia and idiopathic Addison's disease.

## 1. THYROID DISEASE

### *A. Hashimoto's Thyroiditis and Other Forms of Chronic Lymphocytic Thyroiditis*

Hashimoto's thyroiditis is a chronic inflammatory disorder of the thyroid gland characterized histologically by varying degrees of lymphocytic infiltration and clinically by a variable onset and course. It occurs predominantly in

females in the third to sixth decades of life. Characteristically, the gland is firm or hard; it is diffusely enlarged to a slight or moderate degree, and it decreases in size consequent to thyroid therapy. With the exception of the goiter, many patients are asymptomatic, but the initial symptoms may be those of hypo-, or less commonly, hyperthyroidism. Pathologic changes in the thyroid originally described by Hashimoto (1912) include extensive formation of lymphoid follicles, round cell infiltration, epithelial changes and connective tissue formation. In many patients with chronic thyroiditis, the changes may be much less marked and consist essentially of lymphocytic infiltration. Whether these disorders with varying degrees of lymphocytic infiltration, with or without other histologic changes in the thyroid gland, are part of a spectrum of a single pathogenic process is not definitely known; in this discussion, they are grouped together under the heading of chronic lymphocytic thyroiditis (CLT).

On the basis of data accumulated from experiments in lower animals and from clinical, serological and pathological observations in man, CLT is considered by many workers to be the most convincing example of an autoimmune disorder in man. In 1956, Rose and Witebsky produced a disease in rabbits with histological resemblances to some forms of CLT in man by injection of allogeneic (homologous) thyroglobulin or crude thyroid extracts emulsified in complete Freund's adjuvant.* In addition to the morphologic changes, organ-specific thyroid antibodies appeared in the serum (i.e., circulating antibodies) (Witebsky and Rose, 1956). Hemi-thyroidectomized rabbits similarly injected with autologous thyroid extract developed thyroiditis in the remaining portion of the gland and circulating antibodies which reacted with the rabbits' own thyroid glands. Thus, this experimental disease is a true autoimmune thyroiditis, and the associated circulating antibodies are true autoantibodies. Autoimmune thyroiditis has since been induced in a variety of other animals, but the disorder in the monkey bears the closest resemblance serologically and histologically to that seen in man (Rose et al., 1965).

Shortly after the experimental induction of CLT was demonstrated in animals, the detection of thyroid antibodies in the sera of many patients with CLT was reported by Roitt, Doniach and co-workers (1956) and by Witebsky, Rose and associates (1957). Antibodies have been defined which react with at least 3 different thyroid-specific antigens (thyroglobulin, a second antigen in colloid distinct from thyroglobulin, and a component associated

* Complete Freund's adjuvant consists of killed acid-fast bacilli in an emulsion of water in oil. The precise manner in which it potentiates autoimmunization is unknown, but it probably acts both by providing a depot for long-term, continuous release of antigen and by altering systemic reactivity (Rose and Taylor, 1965).

with microsomes) (see Doniach and Roitt, 1963, and Rose and Taylor, 1965, for discussion and references). It is usual to use allogeneic (homologous) or heterologous antigens for antibody detection; however, when the appropriate tissues are available, it can be shown that such antibodies will react with autologous thyroid, indicating that they are autoantibodies.

Thyroid autoantibodies of one or more of these 3 specificities are found in the sera of almost all patients with CLT (Doniach and Roitt, 1963). This finding, in conjunction with the observation that autoimmunization can induce thyroiditis in lower animals, has led to the suggestion that autoimmunity is involved in the pathogenesis of CLT in man. However, the etiologic agents and pathogenic mechanisms whereby the proposed autoimmune reactions bring about the disease remain objects of speculation.

*1. Etiology and Pathogenesis*

The tissue lesions are presumably brought about by classical-type circulating antibodies or by factors more directly associated with immunocytes;* i.e., cell-mediated autoimmunity. The evidence currently available favors the operation of cell-mediated immunity, perhaps through delayed-type hypersensitivity reactions (e.g., see discussion in Rose and Taylor, 1965). Humoral antibodies may then reflect the presence of cell-mediated autoimmunity.

There are many difficulties in determining the factors which initiate the immunopathogenic sequence of CLT in man. Classically, the development of an immune reaction follows an identified immunizing event, e.g., innoculation of animals with thyroid antigens in Freund's adjuvant. In man autoimmunity occurs spontaneously, and it is not known whether an unusual exposure to antigen is required or whether there is some breakdown in immunologic homeostasis such as a loss of tolerance for self-antigens. Thus, hypotheses have evolved along two lines:

*a. Antigen-oriented hypotheses.* One such hypothesis holds that the initial step in the sequence is an abnormal release of normal organ-specific thyroid antigens which might have been hidden from the immunocytes during a critical time in ontogenesis when tolerance was being developed. Upon their subsequent exposure they are not recognized as self, and an immune reaction develops. Examples of proposed events or conditions which might lead to inordinate release of normal antigens include thyroid surgery, pregnancy, thyroid hyperplasia, virus infection, or a defective basement membrane. Genetic factors might operate at this level in several ways. For instance, there could be genetically conditioned susceptibility to viruses or genetically determined defects in the thyroid basement membrane. However, it is unlikely that simple

* The word *immunocyte* was introduced by Dameshek (1966) to replace the longer term "immunologically competent cell."

release of unaltered antigen in an otherwise normal animal precipitates thyroid auoimmunity. In experimental animals, innoculation of unaltered autologous thyroid antigens does not induce thyroiditis; Freund's adjuvant is also necessary (Rose, Kite and Doebbler, 1962). Furthermore, when patients with thyrotoxicosis who lack circulating thyroid antibodies are treated with $I^{131}$, these antibodies do not appear despite tissue destruction and antigen release (Irvine, 1964).

Moreover, it has been shown that thyroglobulin is not a "hidden" or sequestered antigen, since it is found in lymph draining the thyroid gland, in sera from healthy subjects and in cord blood (Roitt and Torrigiani, 1967).

An alternate suggestion is that altered autologous or exogenous antigens (e.g., bacteria) provoke the immune response, which is then directed against both the altered or exogenous and the native antigens. Some support for this hypothesis comes from the observation in animals that heated or chemically substituted thyroglobulin elicits autoimmune reactions even in the absence of Freund's adjuvant (Weigle, 1965).

*b. Altered Immune Response Hypotheses*

This type of hypothesis postulates that the primary event in the initiation of autoimmune disease involves an aberration in the immune response rather than in the antigen. The pathogenic reaction might be consequent to immunologic hyperreactivity or to selective immunologic deficiency. For example, Burnet (1959) suggests the development through somatic mutation of "forbidden clones" of immunocytes which lack the capacity to recognize self, and, therefore, become autoaggressive. Viruses or other microorganisms could conceivably increase immunological reactivity either by a direct effect on immunocytes or by some other mechanism. The genotypic background could also be important at this level through inherited predisposition to the development of "forbidden clones" or to heightened immunologic reactivity from other mechanisms.

On the other hand, it has been suggested that inherited predisposition to autoimmunity may reflect genetically determined selective susceptibility for one or another microorganism rather than genetically determined immunologic hyperreactivity (Fudenberg, 1967). The increased frequencies of autoimmune diseases (primarily of the systemic type) in subjects with generalized immunologic deficiency (agammaglobulinemia) and in mice made immunologically deficient by neonatal thymectomy have been cited in support of this suggestion (Fudenberg, 1967).

It may be necessary to evoke an altered immune response hypothesis to explain some of the non-organ-specific putative autoimmune diseases which occur spontaneously in man and which cannot be reproduced experimentally

in animals, e.g., systemic lupus erythematosus. Furthermore, even though organ-specific autoimmune diseases can be induced experimentally, the injection of Freund's adjuvant is required in addition to the autologous antigen. One action of the adjuvant may be to alter immunologic reactivity. Thus, the antigen-oriented and altered immunity hypotheses are not mutually exclusive. It may be that alterations at both the antigen and immunologic apparatus levels underlie at least some human organ-specific diseases. Inherited factors may operate directly or indirectly. For example, even if a virus proves to be of primary importance, only certain genotypes might be susceptible to the virus or to the effects it may have on immunologic reactivity.

2. *Genetic Studies*

*a. Observation in animals.* For the most part, organ-specific thyroid autoimmunity in animals has only been observed after an immunizing event, and therefore, it has not been possible to investigate the possibility that inherited alterations in immunologic reactivity can bring about similar results (a possible exception in chickens is discussed in the section on hypothyroidism). However, experiments in animals do suggest that genotypic background is important in determining susceptibility to induced autoimmunization. McMaster, Lerner and Mueller (1965) immunized 2 inbred strains of guinea pigs with low doses of guinea pig thyroid extract in complete Freund's adjuvant. The incidence of thyroiditis was significantly higher in the Hartley strain than in the other strain tested. Since environmental factors were well-controlled, the results were ascribed to inherited differences in susceptibility to the immunization or to the consequent tissue damage. No breeding experiments were reported. This same strain develops another experimental autoimmune disease (allergic encephalomyelitis) in response to doses of central nervous system antigen which are insufficient to cause the disease in other strains (Lipton and Freund, 1953; Stone, 1962). These observations may indicate that members of a particular strain have a genetically determined susceptibility to autoimmunization by a number of antigens.

Information relevant to genetic influences on susceptibility to human disease associated with immunologic aberrations cannot be directly obtained. However, twin and family studies have provided some potentially useful information.

*b. Twin studies.* At least 6 instances of Hashimoto's disease in monovular twins have been reported (Irvine et al., 1961; see Diamond and Joffe, 1966, for subsequent references). In view of the possible importance of environmental factors such as virus exposure, and the statistical biases associated with the ascertainment and reporting of individual twin pairs, no firm conclusions about genetic influences can be inferred from these limited observations. Large scale studies of unselected twin pairs using identical diagnostic

criteria along with comparisons of the frequency of concordance in monozygotic as opposed to like-sex dizygotic twin pairs are required. The variable time of onset is a complication, and the evaluation would best be done in elderly twin pairs.

*c. Family studies.* There have been several reports of single families with clinical Hashimoto's thyroiditis in sibs or offspring of affected patients (Craig, Spann and Lowbeer, 1952; Dunning, 1959; Hung and Winship, 1963; Hoffman, 1966), but studies in which asymptomatic relatives have been evaluated for either biochemical or immunologic markers have been more informative. For example, an unusual albumin-like iodoprotein distinct from thyroglobulin is frequently, but not specifically, found in the blood of patients with Hashimoto's thyroiditis (Owen and McConahey, 1956). DeGroot and associates (1962) detected this protein in 2 sibs with histologically confirmed CLT, and in their parents and 3 sibs. The authors suggested that the presence of the unusual protein in the blood might represent leakage through a defective basement membrane of a thyroglobulin metabolic product. A similar protein occurs in one type of presumably inherited congenital goiter (DeGroot and Stanbury, 1959). Conceivably, some patients who are heterozygous for genes causing abnormalities in thyroid metabolism may be more susceptible to CLT.

In another study, the unusual iodoprotein was found in a mother and 2 children with histologically confirmed CLT; the 4 other sibs had goiter and/or circulating thyroid antibodies (Thier et al., 1965). Extensive clinical, biochemical and immunologic investigation of 56 other relatives in 4 generations failed to reveal a clear mode of inheritance, and the authors suggested a viral etiology. However, a thorough search for viruses in the glands of 2 subjects who developed goiter while under observation yielded negative results and viral antibody studies were not conclusive. A transient form of subacute thyroiditis may follow infection with mumps virus (Eylan, Zmucky and Sheba, 1957; Felix-Davies, 1958), but an association between viral infection and *chronic* lymphocytic thyroiditis has not been established, and most patients with CLT do not have a history suggestive of a previous episode of subacute thyroiditis (Doniach and Roitt, 1963). Nonetheless, on the basis of the available data, masked or latent chronic virus infection cannot be excluded. Comparisons of the frequency of concordance of thyroid autoimmunity in monozygotic and dizygotic twins, and studies of spouses would be helpful in evaluating the role of conventionally acquired infectious agents.

*d. Thyroid antibodies in relatives of patients with CLT.* A major limitation of the early family studies was the lack of a simple objective means of detecting relatives with latent or subclinical disease. More recent investigations have utilized the presence of thyroid antibodies to "mark" susceptible rela-

tives. These studies have shown a familial concentration of thyroid antibodies, but full interpretation requires knowledge of the distribution of these antibodies in the general population.

Thyroid antibodies of one or another specificity are found in less than 5 per cent of children, but with adulthood, the frequency increases from about 5 per cent at age 21 to about 30 per cent in post-menopausal women (Doniach and Roitt, 1964; Fialkow, 1966). In adult males the frequency is somewhat less, about 10-15 per cent. No studies have been specifically designed to look for geographical variations in the frequency of thyroid antibodies, but in general, data collected on "normal" subjects from other parts of the world are not significantly different from those gathered in North America. Adequate data regarding possible socio-economic, racial and ethnic differences are not available. Comparison of one study with another is hampered by the fact that the sensitivity and types of tests employed vary between laboratories.

Family studies demonstrating familial occurrence of thyroid autoantibodies were pioneered by Hall, Owen and Smart. In their first study (1960), they detected thyroid antibodies in 56 per cent of 39 siblings; a dominant mode of inheritance was suggested. Subsequently, these antibodies were found in either one or both parents of 19 young patients with Hashimoto's thyroiditis (Hall, Saxena and Owen, 1962), and it was further shown that the tendency to form autoantibodies seemed to be transmitted through fathers as well as mothers (Hall, Owen and Smart, 1964). Similar results were obtained by other investigators (Volpe et al., 1963; Buchanan et al., 1965). Some of these earlier studies have been criticized by Masi, Hartmann and Shulman (1965) for being poorly controlled, but subsequent reports confirm the familial predisposition to form thyroid autoantibodies and are consistent with the hypothesis that this predisposition is genetically determined (Table 1).

For example, Doniach, Roitt and Taylor (1965) evaluated the families of 84 propositi with CLT and compared the findings with those in an unselected series of controls matched with the relatives for age and sex. Forty-six per cent of 269 relatives were seropositive, contrasted to 15 per cent of controls ($p<0.01$) (Table 1). Similar findings were observed when the families of 38 propositi with juvenile CLT were tested (Doniach, Nilsson and Roitt, 1965) (Table 1). Of 29 completely tested families, one parent was positive in 17, both were positive in 6, and both were negative in 6.

Even these later studies, which have included evaluation of healthy controls matched with the relatives for age and sex, are not ideal. It would be desirable to compare the findings in relatives of patients with CLT to the findings in control groups consisting of spouses and families of carefully

matched probands with a similar but etiologically distinct disease. In addition, before definite conclusions about modes of inheritance can be made, evaluation of families should be more complete. Despite the fact that the studies reported thus far are not ideal in all of these respects, they do indicate a familial predisposition to the formation of thyroid antibodies and suggest that it may be genetically determined. The data are still insufficient to allow firm conclusions about a mode of inheritance.

*B. Primary Hypothyroidism (Primary Myxedema)*

Hypothyroidism results from deficient thyroid hormone and may be either primary or secondary. Primary forms are associated with a disorder intrinsic to the thyroid gland, while secondary forms are due to deficiency of pituitary thyrotropic hormone. Among adults, primary hypothyroidism is much more common in women. The true frequency in the population is unknown, but in hospitalized patients it may be in the range of 1 per 1,000 or less (see Macgregor, 1964). Some cases are secondary to surgery or to environmental

TABLE 1. — *Thyroid Antibodies in Families of Probands with Adult Onset CLT (Doniach, Roitt and Taylor, 1965), Juvenile CLT (Doniach, Nilsson and Roitt, 1965) and Thyrotoxicosis (Howell Evans et al., 1967).*

| Investigators | Family members: Relationship | Number tested† | % positive | Controls* % positive | P |
|---|---|---|---|---|---|
| Doniach, Roitt & Taylor, 1965 (Adult Onset CLT) | *Probands:* | 84 | | | |
| | Mothers | 30 | 57% | 30% | $<0.1$ |
| | Fathers | 14 | 43% | 8% | $<0.1$ |
| | Sisters | 79 | 54% | 18% | $<0.001$ |
| | Brothers | 46 | 48% | 15% | $<0.01$ |
| | Daughters | 38 | 47% | 3% | $<0.001$ |
| | Sons | 31 | 19% | 6% | $<0.3$ |
| | Distant | 31 | 23% | 23% | $<0.5$ |
| | *Total* | 269 | 46% | 15% | $<0.001$ |
| Doniach, Nilsson & Roitt, 1965 (Juvenile CLT) | *Probands:* | 38 | 100% | | |
| | Mothers | 35 | 69% | 23% | $<0.001$ |
| | Fathers | 30 | 30% | 20% | $<0.6$ |
| Howel Evans et al., 1967 (Thyrotoxicosis) | *Probands:* | 74 | 50% | | |
| | Mothers | 57 | 35% | 12% | $<0.001$ |
| | Fathers | 39 | 12% | 9% | $<0.6$ |
| | Sisters | 101 | 25% | 9% | $<0.01$ |
| | Brothers | 76 | 15% | 7% | $<0.1$ |

* Controls in each of these studies were matched for age and sex with the relatives.

† Antibodies of all 3 specificities were measured. Data given for Howel Evans and associates' study only include tanned red cell results.

agents such as drugs (e.g., see Macgregor, 1964), but it is now generally believed that the disease in the majority of adult cases, especially those without goiter, is related to CLT. Thyroid antibodies are found in at least 80 per cent of adults with primary myxedema (Owen and Smart, 1958), and focal areas of inflammation are frequently observed in the thyroid glands. Clinically, small goiters in patients with Hashimoto's disease may regress spontaneously and lead to thyroid atrophy indistinguishable from primary hypothyroidism. This regression may also occur in as many as 50 per cent of patients with Hashimoto's thyroiditis and large goiters who are treated with thyroid hormones for many years (Doniach and Roitt, 1963). In addition, many children with CLT become hypothyroid (Saxena and Crawford, 1962; Nilsson and Doniach, 1964). Histologically, the serial biopsy studies of Vickery and Hamlin (1961) suggest that progression to increased fibrosis and atrophy occurs in some Hashimoto glands. Thus, it has been suggested that Hashimoto's thyroiditis and non-congenital primary hypothyroidism without goiter are variants of the same process (Owen and Smart, 1958). This interrelationship is further suggested by family studies *(vide infra)*.

*1. Genetic Studies*

*a. Observations in animals.* Primary hypothyroidism occurs spontaneously in a strain of White Leghorn domestic fowl, and is characterized by obesity, decreased skeletal size, long silky feathers and delayed sexual maturity (Cole, 1966). Females are affected more severely and more frequently than males. All affected fowl have pathologic changes in the thyroid including lymphocytic infiltration, and most glands have some degree of atrophy. Similar but less severe histologic changes are not uncommon in clinically normal fowl from the same obese stock. The clinical and pathologic changes are not present when the chicks hatch, but usually the trait can be recognized at 6 to 8 weeks of age. Thus, this spontaneous disease in fowl bears remarkable clinical and pathologic resemblances to CLT-hypothyroidism in man.

Cole noted that the few chicks in which the disease occurred randomly were all from one of 3 strains and were often sibs; therefore, he instituted genetic studies (Cole, 1966). By selective breeding, the frequency of hypothydroidism in this strain was increased from less than 1 per cent to over 70 per cent in both sexes. In the inbred chicks, there was earlier onset and more severe phenotypic expression, but even after several generations of selection, considerable variation was noted in both individual and family expression. Backcrosses of normal $F_1$ females to affected males resulted in significantly increased incidences in both sexes (42 per cent in females and 23 per cent in males). Outcrosses to fowl from unrelated strains produced low, but significant numbers of affected offspring (6 per cent). It was concluded that the

trait is hereditary and polygenic and that more severe expression occurs in the female (Cole, 1966). Virologic studies were not reported, but if vertically transmitted or neonatally acquired viruses can be excluded, there is presumptive evidence in fowl for the primary importance of genetic factors in CLT-hypothyroidism.

*b. Family studies.* Family studies have led to divergent conclusions. Goldberg (1960) investigated a small number of relatives of 57 probands with primary hypothyroidism in an overseas military population. He found an affected spouse in 13 instances and an affected child in only 2 cases, and suggested that environmental agents such as viruses were operating. The population studied was a restricted one, and many affected relatives had only borderline abnormalities. It is not known how, if at all, this state of "partial or mild hypothyroidism" is related to persistent frank myxedema. Hall (1965) selected for study the families of 38 patients with overt primary myxedema who were treated at an endocrine clinic during an 18 year period. No affected spouses were detected, but among 5 of the families there were 14 additional relatives with myxedema, including 8 parent-child and 2 sib-sib combinations. Furthermore, in one of the 5 families and in a sixth kinship a woman with myxedema had a daughter wth thyrotoxicosis. Hereditary factors in myxedema and a genetic relationship between it and thyrotoxicosis were suggested. Extensive immunologic evaluation of the relatives of probands with primary hypothyroidism per se has not been reported.

*C. Thyrotoxicosis (Graves' Disease)*

The majority of patients with thyrotoxicosis have thyroid antibodies and focal lymphocytic thyroiditis. It was formerly assumed that hyperplasia associated with thyroid overactivity provided the antigenic stimulus responsible for autoimmunity. However, several groups have recently suggested that immunological aberrations are of primary importance in the genesis of this disease (Anderson et al., 1964; Doniach and Roitt, 1964; Fialkow, Fudenberg and Epstein, 1964). In support of this proposal, these workers cited evidence that thyrotoxicosis is found, both in individual patients and in families, in association with other autoimmune disorders, such as pernicious anemia, Hashimoto's thyroiditis, myasthenia gravis and Addison's disease.

Recent work on long-acting thyroid-stimulator (LATS) may lend some support to the hypothesis that autoimmunity is important in initiating and perpetuating Graves' disease (see Brit. Med. J., 1966). The plasma of most thyrotoxic patients contains LATS; both its origin and stimulatory effect on the thyroid gland are distinct from the usual physiologic pituitary thyrotropic hormone (e.g., it has a more prolonged period of activity, hence it is called "long-acting"). Attempts to dissociate LATS from 7S gamma globulin

(IgG) have been uniformly unsuccessful, and its activity can be removed by absorption with thyroid microsomes. These observations have stimulated the belief that LATS is an IgG antibody, and that its corresponding antigen is present in thyroid microsomes (Brit. Med. J., 1966). Further evidence in support of this assumption has not yet been provided (Burke, 1967).

*1. Genetic Studies*

Genetic studies in this disease have largely been confined to observations in man.

*a. Twin studies.* Investigations reported before the immunological associations of thyrotoxicosis were fully appreciated provided strong support for the importance of genetic factors. In one report, 66 per cent of monozygotic twins were concordant as opposed to 9 per cent of dizygotic twins (cited by Vogel, 1959) and in another the figures were 47 per cent and 3.1 per cent respectively (Von Verschuer, 1958). In a large retrospective study of unselected twins born in Denmark, 41 monozygotic pairs with at least one thyrotoxic twin were ascertained. Twelve co-twins were similarly affected, but only 2 of 59 like-sex dizygotic co-twins of probands with thyrotoxicosis had that disease (Harvald and Hauge, 1956; Harvald, cited in Jayson et al., 1967).

*b. Family studies.* Early clinical family studies also provided support for a genetic susceptibility to thyrotoxicosis, but they did not allow firm conclusions regarding the mode of inheritance (Climenko, 1920; Bartels, 1941; Martin and Fisher, 1945). Thus, a significantly increased frequency of thyrotoxicosis in relatives of affected patients was reported, but both autosomal recessive and dominant inheritance were suggested. In many of these studies the diagnoses in affected relatives were based wholly on historical information, and therefore, the divergent conclusions are not surprising. More recently, in an effort to provide objective evidence of subclinical predisposition to thyrotoxicosis, family studies have been conducted using laboratory evidence of aberrant thyroid hormone metabolism as a "marker." For example, Ingbar and associates (1956) demonstrated increased hormonal turnover and $I^{131}$ uptake by the thyroid gland in presumably euthyroid close relatives of affected patients. Expansion of these studies in conjunction with measurement of thyroid antibodies and LATS is needed.

Saxena (1965) studied the parents of 12 children with thyrotoxicosis and found thyroid antibodies in one parent in 10 instances. More recently, in a carefully controlled immuno-clinical investigation, Howel Evans and coworkers (1967) studied 74 families in which the probands had thyrotoxicosis. The frequency of thyroid autoantibodies was significantly higher in mothers and sisters than in controls, but only suggestively increased in brothers and fathers (Table 1). In the 13 instances where both parents were

known to be negative, only 4 of 35 sibs were positive, whereas in the 30 families with at least one seropositive parent, 25 of 79 sibs had antibodies ($p<0.05$). The results of this well-controlled study provide evidence for an aggregation of thyroid antibodies in relatives of probands with thyrotoxicosis, and they suggest an inherited predisposition to form these antibodies, provided environmental factors are not responsible for the familial aggregation. However, as the authors state, a control group consisting of relatives of control probands would be useful for further evaluation.

*D. Relationship Between CLT, Primary Hypothyroidism and Thyrotoxicosis*

Family studies demonstrate that there is an increased frequency of thyroid antibodies in relatives of probands with any one of these thyroid diseases. These observations, in conjunction with investigations of the probands themselves which have revealed immunological components common to the 3 disorders, support the suggestion of early workers (e.g., Bartels, 1941) that CLT, primary hypothyroidism and thyrotoxicosis are etiologically interrelated. Many of the recent immunologic family investigations which included clinical evaluation of relatives add further support.

Jayson and co-workers (1967) recently reported a pair of female monozygotic twins, one with thyrotoxicosis and the other with Hashimoto's thyroiditis. The presence of LATS in the serum of both twins was interpreted as an indication of a common immunological defect in the 2 disorders and a possible genetic factor in their etiology. The authors noted that in Harvald's twin series of 41 monozygotic twin probands with thyrotoxicosis, there were in addition to the 12 co-twins with thyrotoxicosis, 6 with "non-toxic goiter." They suggested that some of these 6 subjects may have had Hashimoto's thyroiditis.

Studies of the families of probands with a specific autoimmune thyroid disorder reveal not only an excess of relatives with that disorder, but an excess of other thyroid disorders as well. For example, Howel Evans and his

TABLE 2.—*Clinical Thyroid Disease in Relatives of Probands with Thyrotoxicosis* (from Howel Evans et al., 1967)*

| Disease | Relationship | | | | |
|---|---|---|---|---|---|
| | Mothers | Sisters | Fathers | Brothers | Total |
| *Number evaluated:* | 57 | 101 | 39 | 76 | 273 |
| Number with thyrotoxicosis | 6 | 6 | — | 1 | 13 |
| Number with myxedema | 1 | 2 | 1 | — | 4 |
| Number with non-toxic goiter | 1 | 1 | — | — | 2 |
| Total wtih thyroid disease | 8 | 9 | 1 | 1 | 19 |

* No cases of overt thyrotoxicosis or myxedema were found in 511 controls (320 females, 191 males), but one female was found to have Hashimoto's thyroiditis.

associates (1967) reported that in an investigation of the families of 74 probands with thyrotoxicosis, 4 of 19 relatives with thyroid disease had myxedema (Table 2). In their study of adults with CLT, Doniach, Roitt and Taylor (1965) found clinical thyroid disease in 52 of 194 relatives. Twentyone had goiter with laboratory evidence of active thyroiditis, but 17 had Graves' disease, 12 had goiter with low titer or negative antibodies, and 2 had myxedema.

*1. Familial Predisposition to Thyroid Autoimmunity*

The immuno-clinical family studies suggest that CLT, primary myxedema and thyrotoxicosis are etiologically interrelated. Clinically, there is an apparent excess of each of these thyroid diseases in relatives of probands with any one of them. This suggests that a component of the familial susceptibility to these diseases is non-specific in the sense that it does not predispose to any one of the 3 disorders in particular. In addition, thyroid antibodies occur with increased frequency in relatives irrespective of the clinical form of the thyroid autoimmunity in the proband. It is tempting to speculate that the familial tendency to thyroid disease is at least in part related to the familial tendency to form thyroid autoantibodies. However, even if for the sake of discussion this familial predisposition to thyroid autoimmunity is accepted, other factors must operate to determine the specific form and severity of the thyroid autoimmunity in a given subject, e.g., mild or severe CLT, CLT or thyrotoxicosis. The nature of such factors is unknown, and only a few possibilities will be discussed.

*2. Factors Which May Influence the Form of Disease*

There is some evidence suggesting the existence of inherited factors predisposing an individual to a specific thyroid disease. For example, as discussed above, when the monozygotic twin of a subject with thyrotoxicosis has thyroid disease, he may have another autoimmune thyroid disease such as CLT (Jayson et al., 1967), but he more frequently also has thyrotoxicosis (Harvald and Hauge, 1956). Similarly, relatives of probands with thyrotoxicosis may have myxedema, but it is more usual for them to have thyrotoxicosis (Howel Evans et al., 1967, Table 2). The same is true for probands with myxedema (i.e., affected relatives have myxedema more frequently than thyrotoxicosis (Hall, 1965).

The ability to taste PTC (phenylthiocarbamide), which is inherited as a dominant trait, may play a role in determining which specific thyroid disease will occur in a subject predisposed to thyroid abnormalities. Since a variety of natural and synthetic anti-thyroid compounds share the chemical grouping to which tasters respond, the tasting response has been studied in patients with various thyroid diseases (Harris, Kalmus and Trotter, 1949). There is

a deficiency of tasters among subjects with nodular goiter (Harris, Kalmus and Trotter, 1949; Kitchin et al, 1959), and one report suggests that there may be an excess of tasters among patients with thyrotoxicosis (Kitchin et al., 1959). If this association of PTC tasting with thyrotoxicosis can be confirmed, it would indicate multifactorial inheritance of that disorder and suggest that PTC tasting may be involved in determining the specific thyroid disease which will occur in a patient with a generalized tendency to thyroid abnormalities.

There is some suggestion that inherited factors may affect the degree of the aberrant immunologic response and thence the clinical manifestations. Juvenile patients with CLT seem to differ from adults with that disorder in several respects. For example, they may have a positive family history of thyroid disease more frequently than adult patients (Doniach, Nilsson and Roitt, 1965). A notable feature in the 2 investigations of CLT with onset before age 20 years is the frequency with which either thyroid antibodies or thyroid diseases are found in both parents, i.e. in 13 of the 44 families completely studied (Hall, Saxena and Owen, 1962; Doniach, Nilsson and Roitt, 1965). Doniach, Nilsson and Roitt (1965) suggest that transmission from both parents could account for the early age of onset of overt CLT. It might also account for the fact that children with CLT seem to have a more widespread immunologic disturbance, as expressed by the high frequency of non-organ-specific antibodies such as antinuclear factors. Antibodies of this kind were found in 9 per cent of adult patients and 28 per cent of juvenile patients, but not in any juvenile controls. However, before this plausible suggestion can be fully accepted, further data must be obtained.

*E. Sex and Age Distribution*

Any genetic hypothesis must take into account the fact that the majority of patients with autoimmune thyroid disorders are females. Accordingly, proponents of single factor inheritance suggested that the disorders are partially sex-limited (e.g., Bartels, 1941; Martin and Fisher, 1945). In the past it has generally been assumed that females are more apt to be affected because their thyroid glands are subjected to greater stresses by cyclical endocrine changes associated with puberty, menstruation, pregnancy and the menopause. It cannot be denied that such events do exert an influence on thyroid homeostasis, but the fact that almost all putative autoimmune disorders occur more frequently in females suggests that factors not unique to the thyroid gland may be operating. The question may be asked whether the distorted sex distribution is at least in part more directly dependent upon genetic differences between the two sexes—perhaps due to loci on the X chromosome involved in immunologic homeostasis.

*1. Comparison of Juvenile and Adult Onset CLT*

If such factors as menstruation and pregnancy are primarily responsible for the excess of thyroid disease in females, it should be less marked or absent in pre-pubertal patients (assuming that the juvenile and adult forms are variants of the same disease). Analysis of published data indicates that this is the case for some putative autoimmune disorders such as myasthenia gravis and idiopathic Addison's disease *(vide infra)*, but not for others. The data from 4 of the largest series of patients with juvenile CLT are summarized in Table 3. The ratio of 7 females to 1 male is still notably elevated and may not be significantly different from the ratio in adults which has been variously estimated at from 4:1 to 19:1 (see Masi, Hartmann and Shulman, 1965). Thus, if juvenile and adult CLT are variants of the same disease, post-pubertal changes cannot entirely explain the excess of females with CLT.

*2. Comparison of Juvenile Myxedema and Juvenile CLT*

It is now generally assumed that juvenile non-congenital myxedema and juvenile CLT are variants of the same underlying autoimmune thyroid disease (Nilsson and Doniach, 1964; Winter, Eberlein and Bongiovanni, 1966). Consequently, it is of some interest to compare the sex distribution in the 2 disorders. Winter, Eberlein and Bongiovanni (1966) compared 17 patients with definite "acquired" juvenile hypothyroidism to 33 patients with juvenile CLT. The sex distribution in the thyroiditis group showed the expected excess of females, 5.5 females to 1 male, but in the hypothyroidism group, it was 1:1. In Nilsson and Doniach's (1964) series of 35 patients with juvenile lymphocytic thyroiditis, the overall ratio was 5:1, but of the 3 patients presenting with frank myxedema, 2 were males. If we accept the hypothesis that juvenile CLT and myxedema with onset in childhood are variants of the same autoimmune thyroid disease, these limited findings suggest that CLT is more common in the female, but more severe in the male. If a locus or loci on the X chromosome at least in part determined susceptibility to CLT, the disorder might be expected to occur with increased frequency in females, but be more severe in males (i.e., have an earlier age of onset of myxedema). However, it can be argued that other factors such as infection play a role, and that boys are generally weaker in their defense mechanisms.

TABLE 3.—*Sex Distribution (Females: Males) in Patients with Juvenile CLT*

| Investigators | Age at onset | | Total |
|---|---|---|---|
| | 0-10 | 11-17 | |
| Saxena and Crawford, 1962 | 15:2 | 14:1 | 29:3 |
| Nilsson and Doniach, 1964 | 8:1 | 21:5 | 29:6 |
| Hahn, Hayles and Woolner, 1965 | 14:1 | 10:2 | 24:3 |
| Winter, Eberlein and Bongiovanni, 1966 | * | * | 28:5 |
| Total | 37:4 | 45:8 | 110:17 |

* Data not presented in this fashion

*3. Analysis of Age- and Sex-Specific Incidence Rates*

Burch and Rowell (1963) presented a mathematical analysis of the age- and sex-specific incidence rates and prevalence of Hashimoto's thyroiditis and concluded that a specific predisposing genotype was a necessary, but not a sufficient cause. Phenotypic expression (disease) would thus depend on interaction between this genotype and somatic mutation at a variable number of autosomal and/or X chromosomal loci. Adopting Burnet's (1959) clonal selection hypothesis, Burch and Rowell (1963) proposed that the somatic mutations give rise to pathogenic "forbidden clones" of immunocytes with specificity for the thyroid gland. They tentatively concluded that as many as 4 inherited mutant genes on the X chromosome and 8 somatic mutations on the autosomes were involved in Hashimoto's thyroiditis. Similar theories were propounded for other putative autoimmune disorders including inflammatory polyarthritis (Burch, 1963). The mathematical analysis was subsequently criticized by the Maynard Smiths (1963), and a lively exchange ensued in the columns of the Letters to the Editor of the Lancet. The sophisticated mathematical arguments are difficult to evaluate; however, if accurate age- and sex-specific incidence rates and prevalence could be obtained for a homogeneous disease, this approach would be useful.

*4. Prevalence of Thyroid Antibodies in Older Age Groups*

One virtue of Burch's hypothesis is that it offers an explanation for the very high frequency of thyroid antibodies in "normal" post-menopausal females which approaches 25 to 30 per cent (Doniach and Roitt, 1964; Fialkow et al., 1965a). It has been shown that the occurrence of these antibodies is associated with histologic evidence of focal thyroiditis (Goudie, Anderson and Gray, 1959). If these mild aberrations are part of the spectrum of "autoimmune thyroiditis," hypotheses propounding single-factor inheritance of the predisposition to form thyroid antibodies will be faced with the task of accounting for the "thyroid antibody polymorphism." It seems more likely that such changes reflect either increasing susceptibility to, or accumulation of, random events with aging.

## II. PERNICIOUS ANEMIA AND IDIOPATHIC CHRONIC GASTRITIS

Pernicious anemia (PA) in adults is a chronic disorder characterized histologically by atrophy with a variable degree of chronic inflammation and lymphocytic infiltration in the gastric mucosa. However, most patients with the histologic changes of chronic idiopathic gastritis do not develop frank PA; deficiency of intrinsic factor (IF) is essential for its appearance. IF is necessary for the absorption of vitamin $B_{12}$, and the hematologic and neurologic abnormalities in PA are secondary to malabsorption of this vitamin.

Many investigators believe that autoimmunity is important in the patho-

genesis of idiopathic chronic gastritis and PA. The following observations have been advanced in support of this hypothesis: 1) The chronic inflammatory changes and atrophy in the gastric mucosa resemble those seen in CLT and primary myxedema. 2) A large proportion of patients with PA have circulating gastric antibodies. 3) PA and autoimmune thyroid disease not infrequently coexist in the same patient. For example, as many as 20 per cent of patients with primary myxedema may have impaired absorption of vitamin $B_{12}$ and 10 per cent may have frank PA (Tudhope and Wilson, 1960; 1962). 4) The suggested relationship between PA and thyroid disorders has been strengthened by the demonstration of immunologic correlations in patients and in their families.

Despite these resemblances and associations with other autoimmune disorders, a primary pathogenic role for autoimmunity in chronic gastritis and PA has not been conclusively established. One of the main obstacles has been the failure to reproduce the disease regularly in animals by autoimmunization.

*A. Circulating Antibodies*

Circulating antibodies to at least 2 different organ-specific gastric antigens have been identified—antibodies to IF (Taylor, 1959; Schwartz, 1960) and antibodies to an antigen associated with a "microsomal" component of gastric parietal cells (Irvine et al., 1962; Markson and Moore, 1962). Circulating IF antibodies can be detected in 60 per cent of patients with PA (Ardeman and Chanarin, 1963), and rarely in patients with other disorders. However, even in these latter cases, the presence of IF antibody is usually correlated with some degree of $B_{12}$ malabsorption (Wright et al., 1966; Fisher et al., 1967). This observation and the fact that IF antibodies can be detected in the gastric juice and saliva of patients with PA (Fisher, Rees and Taylor, 1966; Schade et al., 1966; Carmel and Herbert, 1967) have led some investigators to suggest that IF antibody in the gastrointestinal tract is a critical factor in determining which patients will develop PA. This hypothesis awaits firm documentation.

Parietal cell antibodies are found in about 90 per cent of patients with PA (Taylor et al., 1962), in 50 per cent of patients with idiopathic chronic atrophic gastritis who do not have PA (Mackay, 1964) in about a third of patients with clinical thyroid autoimmunity (Irvine et al., 1962; Taylor et al., 1962), and in increased frequency in patients with diabetes mellitus (Moore and Neilson, 1963), and idiopathic Addison's disease (Irvine, Stewart and Scarth, 1967). The frequency of these antibodies in "normal" subjects increases from about 5 per cent in those less than 60 to about 20 per cent in females over 70 years of age (Doniach and Roitt, 1964). However, even in asymptomatic subjects the presence of parietal cell antibodies is closely corre-

lated with the occurrence of histologic evidence of gastritis, and these antibodies are not found in subjects with proven normal gastric histology (Adams et al., 1964; Wright et al., 1966; Fisher et al., 1967). The association with gastritis is only qualitative, and there is no evidence that these antibodies are directly involved in the pathogenesis of gastritis. It is of some interest that the correlation is not solely with chronic gastritis, but also with the disease in which the gastric changes occur. For example, the sera of patients who have undergone partial gastrectomy for peptic ulcer disease are generally free of gastric antibodies, even though gastric biopsy may reveal histologic changes indistinguishable from those seen in chronic idiopathic gastritis or PA (Wright et al., 1966; Fisher et al., 1967). This finding is not consistent with hypotheses relating the development of gastric autoantibodies simply to release of gastric antigen.

### *B. Genetic Studies*

Atrophic gastritis with or without PA is not known to occur spontaneously in animals nor has it been possible experimentally to reproduce it regularly. Consequently, genetic studies have largely been confined to observations in man.

Early reports suggested a familial predisposition to PA (e.g., Stamos, 1940; Wilkinson, 1949; Mosbech, 1953), but later investigations in which asymptomatic relatives were evaluated for either subclinical aberrant $B_{12}$ absorption or immunological markers have been more informative.

#### *1. Vitamin $B_{12}$ Absorption in Families*

Measurement of $B_{12}$ absorption not only provides a more secure basis for establishing the diagnosis of PA in probands, but it may also be a means of detecting pre-clinical or latent disease in asymptomatic relatives (Callender and Denborough, 1957; McIntyre et al., 1959; McIntyre, 1968). In an extension of an earlier study, McIntyre (1968) found some regression of ability to absorb $B_{12}$ with age in relatives of patients with PA, but she was unable to confirm the greatly increased prevalence of $B_{12}$ malabsorption originally reported in these relatives (McIntyre et al., 1959). This discrepancy appeared to be due to incomplete urine collection by the relatives in the initial study.

#### *2. Parietal Cell Antibodies in Families*

TeVelde and associates (1964) evaluated direct and allied relatives of 21 adult patients with PA ("allied" relatives were the probands' 21 spouses and 57 spouses of the probands' direct relatives). The sex distributions in the first degree relatives and allied relatives were similar, but parietal cell antibodies were found in the sera of 31 of 98 first degree relatives (Table 4) as opposed to only 3 of 78 allied relatives. This study has been expanded to include 529 direct relatives of 60 probands of which 128 were seropositive (in-

TABLE 4.—*Parietal Cell Antibodies in First Degree Relatives of Adult Patients with PA*

| Investigators | Relationship<br>Number tested/Number positive (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Mothers | Fathers | Sisters | Brothers | Daughters | Sons | Total |
| teVelde et al., 1964 | 2/3 | 0/2 | 8/21(38%) | 5/25(20%) | 11/27(41%) | 5/20(25%) | 31/98(32%) |
| Doniach, Roitt and Taylor, 1965 | 1/4 | 0/3 | 18/35(51%) | 7/21(33%) | 9/27(33%) | 2/16(13%) | 37/106(35%) |

cluding 94 of 333 first degree relatives) compared to 5 of 114 allied relatives (te Velde, 1967). This finding indicates a familial predisposition to the formation of these antibodies, and comparison of relatives with spouses suggests that inherited factors may be responsible. Further analysis of the complete pedigrees revealed the following observations which are relevant to possible modes of inheritance: female relatives were seropositive twice as often as male relatives; the antibodies were often present in 2 or more generations; the offspring of seronegative subjects were usually seronegative, whereas their seropositive sibs often had seropositive children; and in the 13 sibships with at least one positive parent, 19 of 44 sibs had gastric antibodies. The authors suggested that autosomal dominant inheritance with partial sex limitation was responsible for the appearance of parietal cell antibodies. They further proposed that the same gene with decreased penetrance and pleiotropic effects was responsible for atrophic gastritis and PA.

TeVelde and co-workers (1964) further demonstrated that most of the parietal cell antibody-positive asymptomatic relatives had histologic evidence of gastritis and many had severe $B_{12}$ malabsorption. It was suggested that antibody determinations should be used to screen adult relatives of patients with PA and that when positive results are obtained, $B_{12}$ absorption studies should follow. In this way, sub-clinical or latent PA could be discovered and treated before clinical illness develops. Thus, detection of pre-clinical PA would seem to provide an excellent opportunity for preventive medicine.

Also summarized in Table 4 are the data of Doniach, Roitt and Taylor (1965) who tested a larger number of first degree relatives of adult patients with PA and found parietal cell antibodies in 35 per cent versus 7 per cent in controls. Irvine and co-workers (1965) also studied asymptomatic relatives and suggested that the predisposition to gastric autoimmunity is inherited as an autosomal dominant characteristic with expression depending upon age and sex. Ardeman and associates (1966) extensively studied a family in which 6 of 9 adult siblings had PA and all 9 had parietal cell antibodies. The latter were also detected in 10 of 17 of the siblings' offspring.

These studies have demonstrated a familial aggregation of parietal cell antibodies, and while there may be some question about the mode of inheritance, comparison of relatives with spouses (TeVelde et al., 1964) suggests that heritable factors and not conventionally acquired viruses or similar agents are involved. Accordingly, it is pertinent to determine whether this familial predisposition to form gastric autoantibodies is related to the familial tendency to produce other autoantibodies.

*3. Overlap Between Gastric and Thyroid Autoimmunity in Families*

Doniach, Roitt and Taylor (1965) found CLT, primary myxedema or thyrotoxicosis in about one third of relatives of adult probands with PA. In

addition, thyroid antibodies were detected in 50 per cent of relatives compared to 15 per cent of age and sex matched controls ($p<0.01$). Approaching the question from the other side, these investigators also found a significantly increased frequency of parietal cell antibodies in relatives of probands with adult or juvenile onset CLT (Doniach, Roitt and Taylor, 1965; Doniach, Nilsson and Roitt, 1965). In these studies, the tendency to form gastric antibodies could not be dissociated from the tendency to form thyroid antibodies (Doniach and Roitt, 1966).

The close relationship between the familial tendency to thyroid and gastric autoimmunity is further illustrated by observations in twins. Of 5 monozygotic twin pairs ascertained and reported because of concordance for autoimmune thyroid disease, both members of 4 twin pairs had gastric parietal cell antibodies. The fifth pair of twins lacked these antibodies (Hassan et al., 1966; Jayson et al., 1967).

These investigations indicate that common factors are at least in part responsible for the familial predisposition to both thyroid and gastric autoimmunity. Later in this review, data will be presented suggesting that the same may be true for adrenal and parathyroid autoimmunity. The data seem compatible with an inherited predisposition to a multiplicity of organ-specific autoimmune reactions. If this is true, other factors must operate in the predisposed subject to determine not only the form and severity of the disease in any one organ, but also to select which organ will be primarily involved, e.g., stomach, thyroid and/or adrenal. Thus, factors discussed in the section on thyroid diseases which may influence the form of the disease in that organ (e.g., CLT or myxedema) may also function in selecting the thyroid as a site of involvement. Some of the many possible factors which could orient the immunologic disease to the stomach or potentiate it can be considered briefly.

### C. *Factors Determining Type and Severity of Disease*

*1. Degree of immunological disturbance.* Doniach, Roitt and Taylor (1965) noted that when PA and CLT were present concurrently in the same subject, the female relatives had a significantly higher frequency of both gastric and thyroid antibodies than did the relatives of probands with either PA or CLT alone. They suggested that a greater immunological disturbance is present in patients having both diseases, and that is finds expression in the more frequent development of antibodies in their relatives.

*2. IF antibodies.* It can be argued that PA is much more likely to develop in a patient with idiopathic chronic gastritis who has IF antibodies in his gastrointestinal tract. If so, the production of these antibodies could depend upon a greater immunological disturbance than is required for parietal cell antibodies, or upon other, possibly heritable, factors.

*3. Genes which cause IF deficiency.* When PA occurs in infancy, the disease differs from the adult form in that gastritis and circulating antibodies are absent (Doniach, Roitt and Taylor, 1965). It is likely that the infant PA represents an autosomal recessive defect in IF formation or selective malabsorption of $B_{12}$ (Waters and Murphy, 1963). Some adults with chronic gastritis may be more prone to PA because they are heterozygous for one of these genes (Waters and Murphy, 1963).

*4. Iron deficiency anemia.* Parietal cell antibodies are relatively common in patients with chronic iron deficiency anemia (Markson and Moore, 1962), especially if they have associated chronic atrophic gastritis (Dagg et al., 1964; Coghill et al., 1965). It is not known whether atrophic gastritis is the cause or the result of iron deficiency anemia (e.g., see Coghill et al., 1965); therefore, it is conceivable that severe iron deficiency may be a factor which either orients the immunologic disease towards the stomach or potentiates it. Limited observations suggest that gastric autoimmunity is more common in relatives of patients with iron deficiency anemia and gastritis who have detectable abnormalities in $B_{12}$ absorption than in relatives of similar patients with normal $B_{12}$ absorption (McFadyen et al., 1966).

*5. Blood group associations.* The relative risk of PA is 20 per cent higher in subjects with blood group A than in subjects with other blood groups (see McConnell, 1966, for discussion and references). Other gastrointestinal disorders such as duodenal ulcer are also seen more frequently in subjects with particular ABO blood groups (see McConnell), but thyrotoxicosis, which displays clinical and serological overlap with PA, apparently has no such association (Jackson, Boyle and Harden, 1965). This suggests that the ABO blood group effect is distinct from the familial predisposition to thyroid and gastric autoimmunity. It may be one heritable factor in determining which patients with this predisposition will get PA. In any event, the association of PA with blood group A indicates with certainty that genetic factors are important in PA and that the inheritance of PA is not determined by a single gene, but is multifactorial. Another of these multiple factors might be the genetic predisposition to form autoantibodies.

## III. IDIOPATHIC ADDISON'S DISEASE (ADRENAL FAILURE)

Primary adrenocortical insufficiency in man (Addison's disease) is the result of progressive destruction of the adrenal cortex and is characterized by pigmentation of the skin, weight loss, weakness, hypotension, and gastro-intestinal disturbances. In previous years, approximately 70 per cent of cases were associated with adrenal tuberculosis, but now the most frequent pathologic finding is idiopathic adrenal atrophy with lymphocytic and plasma cell infiltration.

It has been suggested that adrenal lesions in the idiopathic disease are the result of organ-specific autoimmunity (see Lancet, 1967), and there are some reports of the production of adrenalitis and adrenal antibodies by the injection of adrenal tissue in experimental animals (Colover and Glynn, 1958; Steiner et al., 1960; Witebsky and Milgrom, 1962). However, as in the case of PA, much of the evidence that autoimmunity is involved in the pathogenesis of the human disease is indirect and rests on the following observations: 1) The histologic changes in the adrenal gland resemble those seen in myxedema and PA. 2) Addison's disease is not infrequently accompanied by coexisting clinical and/or serologic evidence of thyroid and gastric autoimmunity (e.g., Irvine, 1963). 3) There is a high frequency of circulating adrenal antibodies in affected patients.

*A. Circulating Antibodies*

Organ-specific circulating adrenal antibodies, which were first described by Anderson and co-workers (1957), are specific for a cytoplasmic antigen in cells of all 3 zones of the adrenal cortex (Blizzard and Kyle, 1963). The antibodies can be detected in over 50 per cent of patients with idiopathic Addison's disease (see Lancet, 1967), but are almost never found in "normal" subjects. In marked contrast, these antibodies are only rarely detected in tuberculous, fungal or neoplastic adrenal destruction. In 4 recent studies, only 4 of 74 such cases were sero-positive (Goudie et al., 1966; Nerup et al., 1966; Blizzard, Chee and Davis, 1967; Irvine, Stewart and Scarth, 1967). This suggests that the antibodies do not arise simply in response to release of adrenal antigens, and it is more likely that they are formed predominantly in patients predisposed to autoimmune reactions. Support for this hypothesis comes from observations of a clinical and serological overlap with other autoimmune diseases and from limited family studies.

A notable difference between adrenal antibodies and those with thyroid or gastric specificity is that the former are almost never demonstrable in asymptomatic "normal" subjects and are only very rarely found in patients with thyroid or gastric autoimmunity. However, serological overlap can be demonstrated by studying the frequency of gastric and thyroid antibodies in patients with idiopathic Addison's disease (Irvine, 1963). In order to minimize selection and ascertainment bias, this frequency has been compared to the frequency found in patients with tuberculous Addison's disease. A significantly higher prevalence of thyroid and gastric antibodies was found among the patients with idiopathic disease (Goudie et al., 1966; Nerup et al., 1966; Blizzard, Chee and Davis, 1967; Irvine, Stewart and Scarth, 1967). For example, in one study, parietal cell antibodies were found in two-fifths of 35 patients (3 of whom had PA) in the idiopathic group and in only 1 of 16

in the tuberculous group; gastric malfunction and gastritis were associated with the presence of the antibodies (Irvine, Stewart and Scarth, 1967). These and other studies (see Carpenter et al., 1964 and Lancet, 1967, for references) suggest that patients with idiopathic Addison's disease have a more generalized predisposition to autoimmunity. By inference from observations in gastric and thyroid diseases, this predisposition is likely to have a familial, possibly genetic basis.

*B. Genetic Studies*

Familial occurrence of uncomplicated idiopathic Addison's disease has only rarely been recorded (Bamatter et al., 1966), and only one immunologic family study has been reported (Wuepper and Fudenberg, 1966). Wuepper and Fudenberg (1966) looked for a variety of antibodies in 70 relatives of 12 probands. At least one first degree relative in each family had immunologic aberrations, although none had adrenal antibodies. These data, in conjunction with the observations of clinical and serological evidence of gastric, thyroid and adrenal autoimmunity in patients with idiopathic adrenal failure, suggest that the familial predisposition to autoimmunity may also manifest in adrenal disease. It is therefore appropriate to consider which factors may be involved in selecting the adrenal gland as the target organ.

The facts that adrenal antibodies are not seen in patients with thyroid or gastric disease, that familial idiopathic Addison's disease is rare, and that adrenal antibodies are not detected in relatives of patients with Addison's disease, suggest that in a subject with the hypothetical genetic predisposition to organ-specific autoimmunity, the factors involved in selecting the adrenal gland are to a large part not genetically determined. However, the syndrome of idiopathic Addison's disease with hypoparathyroidism may be an exception.

*C. Addison's Disease with Idiopathic Hypoparathyroidism*

Patients with this rare disorder have the stigmata of Addison's disease as well as hypocalcemia and tetany. Evidence that hypoparathyroidism may have an immunological basis includes: 1) the finding of circulating parathyroid antibodies in 38 per cent of affected patients versus 6 per cent in controls (Blizzard, Chee and Davis, 1966); 2) the clinical and serologic overlap with thyroid, gastric and adrenal autoimmunity (Blizzard, Chee and Davis, 1966); and 3) the experimental induction of isoimmune hypoparathyroidism (Lupulescu et al., 1965). Hypoparathyroidism is unusual in adult patients with Addison's disease but is not infrequent in the rarer form with onset in childhood (Blizzard, Chee and Davis, 1967). In these cases, mucocutaneous infection with the fungus, *Candida albicans* (moniliasis) is also often found. This syndrome has a familial occurrence and may be found in partial forms

Whitaker et al, 1956; Wuepper and Fudenberg, 1967). Wuepper and Fudenberg (1967) found autoantibodies in 17 of 28 relatives of a patient with this disorder. This finding, as well as the increased frequency of gastric and thyroid autoimmunity in patients with this rare syndrome, suggests that it is part of the spectrum of diseases associated with familial predisposition to autoimmunity.

The familial distribution and occurrence of consanguinity in this syndrome (see Motulsky and Epstein, 1967) suggests that a major factor in selecting the adrenal and parathyroid glands for involvement is inherited as an autosomal recessive character. One of the more provocative possibilities is that these patients have a genetic defect which predisposes them to moniliasis, and subsequently an absorption product of the fungus acting directly as a toxin or indirectly as a cross-reacting antigen might be responsible for the parathyroid and/or adrenal disease (Shannon, Johnson and Austen, 1966). In any event, familial Addison's disease with hypoparathyroidism is rare in adults, but is not infrequent in children. This suggests that in patients with the familial predisposition to autoimmunity, acquired factors are more important in determining that the adrenal gland will be involved in the more common cases with adult onset, and no parathyroid disease, while genetic factors are important in the childhood form.

*D. Sex Distribution*

In adults, females may be affected twice as frequently with idiopathic Addison's disease as males (Irvine, Stewart and Scarth, 1967; Lancet, 1967). The limited data available suggest a much different ditribution in children. In the 47 cases of Addison's disease with juvenile onset reported by Blizzard, Chee and Davis (1967), the sex distribution of 17 females to 30 males was significantly different from the 37 to 25 distribution found among adult cases in the same series. Thus, the sex distribution among young patients with this disease resembled primary myxedema and is notably different from the distribution in CLT. As in the case of CLT-myxedema, one may speculate that if a locus or loci on the X chromosome at least in part determines adrenalitis, the disorder might be more frequent in females, but more severe in males, e.g., have an earlier age of onset. A comparative study of the prevalence of familial autoimmunity in juvenile and adult patients would be of interest.

## IV. DIABETES MELLITUS

A discussion of thyroid-gastric-adrenal-parathyroid autoimmunity would not be complete without some remarks about diabetes mellitus. There is no direct evidence to indicate that autoimmunity is important in the pathogenesis of this disease, but some cases do show immunological associations com-

mon to some of the disorders discussed above. Thus, diabetes is not infrequently found in patients with PA (Arapakis et al., 1963) and Schmidt's syndrome (hypothyroidism concurrent with Addison's disease) (Carpenter et al., 1964). Carpenter and associates (1964) reviewed the literature on Schmidt's syndrome and added 15 cases of their own with Addison's disease and thyroid dysfunction. Ten of these also had diabetes mellitus, and the authors suggested a polyendocrinopathy with an immunological basis to explain the association. An increased prevalence of diabetes mellitus has also been observed in thyrotoxicosis (Kreines, Jett and Knowles, 1965) and in 17 of 172 patients with idiopathic Addison's disease (combined data from Irvine, Stewart and Scarth, 1967, and Blizzard, Chee and Davis, 1967). In addition, a relatively high frequency of other autoantibodies such as those with parietal cell and thyroid specificity has been found in patients with diabetes mellitus (Moore and Neilson, 1963; Irvine and Davies, 1963; Landing et al., 1963). More recently, an impressive frequency of intrinsic factor (IF) antibodies has also been reported (Ungar et al, 1967). Diabetes mellitus is clearly a heritable phenotype, but even after years of intensive study, the mode of inheritance remains obscure; both single factor and multifactorial hypotheses have been advanced (see Motulsky and Epstein, 1967, for discussion). It is conceivable that a genetic predisposition to autoimmunity could be among the multigenic factors, or that if diabetes were determined by a single major genotype, autoimmunity might alter phenotypic expression. Thus far, no systematic, well-controlled, immunologically-oriented studies of families of patients with diabetes mellitus have been reported.

## V. MYASTHENIA GRAVIS

Myasthenia gravis, a disorder characterized by weakness and rapid fatigability of voluntary muscles, results from impaired transmission of nerve impulses across the myoneural junction. Thymic hyperplasia is found in 70 per cent and thymoma in 10 per cent of affected patients (Castleman, 1966); focal aggregations of lymphocytes in muscles are also commonly seen. In 1960 Simpson, as well as Nastuk, Plescia and Osserman, suggested that autoimmunity plays an underlying role in myasthenia gravis. A brief summary of the evidence favoring this view is given here.

Circulating antibodies to skeletal muscle, first reported by Strauss et al., (1960), can be detected in 30 to 50 per cent of affected patients, but they are especially prevalent in patients who are severely ill or who have a thymoma (Weiner and Osserman, 1966). They are almost never detected in subjects who are asymptomatic or who have disorders other than thymoma or myasthenia gravis (e.g. Strauss et al., 1966). The sera of most patients with antimuscle antibodies display concurrent reactivity with thymic "epithelial" cells

*in vitro* (van der Geld, Feltkamp and Oosterhuis, 1964). However, there is no evidence to suggest a pathogenic role for these circulating antibodies.

Goldstein and Whittingham (1966) immunized guinea pigs with heterologous thymus or muscle extracts in complete Freund's adjuvant; a "myasthenic" neuromuscular block, collections of lymphocytes in the thymic medulla and circulating muscle and thymus antibodies were observed in some animals. The authors suggest that an autoimmune reaction in the thymus in patients with myasthenia gravis liberates a humoral neuromuscular inhibitor substance which is responsible for the clinical symptoms.

A relatively high frequency of thyroid and gastric antibodies is found among patients with myasthenia gravis (van der Geld et al., 1963; Simpson, 1964; Feltkamp, 1966). In addition, in contrast to adult-onset CLT and PA, patients with myasthenia gravis have a notably elevated frequency of non-organ-specific antibodies such as antinuclear factors (White and Marshall, 1962; van der Geld et al., 1963; Djanian, Beutner, and Witebsky, 1964; Feltkamp, 1966). Thus, patients with myasthenia gravis, like those with Sjogren's syndrome (Bunim, 1961) and juvenile CLT (Doniach, Nilsson and Roitt, 1965) have features of both organ-specific and non-organ-specific autoimmunity.

Familial occurrence of adult-onset myasthenia gravis has only rarely been recorded (see Pratt, 1967), but when the disorder occurs in infancy or childhood, as many as 25 per cent of patients may have an affected relative (Osserman, 1958). The sex distribution, considering cases of all ages, is approximately 2 females to one male, but in juvenile patients and in those cases with onset after 50 years of age, the sex distribution is nearly equal (Osserman, 1958). Thus, there may be a fundamental difference between the juvenile and adult forms; alternatively, such factors as menstruation and pregnancy may be responsible for the overall excess of females. Some investigators have noted an exacerbation of symptoms in females during menstruation (Keynes, 1954; Osserman, 1958).

In an immuno-clinical study, Wright and Kerr (1967) evaluated 106 close relatives and 20 spouses of 32 probands. Muscle antibodies were found in only one of the relatives, but in some families there was a notable increase in the frequency of thyroid and gastric antibodies and antinuclear factors in first degree relatives. However, thyroid antibodies also occurred slightly more frequently in spouses than in matched controls. Before any firm conclusions can be drawn, these studies need to be extended and should include comparison between the families of probands with juvenile and adult onset.

## VI. AUTOIMMUNE HEMOLYTIC ANEMIA

The occurrence of autoagglutinins in patients with acquired hemolytic

anemia was reported 60 years ago by Chauffard and Troisier (1908) and Widal, Abrami and Brule (1908). Thirty years later Dameshek and Schwartz (1938) emphasized the potential importance of these findings and called attention to the similarity of acquired hemolytic anemia in man to experimental hemolytic disease in guinea pigs produced by injection of anti-guinea pig erythrocyte serum.

*A. Autoimmune Hemolytic Anemia in Man*

Circulating autoantibodies to erythrocytes can be demonstrated in most cases of "acquired" hemolytic anemia in man. Occasionally these antibodies directly agglutinate or hemolyze red cells *in vitro,* but most often they only coat (sensitize) them. These sensitizing antibodies can be detected by the antiglobulin test (Coombs test).

The antibody-positive hemolytic anemias may be divided into 2 clinical groups: primary (or idiopathic) in which no underlying disease is known to be present, and secondary (or "symptomatic"). The secondary hemolytic anemias are most frequently found in diseases associated with immunologic aberrations (e.g., systematic lupus erythematosus), lymphoid neoplasia, or infections (e.g., Mycoplasma pneumonia). The cases can also be classified into 2 types depending upon the immunoglobulin class and the thermal range (Dacie, 1962) in which the antibodies are most reactive with erythrocytes. Warm-type antibodies are nearly always γG globulins which are optimally active at body temperature and have specificities related to the Rh and LW antigens. (Weiner et al., 1953; Weiner, Gordon and Gallop, 1953; Weiner and Vos, 1963; Celano and Levine, 1967). Cold-type antibodies are γM macroglobulins which react optimally at 4 to 10° C. and usually have I or i specificity (Wiener et al., 1956; Weiner, Shinton and Gray, 1960; Jenkins et al., 1960; Marsh, 1961; Calvo et al., 1965; Troxel, Innella and Cohen, 1966).

Examples of acquired autoimmune hemolytic anemia in more than one member of the same family are rare, and only 6 such kinships are known to the author (Kissmeyer-Nielsen, Bent-Hansen and Kieler, 1952; Vajda et al., 1960; Fialkow, Fudenberg and Epstein, 1964; Dobbs, 1965; Cordova et al., 1966; Shapiro, 1967). Since considerable bias was involved in the ascertainment and reporting of these families, no firm conclusions can be made about familial predisposition until a well-defined, large series of unselected patients is studied.

The pedigree of a proband who had thyrotoxicosis and carditis in addition to autoimmune hemolytic anemia is illustrated in Figure 1. Thyroid antibodies and/or antinuclear (ANF) and rheumatoid factors were detected in several asymptomatic relatives (Fialkow, Fudenberg and Epstein, 1964). Subsequent to the original report, a sister (II-5) and a niece (III-3) devel-

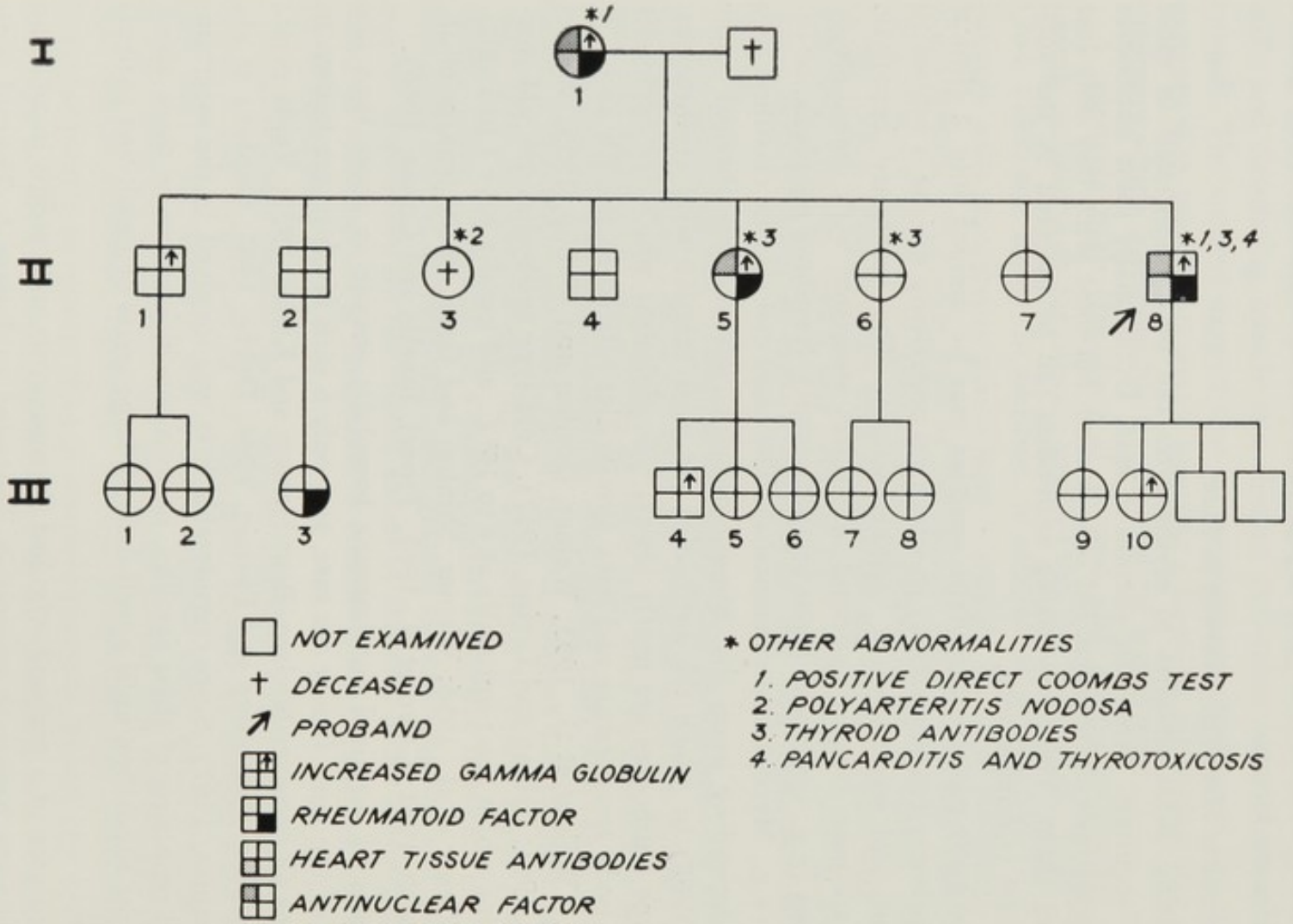



FIG. 1—Pedigree of patient with autoimmune hemolytic anemia, carditis, and thyrotoxicosis (reproduced from Fialkow, Fudenberg and Epstein, 1964, with permission of the publisher).

oped CLT and ulcerative colitis respectively (Fudenberg, 1967). The findings in such families suggest an overlap between organ-specific and non-organ-specific autoimmunity. As discussed in the preceding section, further support for this relationship comes from observation of both localized and systemic autoimmune reactions in patients with diseases like Sjogren's syndrome, juvenile CLT, and myasthenia gravis. Moreover, ANF is twice as prevalent in first degree relatives of adult patients with CLT or PA as in age and sex-matched controls (Doniach, Roitt and Taylor, 1965). These observations suggest that to some extent organ-specific and non-organ-specific forms of autoimmunity are interrelated, perhaps through an inherited tendency to aberrant immunologic reactions.

*B. Autoimmune Hemolytic Anemia in NZB/Bl Mice*

Autoimmune hemolytic anemia has not been induced experimentally, but in 1959, Bielschowsky, Helyer and Howie reported that members of one of their local mouse strains, NZB/Bl, consistently developed that disorder. The potential importance of these animals was recognized immediately, and a large body of data has been accumulated. Only a brief summary can be given here; the reader is referred to recent publications for more details (e.g., Burnet, 1966; Howie and Helyer, 1966; Holmes and Burnet, 1966a).

The outstanding feature of the immunological disease in NZB mice is autoimmune hemolytic anemia which develops from age 4-6 months and is characterized by a positive antiglobulin (Coombs) test (Holmes and Burnet, 1963; Helyer and Howie, 1963a). The anemia closely resembles the warm antibody type of acquired hemolytic anemia in man. Circulating ANF can also be detected (Norins and Holmes, 1964) and kidney disease which resembles chronic membranous glomerulonephritis or lupus nephritis is an important cause of death (Helyer and Howie, 1963a; Mellors, 1965; Hicks and Burnet, 1966). Other mice may die with lymphoid tumors (Mellors, 1966). Thymic lesions are also present in these mice, as they are in some patients with autoimmune disorders, and it has been postulated that the thymus plays an important primary role in autoimmunity (Burnet and Holmes, 1964).

Genetic studies have been mainly concerned with variations in onset, as judged by conversion to a positive antiglobulin test, and with the patterns of renal disease in $F_1$ hybrids. Holmes and Burnet (1964) found that, in $F_1$ hybrids of NZB x $C_3H$, the time of conversion to a positive antiglobulin test was greatly delayed compared to the NZB parents ($C_3H$ parents did not develop positive tests). The results were similar with both NZB female x $C_3H$ male and $C_3H$ female x NZB male matings. Back crosses to NZB gave an intermediate and irregular distribution of ages of conversion. The results were not what would be expected if a single locus determined the time of onset of

the antiglobulin conversion, and it was concluded that this autoimmune manifestation was under multigenic control. Similar findings were obtained with AKR x NZB $F_1$ and back crosses and with other $F_1$ hybrids (Burnet and Holmes, 1965). Sex influences the time of conversion; males convert earlier in NZB mice, and females earlier in $F_1$ hybrids of NZB with $C_3H$, AKR, NZW and $T_6$. However, no significant sex differences were observed in $C_{57}Bl$ $F_1$ hybrids. Thus it appears that while the $F_1$ hybrid may inherit the "autoimmune" factors from his NZB parent, the effects of these factors are modified to some extent by the genetic contribution of the non-autoimmune parent. This assumption is supported by the observations of Howie and Helyer (1965) in crosses of NZB to 7 different local inbred strains. Variations in the frequency, severity and age of onset of hemolytic disease between the 7 classes of $F_1$ hybrids were significant.

The renal lesions in $F_1$ hybrids were first described by Helyer and Howie (1963b), who noted that the $F_1$ hybrids NZB x NZW and NZB x NZY were even more susceptible to kidney disease than NZB parents. The NZB x NZW $F_1$ hybrids developed a disorder after 6 months of age which closely resembled lupus nephritis in man. The 7 classes of $F_1$ hybrids produced by crossing the NZB with 7 local strains differed markedly in the time of onset and death from kidney disease (Howie and Helyer, 1965). Thus, genetic contributions from the nonautoimmune parent also have an effect on the pattern of expression of renal disease.

Burnet and his colleagues have also studied renal disease in $F_1$ and back crosses (Hicks and Burnet, 1966; Holmes and Burnet, 1966b); their results have been recently summarized (Burnet, 1966). They confirmed Helyer and Howie's observation (1963b) that a lethal renal disease occurred in NZB x NZW $F_1$ hybrids. Since renal lesions in old NZW mice were minor, it was concluded that genetic factors in parent strains cooperate to produce the much more severe lesions in their offspring. In order to gain further information, the life histories of animals produced from backcrosses of the NZB x NZW $F_1$ hybrids to both NZB and NZW parents were studied, and the results were not consistent with a single gene difference (Burnet, 1966). However, the results of crossing NZB with $C_3H$ and AKR mice were compatible with single factor inheritance of a condition which was further dependent on a number of genetic and physiological characters for its expression (Burnet, 1966; Holmes and Burnet, 1966b). There is insufficient data for evaluating the extent to which the renal disease is influenced by the factors controlling the antiglobulin reaction.

Burnet (1966) suggests that the pathogenic agent in the murine autoimmune hemolytic anemia is a forbidden clone of immunocytes. Following the suggestion of Burch (1963), mutations, both germinal and somatic, are pro-

visionally postulated by Burnet (1966) to occur in the following sequence: (a) a germinal mutation incorporated into the NZB genome, which allows (b) a common somatic mutation to occur, endowing the cell with resistance to the homeostatic controls that ordinarily ensure elimination or inactivation of autoreactive immunocytes, and (c) a second sequential mutation (or mutations) in cells already a b to give the full pathogenic activity a b c. Since sex markedly influences the time of conversion of the antiglobulin reaction, some of the genetic components are presumed to be on the X chromosome (Burnet and Holmes, 1965).

Other investigators have suggested that the findings in the NZB mice might be explained by a vertically transmitted virus. Virus-like particles have been observed in cells of conventional (Mellors and Huang, 1966) and germ-free (East et al., 1967) NZB animals with positive antiglobulin tests. This observation, together with the finding of similar particles in the thymus of a conventional NZB embryo, suggested to East and her co-workers (1967) that a virus was passed vertically via the germ-cells or placenta.

Recently, Mellors and Huang (1917) reported the production of hemolytic and renal disease in Swiss mice by neonatal intraperitoneal inoculation of cell-free filtrates prepared from the spleens of old NZB/Bl mice. Virus-like particles were demonstrated in one such Swiss mouse. Mice inoculated at age 6 weeks did not display hematologic or renal manifestations. The authors suggest that the clinical manifestations in NZB animals may require viral infection of newborn or infant mice and persistent, possibly tolerant, infection of adult mice.

In any event, the patterns of disease in $F_1$ hybrids and backcrosses do not suggest simple direct vertical transmission of a single factor. Moreover, the variations in time of onset and patterns of disease observed in the offspring when NZB animals are mated with mice from other strains suggest that genetic contributions from the nonautoimmune parent have an important effect on disease expression. Thus, even assuming that viruses play a role in the initiation, expression or propagation of autoimmune disease in NZB mice and their offspring, genetic factors are also likely to be important. For example, the animal's genotype could affect 1) whether or not he will be susceptible to the virus; 2) if he is, which organs the virus will attack; and/or 3) what the pattern of the resulting tissue response will be.

## VII. AUTOIMMUNITY AND CHROMOSOMAL ABERRATIONS

In recent years, a number of studies have indicated a relationship between thyroid autoimmunity and some congenital chromosomal aberrations such as those seen in patients with Down's syndrome and gonadal dysgenesis. This topic has been reviewed recently (Fialkow, 1966; Fialkow and Uchida, 1966; Vallotton and Forbes, 1967); and only a brief account will be given here.

*A. Down's Syndrome*

*1. Probands.* The first case of Down's syndrome with Hashimoto's thyroiditis was recorded by Benda in 1946, and an association with thyroid antibodies was first reported by Mellon, Pay and Green (1963). The data in this and 7 later reports are summarized in Table 5. With one exception, the results show a notably higher frequency of thyroid antibodies in patients with Down's syndrome than in controls.

The basis for this relationship must be either that chromosomal aberrations predispose to thyroid antibody formation or that a factor related to the antibodies predisposes to chromosomal errors. In the latter case, the antibodies or related factors would be present in the parent. This was adopted as a working hypothesis (Fialkow, 1964), and an investigation of the parents of children with Down's syndrome was instituted.

*2. Parents.* An initial approach was to evaluate prospectively parents of patients with Down's syndrome for clinical thyroid disease (Fialkow et al., 1965b). Among the 9 affected out of 44 mothers of children with trisomy-21 syndrome, 3 had Hashimoto's thyroiditis, 1 had thyrotoxicosis, and 5 had nontoxic goiters (Fialkow, 1966). In contrast, 3 of 44 age-matched mothers of control children had clinical thyroid disease (one with primary myxedema and 2 with goiters).

Subsequently, thyroid antibodies were measured in families of children with Down's syndrome in Seattle and Winnipeg (Fialkow et al., 1965a), and this study now includes 242 families (Fialkow and Uchida, 1966; Fialkow, 1967a). Fathers did not have increased prevalence of thyroid anti-

TABLE 5. — *Thyroid Antibodies in Patients with Down's Syndrome and Controls*

| Investigators | Down's syndrome | | Controls | | |
|---|---|---|---|---|---|
| | Number tested | Number positive (%) | Number tested | Number positive (%) | P* |
| Mellon, Pay and Green, 1963. | 35 | 7(20%) | 35 | 0 | $< 0.05$ |
| Burgio et al., 1965, 1966. | 65 | 21(32%) | 101 | 9(9%) | $< 0.001$ |
| Saxena and Pryles, 1965. | 50 | 14(28%) | 30 | 2(7%) | $< 0.05$ |
| Richards et al., 1965. | 174 | 8( 5%) | 90 | 0 | $< 0.1$ |
| Fialkow, 1966; Fialkow and Uchida, 1966, 1967a. | 126 | 32(25%) | 126 | 4(3%) | $< 0.001$ |
| Wren et al., 1967. | 44 | 7(16%) | 60 | 6(10%) | $< 0.6$ |
| Engel, 1967. | 37 | 16(43%) | None reported | | — |
| Engelberth et al., 1967. | 35 | 16(46%) | 58 | 6(10%) | $< 0.001$ |

* The $\chi^2$ used to obtain the P value was calculated using Yates' correction for continuity.

bodies, but 29 per cent of the 242 mothers of affected children were seropositive, compared with 16 per cent of an equal number of age matched control women ($p<0.005$) (Table 6). Similar results have been reported by Burgio et al. (1965, 1966) and Dallaire and Flynn (1967) and Engelberth et al. (1967) (Table 6). In Dallaire and Flynn's study, the occurrence of antibodies in mothers of children with Down's syndrome was twice as prevalent as that seen in mothers matched for age and parity who had children with non-specific multiple congenital anomalies. The suggested (but not statistically significant) correlation between seropositivity and parity in both the mothers of children with Down's syndrome and control mothers reported by Dallaire and Flynn (1967) requires further investigation. Wren et al. (1967) also found an apparent increase of significant clinical thyroid disease in mothers of children with Down's syndrome, but were nonetheless unable to confirm an increased prevalence of thyroid antibodies. Their study was also exceptional in that an increased antibody prevalence was not observed in probands, and they did not find a rising frequency of seropositivity in women with advancing age. This latter relationship has been almost universally noted (e.g., see Hill, 1961; Doniach and Roitt, 1964; Fialkow et al., 1965a; Dingle et al., 1966).

*B. X Chromosomal Disorders*

The published data on gonadal dysgenesis are more limited. Hashimoto's thyroiditis in patients with isochromosome-X, first reported by Engel and Forbes (1961) and Sparkes and Motulsky (1963), may occur in over 20 per cent of cases with this chromosomal abnormality (Sparkes and Motulsky, 1967). Although Hashimoto's thyroiditis may be more common in patients with an isochromosome-X, primary myxedema and CLT have been noted in

TABLE 6.—*Thyroid Antibodies in Mothers and Patients with Down's Syndrome and Control Women*

| Investigators | Mothers of patients with Down's syndrome | | Control women | | P* |
|---|---|---|---|---|---|
| | Number tested | Number (%) positive | Number tested | Number (%) positive | |
| Fialkow et al., 1965a, Fialkow, 1967a. | 242 | 69(29%) | 242 | 39(16%) | <0.005 |
| Burgio et al., 1965, 1966. | 50 | 22(44%) | 80 | 11(14%) | <0.001 |
| Wren et al., 1967 | 59 | 9(15%) | 68 | 14(21%) | <0.6 |
| Dallaire and Flynn, 1967. | 44 | 12(27%) | 101 | 12(12%) | <0.05 |
| Engelberth et al., 1967 | 24 | 12(50%) | 24 | 6(25%) | <0.15 |

* The $\chi^2$ used to obtain the P value was calculated using Yates' correction for continuity.

patients with gonadal dysgenesis who have other karyotypes (Williams, Engel and Forbes, 1964; Grumbach and Morishima, 1964). Moreover, a high frequency of thyroid antibodies has been detected in such patients as well as in their mothers (Williams, Engel and Forbes, 1964; Grumbach and Morishima, 1964; Fialkow and Uchida, 1966; Fialkow, 1967a; Vallotton and Forbes, 1967; Engel, 1967; Engelberth, 1968), but in one study this was found in propositi and not in their parents (Doniach et al., 1968). Increased frequencies of diabetes mellitus (Forbes and Engel, 1965) and of circulating antibodies to ovarian cytoplasm (Valloton and Forbes, 1967) in affected patients and their close relatives have also been reported. On the other hand, subjects with Klinefelter's syndrome and their parents do not have increased frequency of antithyroid-micosomal or antithyroglobulin antibodies (Day and Wright, 1964; Ferguson-Smith et al., 1966; Vallotton and Forbes, 1967) although one report suggests that they may have a relatively high frequency of other antibodies (Engleberth et al., 1966).

*C. Discussion*

It has been suggested that the development of autoimmune disease in patients with gonadal dysgenesis who have an isochromosome-X may be related to reactivation of an X chromosome in the target organ (Williams et al., 1966). Thus, if the isochromosome-X were inactive in all cells during development, immunological tolerance would only develop to proteins coded by the remaining normal active X chromosome. If in such patients the inactivated X chromosome should later acquire the ability to code for protein synthesis, the resulting proteins would be recognized as foreign and an autoimmune disease could ensue. This hypothesis does not explain the relatively high prevalence of thyroid antibodies in the patients' mothers or in subjects whose cells are uniformly XO.

Some understanding of the association between thyroid autoimmunity in mothers and the occurrence of gonadal dysgenesis or Down's syndrome in their offspring might be gained if the maternal immunologic aberrations could be correlated with a sub-classification within the children's chromosomal abnormalities. Contrary to initial expectation, when the results of the antibody tests were analyzed in relation to maternal age at the time of birth of the child with Down's syndrome, the most prominent increase was found in those women who were less than 35 years of age when the child was born (Fialkow, 1967b), i.e., in the maternal age-independent group (Penrose, 1961). Dallaire and Kingsmill-Flynn (1967a) and Engelberth (personal communication) also reported that the increased frequency of thyroid antibodies in mothers of children with Down's syndrome compared with age matched female controls was more striking in younger than in older mothers. In gona-

dal dysgenesis, seropositivity was more frequent in patients with mosaicism or an isochromosome-X in two studies (Fialkow and Uchida, 1966; Fialkow, 1967a; Doniach et al., 1968), but not in another (Engel, 1967). Moreover, higher frequencies of thyroid antibodies were found in mothers of patients with mosaicism or an isochromosome-X than in mothers of patients with an XO karyotype (Fialkow, 1967a). The absence of an increased frequency of thyroid antibodies in mothers of patients with Klinefelter's syndrome as well as the apparent increased prevalence of these antibodies in younger mothers of children with Down's syndrome and in mothers of children with mosaicism and isochromosome-X may offer some insight into the pathogenic mechanisms.

It could be argued that if women with thyroid antibodies are predisposed to have children with chromosomal aberrations, a higher familial incidence of Down's syndrome should be observed. On the basis of the data presented above, this should be most frequent in seropositive women who have had one affected child when they were less than 35 years of age. Mean maternal age is lower in instances of familial Down's syndrome than it is in cases of the isolated occurrence of this disorder (Penrose and Smith, 1966). Moreover, recent studies of spontaneous abortions may explain why familial chromosomal anomalies in this group are not even more frequent. Major chromosomal abnormalities underlie at least approximately 25 per cent of spontaneous abortions occurring in the first trimester (Carr, 1967), and the great majority of fetuses destined to have Down's syndrome or gonadal dysgenesis may be aborted spontaneously (Edwards, 1965). Dallaire and Kingsmill-Flynn (1967a) reported that the frequency of antecedent spontaneous abortions was suggestively higher in seropositive than in seronegative mothers of children with Down's syndrome. Our own findings (unpublished) tend to confirm this. Forty-six seropositive mothers of children with Down's syndrome had a mean number of 0.804 spontaneous abortions, contrasted with 0.488 in the 121 seronegative mothers. Thus, seropositive women may in fact have more than one chromosomally abnormal fetus more frequently than seronegative women, but many such fetuses may be aborted spontaneously.

Several possibilities could explain the relationship between maternal thyroid antibodies and chromosomal aberrations in the offspring (Fialkow, 1964; Fialkow and Uchida, 1966), but firm conclusions must await further study. However, the available data are compatible with the hypothesis that the antibodies or related factors are predisposing agents in the development of chromosomal aberrations (Fialkow, 1964). The relationship need not be direct since an agent such as a virus or an abnormality in thyroid structure or function might lead to both maternal antibodies and child's chromosomal abnormality. The increased frequency of thyroid antibodies in patients with

infectious hepatitis reported in one study (Dallaire and Kingsmill-Flynn, 1967b) may be relevant in this regard. Alternately immunologic reactions could directly interfere with cell division. It seems unlikely that humoral thyroid antibodies themselves are the pathogenic factors and only slight increases in the prevalence of other circulating antibodies such as antinuclear factor have been found in mothers of patients with Down's syndrome (Fialkow and Uchida, 1966) or gonadal dysgenesis (Vallotton and Forbes, 1967). It is possible that cell-mediated immunologic reactions induce chromosomal aberrations. *In vitro,* the addition of extracts of lymphocytes to genetically foreign fibroblasts results in chromosomal breakage, rearrangement and hyperploidy including endoreduplication (Fialkow and Gartler, 1966; Fialkow, 1967c). These changes are not observed when the lymphocytes from which the extracts are derived and the fibroblasts on which they are tested are obtained from the same donor. Thus, the mechanisms responsible for the induction of the chromosomal aberrations involve discrimination between "self" and "not-self," but the relationship of these observations *in vitro* to chromosomal abnormalities *in vivo* is unknown.

## VIII. Summary and Concluding Remarks

Some types of organ-specific autoimmune diseases can be produced experimentally by a defined immunizing event, e.g., chronic lymphocytic thyroiditis (CLT) following injection of autologous thyroid antigen in Freund's adjuvant. In these instances, there is evidence suggesting that the animal's genotype affects his susceptibility to the immunization. On the other hand, there is one example in animals where a disease resembling CLT-myxedema occurs spontaneously (in fowl) and seems to be dependent upon multifactorial inheritance.

In man, CLT and similar diseases are characterized by the presence of circulating antibodies, and although they are probably not the cytopathogenic agents, the presence of these antibodies can be utilized as a marker for a defect in immunologic homeostasis. Serologic investigations of the asymptomatic relatives of probands have been limited in scope and in choice of controls, but they do indicate a familial predisposition to form such antibodies and suggest that it is at least in part genetically determined. Moreover, these studies add weight to the suggestion that the thyroid disorders most intimately associated with immunologic aberrations, CLT, primary non-congenital myxedema and thyrotoxicosis, are etiologically interrelated. The clinical counterparts of these and other studies suggest a familial predisposition not only to the formation of thyroid antibodies, but also to the clinical thyroid diseases themselves. The susceptibilities to antibody formation and to disease may be interrelated through a familial predisposition to autoim-

munity.

Extension of these studies indicates an overlap of thyroid and other types of organ-specific autoimmunity in patients, suggesting more complex etiologic relationships. The relationship between thyroid and gastric autoimmunity is most convincing, but adrenal and parathyroid autoimmunity may also share in this predisposition. In the patients' families, there is also a relationship between several distinct non-cross-reacting types of organ-specific autoantibodies, and this has implications for the etiology and pathogenesis of the disease which the antibodies characterize.

Conceivably, the immunologic aberrations could arise as the consequence of a familial susceptibility to leakage of normal antigens from several organs. There is no evidence to support a genetically determined generalized defect in basement membranes or a familial susceptibility to an acquired agent like a virus which has a predilection for these many tissues. The possibility that the antibodies arise in response to genetically influenced alterations in antigens in several organs cannot be excluded. Although the evidence is far from complete, it seems at least as likely that one basic abnormality is in the immunologic apparatus itself, and that it effects a predisposition to the formation of multiple autoantibodies (Holman, 1962; Fialkow, Fudenberg and Epstein, 1964; Irvine, 1964). The evidence suggests that this predisposition is inherited and that genetic factors are important, but as yet the data do not allow firm conclusions about possible modes of inheritance. Analyses of the sex and age distributions of some organ-specific autoimmune diseases indicate the likelihood that loci on the X chromosome or other prepubertal differences between the sexes are involved in some instances.

The interrelationship between the various forms of organ-specific immunologic responses is most striking, but some disorders combine features of this type of autoimmunity with those of non-organ-specific immunologic aberrations (e.g., juvenile CLT, Sjogren's syndrome, myasthenia gravis and autoimmune hemolytic anemia). Moreover, both types of immunologic response can occur concomitantly in some families (e.g., see Fialkow, Fudenberg, and Epstein, 1964, and Doniach, Nilsson and Roitt, 1965). These as yet fragmentary data suggest that no sharp dividing line can be drawn between organ-specific and non-organ-specific immunologic aberrations.

There are some observations in experimental animals which indicate that the presence of a virus in the neonatal period may be associated with the delayed onset of systemic "autoimmune" disease in adult animals (Hotchin, 1962; Mellors and Huang, 1967), but only particular genotypes are susceptible. The relevance of these observations in experimental non-organ-specific diseases to primarily organ-specific autoimmune disorders requires further study.

In any event, it seems likely that multifactorial inheritance underlies the organ-specific autoimmune diseases in men. This interpretation is in keeping with what occurs in most other common diseases (Roberts, 1963). One of these multiple factors seems to be a genetic predisposition to immunologic hyperreactivity, but it is likely that intermediate steps involving, for example, antigenic alteration, viruses or somatic mutations are required for phenotypic expression.

Finally, organ-specific antibodies are associated with some congenital chromosomal abnormalities. The observations in most studies of an increased frequency of these antibodies in parents of affected offspring is compatible with the suggestion that a factor related to the antibodies predisposes to the chromosomal aberrations. However, the data are not sufficient to conclude that parental autoimmunity itself is the agent directly responsible for the error in cell division. Alternate possibilities include an intermediate agent such as a virus which may be required for the phenotypic expression of autoimmunity, or a factor related to the genotype in the parent which makes him susceptible to autoimmunity.

Clarification of these and other aspects of the relationship between genes, chromosomes and autoimmunity will require the combined techniques of immunology, virology, biochemistry and related fields, but the potential contributions of well-controlled and thorough immuno-clinical family studies have not been exhausted.

## ACKNOWLEDGEMENT

It is a pleasure to thank Dr. Eloise Giblett for critically reviewing this manuscript and making many valuable suggestions.

## REFERENCES

Adams, J. F., A. I. M. Glen, E. H. Kennedy, I. L. Mackenzie, J. M. Morrow, J. R. Anderson, K. G. Gray and D. G. Middleton. 1964. The histological and secretory changes in the stomach in patients with autoimmunity to gastric parietal cells. Lancet 1: 401-403.

Anderson, J. R., R. B. Goudie, K. G. Gray and G. C. Timbury. 1957. Autoantibodies in Addison's disease. Lancet 1: 1123-1124.

—, K. G. Gray, D. G. Middleton and J. A. Young. 1964. Autoimmunity and thyrotoxicosis. Brit. Med. J. 2: 1630-1632.

Arapakis, G., O. A. Bock, D. L. Williams and L. J. Witts. 1963. Diabetes mellitus and pernicious anaemia. Brit. Med. J. 1: 159-161.

Ardeman, S., and I. Chanarin. 1963. Method for assay of human gastric intrinsic factor and for detection and titration of antibodies against intrinsic factor. Lancet 2: 1350-1354.

—, —, A. Jacobs and L. Griffiths. 1966. Family study in Addisonian pernicious anemia. Blood 27: 599-610.

Bamatter, F., R. Koegel, J. de Haller and A. F. Muller. 1966. Maladie d'Addison familiale. Helv. Paediat. Acta 21: 109-152.

Bartels, E. D. 1941. Heredity in Graves' Disease. Copenhagen: Munksgaard.

Benda, C. E. 1946. Mongolism and Cretinism. New York: Grune and Stratton.

Bielschowsky, M., B. J. Helyer and J. B. Howie. 1959. Spontaneous hemolytic anemia in mice of the NZB/B1 strain. Proc. Univ. Otago Med. Sch. 37: 9-11.

Blizzard, R. M., D. Chee and W. Davis. 1966. The incidence of parathyroid and other antibodies in the sera of patients with idiopathic hypoparathyroidism. Clin. Exp. Immun. 1: 119-128.

—, — and —. 1967. The incidence of adrenal and other antibodies in the sera of patients with idiopathic adrenal insufficiency. (Addison's disease). Clin. Exp. Immun. 2: 19-30.

— and M. Kyle. 1963. Studies of the adrenal antigens and antibodies in Addison's disease. J. Clin. Invest. 42: 1653-1660.

Brit. Med. J. 1966. Placental transmission of autoimmune diseases. 1: 1554-1555.

Buchanan, W. W., J. S. Beck, J. R. Anderson, R. B. Goudie, K. G. Gray and D. G. Middleton. 1965. Family studies in auto-immune thyroiditis and connective-tissue diseases. Scot. Med. J. 10: 70-74.

Bunin, J. 1961. A broader spectrum of Sjögren's syndrome and its pathogenic implications. Ann. Rheum. Dis. 20: 1-10.

Burch, P. R. J. 1963. Autoimmunity: Some aetiological aspects. Inflammatory polyarthritis and rheumatoid arthritis. Lancet 1: 1253-1257.

—, and N. R. Rowell. 1963. Autoimmunity. Aetiological aspects of chronic discoid and systemic lupus erythematosus, systemic sclerosis, and Hashimoto's thyroiditis. Some immunological implications. Lancet 2: 507-513.

Burgio, G. R., F. Severi, R. Rossoni and R. Vaccaro. 1965. Mongolism and thyroid autoimmunity. Lancet 1: 166-167.

—, —, — and —. 1966. Autoantibodies in Down's syndrome. Lancet 1: 497-498.

Burke, G. 1967. Failure of immunologic reaction of long acting thyroid stimulation (LATS) to thyroid components, and the demonstration of a plasma inhibitor of LATS. J. Lab. Clin. Med. 69: 713-727.

Burnet, F. M. 1959. The Clonal Selection Theory of Acquired Immunity. London: Cambridge University Press.

—. 1966. Implications for autoimmune disease in man of studies on NZB mice and their hybrids. I. Autoimmune hemolytic anemia. Royal Institute of Public Health and Hygiene Journal. 29: 87-94. II. Renal and thymic disease. ibid. 29: 95-103. III. Genetics and pathogenesis of autoimmune disease. ibid. 29: 104-112.

— and F. Fenner. 1949. The Production of Antibodies. London: Macmillan.

— and M. C. Holmes. 1964. Thymic changes in the mouse strain NZB in relation to the auto-immune state. J. Path. Bact. 88: 229-241.

— and —. 1965. Genetic investigations of autoimmune diesase in mice. Nature (Lond.) 207: 368-371.

Callender, S. T. and M. A. Denborough. 1957. A family study of pernicious anaemia. Brit. J. Haemat. 3: 88-106.

Calvo R., W. Stein, S. Kochwa and R. E. Rosenfield. 1965. Acute hemolytic anemia due to anti-i: frequent cold agglutinins in infectious mononucleosis. J. Clin. Invest. 44: 1033.

Carmel, R., and V. Herbert. 1967. Intrinsic-factor antibody in the saliva of a patient with pernicious anemia. Lancet 1: 80-81.

Carpenter, C. C. J., N. Solomon, S. G. Silverberg, T. Bledsoe, R. C. Northcutt, J. R. Klinenberg, I. L. Bennett Jr., and A. McGehee Harvey. 1964. Schmidt's syndrome (thyroid and adrenal insufficiency). A review of the literature and a report of fifteen new cases including ten instances of coexistent diabetes mellitus. Medicine 43: 153-180.

Carr, D. H. 1967. Chromosome anomalies as a cause of spontaneous abortion. Amer. J. Obstet. Gynec. 97: 283-293.

Castleman, B. 1966. The pathology of the thymus gland in myasthenia gravis. Ann. N. Y. Acad. Sci. 135: 496-503.

Chauffard, A. and J. Troisier. 1908. Anaemie grave avec haemolysine dans le sérum; ictère haemolysinique. Semaine Méd. 28: 345.

Celano, M. J. and P. Levine. 1967. Anti-LW specificity in autoimmune acquired hemolytic anemia. Transfusion 7: 265-268.

Climenko, H. 1920. Heredity in exophthalmic goiter. Arch. Neurol. Psychiat. 3: 530-535.

Coghill, N. F., D. Doniach, I. M. Roitt, D. L. Mollin and A. Wynn Williams. 1965. Autoantibodies in simple atrophic gastritis. Gut 6: 48-56.

Cole, R. K. 1966. Hereditary hypothyroidism in the domestic fowl. Genetics 53: 1021-1033.

Colover, J., and L. E. Glynn. 1958. Experimental iso-immune adrenalitis. Immunology 1: 172-178.

Cordova, M. S., J. Baez-Villasenor, J. J. Mendez and E. Campos. 1966. Acquired hemolytic anemia with positive antiglobulin (Coombs' test) in mother and daughter. Arch. Intern. Med. 117: 692-695.

Craig, P. E., J. L. Spann and L. Lowbeer. 1952. Hashimoto's disease (struma lymphomatosa): A familial incidence of 3 cases. Amer. J. Surg. 84: 286-292.

Dacie, J. V. 1962. The Haemolytic Anaemias, Part 2. 2nd Ed. London: Churchill.

Dagg, J. H., A. Goldberg, J. R. Anderson, J. S. Beck and K. G. Gray. 1964. Autoimmunity in iron-deficiency anaemia. Brit. Med. J. 1: 1349-1350.

Dallaire, L., and D. Flynn. 1967. Frequency of antibodies to thyroglobulin in relation to gravidity and to Down's syndrome. Canad. Med. Assoc. J. 97: 209-212.

Dallaire, L., and D. Kingsmill-Flynn. 1967a. Auto-immunité de la thyroide et aberrations chromosomiqués. L'Union Méd. du Canada 96: 131-1375.

—, and D. Kingsmill-Flynn. 1967b. Infectious hepatitis and Down's syndrome. Lancet 2: 467.

Damashek, W. 1966. Immunocytes and immunoproliferative disorders. *In* Wolstenholme, G. E. W. and R. Porter (Eds.): Ciba Foundation Symposium: The Thymus: Experimental and Clinical Studies. Boston: Little, Brown and Company. pp. 399-415.

—, and S. O. Schwartz. 1938. Hemolysins as the cause of clinical and experimental hemolytic anemias with particular reference to the nature of spherocytosis and increased fragility. Amer. J. Med. Sci. 196: 769-792.

Day, R. W., and S. W. Wright. 1964. Thyroid autoantibodies and sex-chromosome abnormalities. Lancet 1: 667.

DeGroot, L. J., R. Hall, W. V. McDermott Jr. and A. M. Davis. 1962. Hashimoto's thyroiditis: A genetically conditioned disease. New Eng. J. Med. 267: 267-273.

—, and J. B. Stanbury. 1959. Syndrome of congenital goiter with butanol-insoluble serum iodine. Amer. J. Med. 27: 586-595.

Diamond, M. T., and B. Joffe. 1966. Monozygotic twins with chronic lymphocytic thyroiditis (Hashimoto's disease). J. A. M. A. 198: 182-183.

Dingle, P. R., A. Ferguson, D. B. Horn, J. Tubmen and R. Hall. 1966. The incidence of thyroglobulin antibodies and thyroid enlargement in a general practice in north-east England. Clin. Exp. Immun. 1: 277-284.

Djanian, A. Y., E. H. Beutner and E. Witebsky. 1964. Tanned-cell hemagglutination test for detection of antibodies in sera of patients with myasthenia gravis. J. Lab. Clin. Med. 63: 60-70.

Dobbs, C. E. 1965. Familial auto-immune hemolytic anemia. Arch Intern. Med. 116: 273-276.

Doniach, D., L. R. Nilsson and I. M. Roitt. 1965. Autoimmune thyroiditis in children and adolescents. II. Immunological correlations and parent study. Acta Paediat. Scand. 54: 260-274.

— and I. M. Roitt. 1963. Clinical application of thyroid auto-antibody tests. *In* Gell, P. G. H. and R. R. A. Coombs (Eds.): Clinical Aspects of Immunology. Philadelphia: F. A. Davis Co., pp. 611-632.

—, and —. 1964. An evaluation of gastric and thyroid auto-immunity in relation to hematologic disorders. Seminars Hemat. 1: 313-343.

—, and —. 1966. Family studies on gastric autoimmunity. Proc. Roy. Soc. Med. 59: 691-694.

—, —, and P. E. Polani. 1968. Thyroid antibodies and sex-chromosome anomalies. Proc. Roy. Soc. Med. 61: 278-280.

—, — and K. B. Taylor. 1965. Autoimmunity in pernicious anemia and thyroiditis: a family study. Ann. N. Y. Acad. Sci. 124: 605-625 (part II).

Dunning, E. J. 1959. Struma lymphomatosa: a report of three cases in one family. J. Clin. Endocr. 19: 1121-1125.

East, J., P. R. Prosser, E. J. Holborow and H. Jaquet. 1967. Autoimmune reactions and virus-like particles in germ-free NZB mice. Lancet 1: 755-757.

Edwards, J. H. 1965. The application of knowledge. *In* D. Bergsma and J. German, (Eds.): New Directions in Human Genetics. Birth defects: Original Article Series 1: 75-84. New York: The National Foundation.

Ehrlich, P., and J. Morgenroth. 1900. Ueber Haemolysine. Dritte Mittheilung. Berlin Klin. Wochschr. 37: 453-458.

Engel, E. 1967. Autoantibodies and chromosomal aberrations. Lancet 1: 1271-1272.

—, and A. P. Forbes. 1961. An abnormal medium-sized metacentric chromosome in a woman with primary gonadal failure. Lancet 2: 1004.

Engelberth, O. 1968. Sborn. lék. In press.

Engelberth, O., Z. Jezková, H. Belíkovová and J. Kucera. 1967. Autoantibodies in Down's disease. Sborn. Lék. 69: 232-238.

Engleberth, O., J. Chervat, Z. Jezokva and J. Raboch. 1966. Autoantibodies in chromatin positive men. Lancet 2: 1194.

Eylan, E., R. Zmucky and Ch. Sheba. 1957. Mumps virus and subacute thyroiditis. Evidence of a causal association. Lancet 1: 1062-1063.

Felix-Davies, D. 1958. Autoimmunisation in subacute thyroiditis associated with evidence of infection by mumps virus. Lancet 1: 880-883.

Feltkamp, T. E. W. 1966. Idiopathic Autoimmune Diseases. Amsterdam: Univ. of Amsterdam (Thesis).

Ferguson-Smith, M. A., J. R. Anderson, A. Froland and K. Gray. 1966. Frequency of autoantibodies in patients with chromatin-positive Klinefelter's syndrome and their parents. Lancet 2: 566-568.

Fialkow, P. J. 1964. Autoimmunity: A predisposing factor to chromosomal aberrations? Lancet 1: 474-475.

—. 1966. Autoimmunity and chromosomal aberrations. Amer. J. Hum. Genet. 18: 93-108.

—. 1967a. Autoantibodies and chromosomal aberrations. Lancet 1: 1106.

—. 1967b. Thyroid antibodies, Down's syndrome and maternal age. Nature (Lond.) 214: 1253-1254.

—. 1967c. Chromosomal breakage induced by extracts of human allogeneic lymphocytes. Science 155: 1676-1677.

—, H. Fudenberg and W. V. Epstein. 1964. "Acquired" antibody hemolytic anemia and familial aberrations in gamma globulins. Amer. J. Med. 36: 188-199.

—, and S. M. Gartler. 1966. Hyperploidy effect of lymphocyte extract on fibroblasts in vitro. Nature (Lond.) 211: 713-715.

—, F. Hecht, J. Bryant and A. G. Motulsky. 1965b. Familial predisposition to chromosomal aberrations. Clin. Res. 13: 124.

—, and I. A. Uchida. 1966. Autoantibodies in Down's syndrome and gonadal dysgenesis. Presented at Symposium on Leukocyte Chemistry and Morphology Correlated with Chromosomal Anomalies, New York, Nov. 3, 1966. Ann. N. Y. Acad. Sci. In press.

—, —, F. Hecht and A. G. Motulsky. 1965a. Increased frequency of thyroid autoantibodies in mothers of children with Down's syndrome. Lancet 2: 868-870.

Fisher, J. M., I. R. MacKay, K. B. Taylor and B. Ungar. 1967. An immunological study of categories of gastritis. Lancet 1: 176-180.

—, C. Rees and K. B. Taylor. 1966. Intrinsic-factor antibodies in gastric juice of pernicious-anaemia patients. Lancet 2: 88-89.

Forbes, A. P. and E. Engel. 1963. The high incidence of diabetes mellitus in 41 patients with gonadal dysgenesis, and their close relatives. Metabolism 12: 428-439.

Fudenberg, H., J. L. German and H. G. Kunkel. 1962. The occurrence of rheumatoid factor and other abnormalities in families of patients with agammaglobulinemia. Arthritis Rheum. 5: 565-588.

Fudenberg, H. H. 1967. Genetically determined abnormalities in antigen-antibody interaction. *In* J. F. Crow and J. V. Neal, (Eds.): Proceedings of the Third International Congress of Human Genetics. Baltimore: Johns Hopkins Press. pp. 233-246.

Goldberg, M. 1960. Incidence of hypothyroidism in more than one member of a family. Amer. J. Med. Sci. 239: 562-570.

Goldstein, G. and S. Whittingham. 1966. Experimental autoimmune thymitis. An animal model of human myasthenia gravis. Lancet 2: 315-318.

Goudie, R. B., J. R. Anderson and K. G. Gray. 1959. Complement-fixing antithyroid antibodies in hospital patients with asymptomatic thyroid lesions. J. Path. Bact. 77: 389-400.

—, —, — and W. G. Whyte. 1966. Autoantibodies in Addison's disease. Lancet 1: 1173-1176.

Grumbach, M. M., and A. Morishima. 1964. X chromosome abnormalities in gonadal dysgenesis: DNA replication of structurally abnormal X chromosomes; relation to thyroid disease. J. Pediat. 65: 1087-1088.

Hahn, H. B. Jr., A. B. Hayles and L. B. Woolner. 1965. Lymphocytic thyroiditis in children. J. Pediat. 66: 73-78.

Hall, P. F. 1965. Familial occurrence of myxoedema. J. Med. Gent. 2: 173-180.

Hall, R., S. G. Owen and G. A. Smart. 1960. Evidence for genetic predisposition to formation of thyroid autoantibodies. Lancet 1: 187-188.

—, — and —. 1964. Paternal transmission of thyroid autoimmunity. Lancet 2: 115.

—, K. M. Saxena and S. G. Owen. 1962. A study of the parents of patients with Hashimoto's disease. Lancet 2: 1291-1292.

Harris, H., H. Kalmus and W. R. Trotter. 1949. Taste sensitivity to phenylthiourea in goitre and diabetes; preliminary communication. Lancet 2: 1038-1039.

Harvald, B., and M. Hauge. 1956. A catamnestic investigation of Danish twins; a preliminary report. Danish Med. Bull. 3: 150-158.

Hashimoto, H. 1912. Zur Kenntnis der lymphomatoesen Veraenderung der Schilddruese (Struma Lymphomatosa). Arch. Klin. Chir. 97: 219-248.

Hassan, T. H. A., W. R. Grieg, J. A. Boyle, I. T. Boyle and J. T. Wallace. 1966. Toxic diffuse goitre in monozygotic twins. Lancet 2: 306-308.

Helyer, B. J., and J. B. Howie. 1963a. Spontaneous auto-immune disease in NZB/BL mice. Brit. J. Haemat. 9: 119-131.

—, and —. 1963b. Renal disease associated with positive lupus erythematosus tests in a cross-bred strain of mice. Nature (Lond.) 197: 197.

Hicks, J. D., and F. M. Burnet. 1966. Renal lesions in the "auto-immune" mouse strains NZB and $F_1$ NZB x NZW. J. Path. Bact. 91: 467-476.

Hill, O. W. 1961. Thyroglobulin antibodies in 1,297 patients without thyroid disease. Brit. Med. J. 1: 1793-1796.

Hoffman, E. 1966. Hashimoto's thyroiditis (struma lymphomatosa). Arch. Surg. 92: 865-867.

Holman, H. R. 1962. Clinical and immunological evidence for a predisposition to rheumatic disease in certain families. *In* Steinberg, A. G. and A. G. Bearn (Eds.): Progress in Medical Genetics. New York: Grune & Stratton. 5: 210-229.

Holmes, M. C., and F. M. Burnet. 1963. The natural history of auto-immune disease in NZB mice. Ann. Intern. Med. 59: 265-276.

—, and —. 1964. The inheritance of autoimmune disease in mice: a study of hybrids of the strains NZB and $C_3H$. Heredity 19: 419-434.

—, and —. 1966a. Thymic changes in NZB mice and hybrids. *In* G. E. W. Wolstenholme and R. Porter (Eds.): The Thymus: Experimental and Clinical Studies. Boston: Little, Brown and Company. pp. 381-390.

—, and —. 1966b. The characteristics of $F_1$ and back cross hybrids between "high leukemia" (AKR) and "autoimmune" (NZB) mouse strains. Aust. J. Exp. Biol. Med. Sci. 44: 235-250.

Hotchin, J. 1962. The biology of lymphocytic choriomeningitis infection: virus-induced immune disease. Cold Spring Harbor Symp. Quant. Biol. 27: 479-499.

Howel Evans, A. W., J. C. Woodrow, C. D. M. McDougall, A. R. Chew and R. Winston Evans. 1967. Antibodies in the families of thyrotoxic patients. Lancet 1: 636-641.

Howie, J. B., and B. J. Helyer. 1965. Autoimmune disease in mice. Ann. N. Y. Acad. Sci. 124: 167-177.

—, and —. 1966. The influence of neonatal thymectomy and thymus grafting on spontaneous auto-immune disease in mice. *In* Wolstenholme, G. E. W., and R. Porter (Eds.): Ciba Foundation Symposium: The Thymus: Experimental and Clinical Studies. Boston: Little, Brown and Company. pp. 360-380.

Hung W., and T. Winship. 1963. Struma lymphomatosa in mother and daughter with serologic and histologic evidence. J. Clin. Endocr. 23: 465-469.

Ingbar, S. H., N. Freinkel, J. T. Dowling and L. F. Kumagai. 1956. Abnormalities of iodine metabolism in euthyroid relatives of patients with Grave's disease. J. Clin. Invest. 35: 714.

Irvine, W. J. 1963. A clinical and immunological study of adrenal insufficiency. J. Endocr. 26: xxxii-xxxiii.

—. 1964. Thyroid auto-immunity as a disorder of immunological tolerance. Quart. J. Exp. Physiol. 49: 324-337.

—, and S. H. Davies. 1963. Gastric antibodies in diabetes mellitus. Lancet 2: 838-839.

—, —, S. Teitelbaum, I. W. Delamore and A. Wynn Williams. 1965. The clinical and pathological significance of gastric parietal cell antibody. Ann. N. Y. Acad. Sci. 124: 657-691.

—, —, I. W. Delamore and A. Wynn Williams. 1962. Immunological relationship between pernicious anaemia and thyroid disease. Brit. Med. J. 2: 454-456.

—, A. G. MacGregor, A. E. Stuart and G. H. Hall. 1961. Hashimoto's disease in uniovular twins. Lancet 2: 850-853.

—, A. G. Stewart and L. Scarth. 1967. A clinical and immunological study of adrenocortical insufficiency (Addison's disease). Clin. Exp. Immunol. 2: 31-69.

Jackson, I. M. D., J. A. Boyle and R. Mc G. Harden. 1965. Distribution of ABO blood groups in thyrotoxicosis. Vox Sang. 10: 246-248.

Jayson, M. I. V., D. Doniach, N. Benhamou-Glynn, I. M. Roitt and D. J. El Kabir. 1967. Thyrotoxicosis and Hashimoto goitre in a pair of monozygotic twins with long-acting thyroid stimulator. Lancet 2: 15-18.

Jenkins, W. J., W. L. Marsh, J. Noades, P. Tippett, R. Sangar and R. R. Race. 1960. The I antigen and antibody. Vox Sang. 5: 97-106.

Keynes, G. 1954. Surgery of the thymus gland; Second (and third) thoughts. Lancet 1: 1197-1202.

Kissmeyer-Nielsen, F., K. Bent-Hansen and J. Kieler. 1952. Immunohemolytic anemia with familial occurrence. Acta Med. Scand. 144: 35-39.

Kitchin, F. D., W. Howel Evans, C. A. Clarke, R. B. McConnell and P. M. Sheppard. 1959. P. T. C. taste response and thyroid disease. Brit. Med. J. 1: 1069-1074.

Kreines, K., M. Jett and H. C. Knowles Jr. 1965. Observations in hyperthyroidism of abnormal glucose tolerance and other traits related to diabetes mellitus. Diabetes 14: 740-744.

Lancet. 1967. Autoimmunity in idiopathic Addison's disease. 1:1040-1042.

Landing, B. H., M. D. Pettit, R. L. Wiens, H. Knowles and G. M. Guest. 1963. Antithyroid antibody and chronic thyroiditis in diabetes. J. Clin. Endocr. 23: 119-120.

Lipton, M. M., and J. Freund. 1953. The efficacy of the intracutaneous route of injection and susceptibility of the Hartley strain of guinea pigs in experimental allergic encephalitis. J. Immun. 70: 326-329.

Lupulescu, A., A. Pop, E. Merculiev, C. Neascu and C. Heithmanek. 1965. Experimental iso-immune hypoparathyroidism in rats. Nature (Lond.) 206: 415-416.

Macgregor, A. R. 1964. Hypothyroidism. *In* Pitt-Rivers, R., and W. R. Trotter (Eds.): The Thyroid Gland; Vol. 2. Washington: Butterworth Inc., pp. 112-129.

Mackay, I. R. 1964. Autoimmune serological studies in chronic gastritis and pernicious anaemia. Gut 5: 23-26.

Markson, J. L., and J. M. Moore. 1962. Autoimmunity in pernicious anaemia and iron-deficiency anaemia. A complement-fixation test using human gastric mucosa. Lancet 2: 1240-1243.

Marsh, W. L. 1961. Anti-i: a cold antibody defining the Ii relationship in human red cells. Brit. J. Haemat. 7: 200-209.

Martin, L., and R. A. Fisher. 1945. Hereditary and familial aspects of exophthalmic goitre and nodular goitre. Quart. J. Med. 14: 207-219.

Masi, A. T., W. H. Hartmann and L. E. Shulman. 1965. Hashimoto's disease. An epidemiological critique. J. Chron. Dis. 18: 1-22.

Maynard Smith, J., and S. Maynard Smith. 1963. A genetic theory of inflammatory polyarthritis. Lancet 2: 357-359.

McConnell, R. B. 1966. The Genetics of Gastro-Intestinal Disorders. London: Oxford University Press.

McFadyen, I. J,, A. Goldberg, J. H. Dagg and J. R. Anderson. 1966. Incidence of gastric parietal cell antibody in families of patients with iron deficiency anaemia. Brit. J. Haemat. 12: 697-703.

McIntyre, P. A. 1968. Genetic and autoimmune features of pernicious anemia: I. Unreliability of the Schilling test in detecting genetic predisposition to disease. Johns Hopkins Med. J. 122: 181-183.

McIntyre, P. A., R. Hahn, C. L. Conley and B. Glass. 1959. Genetic factors in predisposition to pernicious anemia. Bull. Hopkins Hosp 104: 132-142.

McMaster, P. R. B., E. M. Lerner and P. S. Mueller. 1965. Genetic influence on experimental allergic thyroiditis in guinea pigs. Science 147: 157-158.

Mellon, J. P., B. Y. Pay and D. M. Greene. 1963. Mongolism and thyroid autoantibodies. J. Ment. Defic. Res. 7: 31-37.

Mellors, R. C. 1965. Autoimmune disease in NZB/Bl mice. I. Pathology and pathogenesis of a model system of spontaneous glomerulonephritis. J. Exp. Med. 122: 25-40.

—. 1966. Autoimmune disease in NZB/Bl mice. II. Autoimmunity and malignant lymphoma. Blood 27: 435-448.

—, and C. Y. Huang. 1966. Immunopathology of NZB/Bl mice. V. Virus-like (filtrable) agent separable from lymphoma cells and identifiable by electron microscopy. J. Exp. Med. 124: 1031-1038.

—, and —. 1967. Immunopathology of NZB/Bl mice. VI. Virus separable from spleen and pathogenic for Swiss mice. J. Exp. Med. 126: 53-62.

Moore, J. M., and J. M. Neilson. 1963. Autoantibodies to gastric mucosa and thyroid in diabetes mellitus. Lancet 2: 645-647.

Mosbech, J. 1953. Heredity in Pernicious Anaemia: A Proband Study of the Heredity and Relationship to Cancer of the Stomach. Copenhagen: Ejnar Munksgaard.

Motulsky, A. G., and C. J. Epstein. 1968. Genetics and endocrinology. *In* Williams, R. H. (Ed.): Textbook of Endocrinology, 4th edition. Philadelphia: W. B. Saunders.

Nastuk, W. L., O. J. Plescia and K. E. Osserman. 1960. Changes in serum complement activity in patients with myasthenia gravis. Proc. Soc. Exp. Biol. Med. 105: 177-184.

Nerup, J., M. Søberg, P. Halberg and K. Brøchner-Mortensen. 1966. Organ specific antibodies in Addison's disease. Acta Med. Scand. (suppl.) 445: 383-388.

Nilsson, L. R., and D. Doniach. 1964. Auto-immune thyroiditis in children and adolescents. I. Clinical studies. Acta Paediat. Scand. 53: 255-268.

Norins, L. C., and M. C. Holmes. 1964. Antinuclear factor in mice. J. Immun. 93: 148-154.

Osserman, K. E. 1958. Myasthenia Gravis. New York: Grune & Stratton.

Owen, C. A. Jr., and W. M. McConahey. 1956. An unusual iodinated protein of the serum in Hashimoto's thyroiditis. J. Clin. Endocr. 16: 1570-1579.

Owen, S. G., and G. A. Smart. 1958. Thyroid antibodies in myxoedema. Lancet 2: 1034-1036.

Penrose, L. S. 1961. Mongolism. Brit. Med. Bull. 17: 184-189.

Peterson, R. D. A., M. D. Cooper and R. A. Good. 1965. Disorders of the thymus and other lymphoid tissues. *In* Steinberg, A. G., and A. G. Bearn (Eds.): Progress in Medical Genetics. New York: Grune & Stratton 4: 1-31.

Pratt, R. T. C. 1967. The Genetics of Neurological Disorders. London: Oxford University Press.

Richards, B. W., A. Stewart, P. E. Sylvester and V. Jasiewicz. 1965. Cytogenetic survey of 225 patients diagnosed clinically as mongols. J. Ment. Defic. Res. 9: 245-259.

Roberts, J. A. F. 1963. An Introduction to Medical Genetics. London: Oxford University Press.

Roitt, I. M., D. Doniach, P. N. Campbell and R. V. Hudson. 1956. Autoantibodies in Hashimoto's disease (lymphadenoid goitre). Lancet 2: 820-821.

Roitt, I. M., and G. Torrigiani. 1967. Extrathyroidal thyroglobulin. J. Clin. Path. Suppl. 20: 391-394.

Rose, N. R., and K. B. Taylor. 1965. The autoimmune diseases. Med. Clin. N. Amer. 49: 1675-1716.

—, and E. Witebsky. 1956. Studies on organ specificity: V. Changes in the thyroid glands of rabbits following active immunization with rabbit thyroid extracts. J. Immun. 76: 417-427.

—, J. H. Kite Jr. and T. K. Doebbler. 1962. Experimental autoimmune thyroiditis. *In* Miescher, P., and P. Grabar (Eds.): Mechanisms of Cell and Tissue Damage Produced by Immune Reactions. Basel: Benno Schwabe & Co., pp. 161-173.

—, —, —, R. Spier, F. R. Skelton and E. Witebsky. 1965. Studies on experimental thyroiditis. Ann. N. Y. Acad. Sci. 124: 201-230.

Saxena, K. M., and J. D. Crawford. 1962. Juvenile lymphocytic thyroiditis. Pediatrics 30: 917-926.

—. 1965. Inheritance of thyroglobulin antibody in thyrotoxic children. Lancet 1: 583.

—, and C. V. Pryles. 1965. Thyroid function in mongolism. J. Pediat. 67: 363-370.

Schade, S. G., P. Feick, M. Muckerheide and R. F. Schilling. 1966. Occurrence in gastric juice of antibody to a complex of intrinsic factor and vitamin $B_{12}$. New Eng. J. Med. 275: 528-531.

Schwartz, M. 1960. Intrinsic factor antibody in serum from patients with pernicious anaemia. Lancet 2: 1263-1267.

Shannon, D. C., G. Johnson and K. F. Austen. 1966. Genetic and clinical aspects of the syndrome of chronic moniliasis and endocrine deficits. Soc. for Pediat. Research. Apr. 29-30, 1966. *Cited in* McKusick, V. 1966. Mendelian Inheritance in Man. Catalogs of Autosomal Dominant, Autosomal Recessive and X-linked Phenotypes. Baltimore: Johns Hopkins Press. p. 195.

Shapiro, M. 1967. Familial autohemolytic anemia and runting syndrome with Rhô-specific autoantibody. Transfusion 7: 281-296.

Simpson, J. A. 1960. Myasthenia gravis: A new hypothesis. Scot. Med. J. 5: 419-436.

—. 1964. Immunological disturbances in myasthenia gravis with a report of Hashimoto's disease developing after thymectomy. J. Neurol. Neurosurg. Psychiat. 27: 485-492.

Sparkes, R. S., and A. G. Motulsky. 1963. Hashimoto's disease in Turner's syndrome with isochromosome X. Lancet 1: 947.

—, and —. 1967. The Turner syndrome with isochromosome X and Hashimoto's thyroiditis. Ann. Intern. Med. 67: 132-144.

Stamos, H. F. 1940. Heredity in pernicious anemia. Amer. J. Med. Sci. 200: 586-590.

Steiner, J. W., B. Langer, D. L. Schatz and R. Volpe. 1960. Experimental immunologic adrenal injury. A response to injections of autologous and homologous adrenal antigents in adjuvant. J. Exp. Med. 112: 187-201.

Stone, S. H. 1962. Differences in reactivity associated with sex or strain of inbred or random-bred guinea pigs in the massive hemorrhagic reaction and other manifestations of delayed hypersensitivity. Int. Arch. Allerg. 20: 193-202.

Strauss, A. J. L., B. C. Seegal, K. C. Hsu, P. M. Burkholder, W. L. Nastuk and K. E. Osserman. 1960. Immunofluorescence demonstration of a muscle binding, complement-fixing serum globulin fraction in myasthenia gravis. Proc. Soc. Exp. Biol. Med. 105: 184-191.

—, C. W. Smith, G. W. Cage,, H. W. R. Van der Geld, D. E. McFarlin and M. Barlow. 1966. Further studies on the specificity of presumed immune associations of myasthenia gravis and consideration of possible pathogenic implications. Ann. N. Y. Acad. Sci. 135: 557-579.

Taylor, K. B. 1959. Inhibition of intrinsic factor by pernicious anaemia sera. Lancet 2: 106-108.

—, I. M. Roitt, D. Doniach, K. G. Couchman and C. Shapland. 1962. Autoimmune phenomena in pernicious anaemia: gastric antibodies. Brit. Med. J. 2: 1347-1352.

te Velde, K. 1967. Antibodies against parietal cells, chronic gastritis and pernicious anemia. Thesis, State University, Groningen, The Netherlands.

TeVelde, K., J. Abels, G. J. P. A. Anders, A. Arends, Ph. J. Hoedemaeker and H. O. Nieweg. 1964. A family study of pernicious anemia by an immunologic method. J. Lab. Clin. Med. 64: 177-187.

Thier, S. O., P. Black, H. E. Williams and J. Robbins. 1965. Chronic lymphocytic thyroiditis: Report of a kindred with viral, immunological and chemical studies. J. Clin. Endocr. 25: 65-75.

Troxel, D. B., F. Innella and R. J. Cohen. 1966. Infectious mononucleosis complicated by hemolytic anemia due to anti-i. Amer. J. Clin. Path. 46: 625-631.

Tudhope, G. R., and G. M. Wilson. 1960. Anemia and hypothyroidism. Quart. J. Med. 29: 513-538.

—, and —. 1962. Deficiency of vitamin $B_{12}$ in hypothyroidism. Lancet 1: 703-706.

Ungar, B., A. E. Stocks, F. I. R. Martin, S. Whittingham and I. R. Mackay. 1967. Intrinsic-factor antibodies in diabetes mellitus. Lancet 2: 77-78.

Vajda, I., L. Aszodi, B. Hajdu, E. Strenszky, P. Barzo, and E. Horvath. 1960. Familial relationships in acquired hemolytic anemia. Magy. Belorv. Belorv. Arch. 13: 121-124.

Vallotton, M. B., and A. Forbes. 1967. Autoimmunity in gonadal dysgenesis and Klinefelter's syndrome. Lancet 1: 648-651.

Van der Geld, H., T. E. Feltkamp, J. J. van Loghem, H. J. G. H. Oosterhuis and A. Biemond. 1963. Multiple antibody production in myasthenia gravis. Lancet 2: 373-375.

—, —, and H. J. G. H. Oosterhuis. 1964. Reactivity of myasthenia gravis serum γ-globulin with skeletal muscle and thymus demonstrated by immunofluorescence. Proc. Soc. Exp. Biol. Med. 115: 782-785.

Vickery, A. L., and E. Hamlin Jr. 1961. Struma Lymphomatosa (Hashimoto's thyroiditis). Observations on repeated biopsies in sixteen patients. New Eng. J. Med. 264: 226-229.

Vogel, F. 1959. Moderne Probleme der Humangenetik. Ergebn. Inn. Med. K. u. Kinderheilk. 12: 52-125.

Volpe, R., C. Ezrin, M. W. Johnston and J. W. Steiner. 1963. Genetic factors in Hashimoto's struma. Canad. Med. Ass. J. 88: 915-919.

Von Verschuer, O. 1958. Die Zwillingforschung im Dienste de inneren Medizin. Verh. Deutsch. Ges. Inn. Med. 64: 262-273.

Waters, A. H., and M. E. Murphy. 1963. Familial juvenile pernicious anaemia: a study of the hereditary basis of pernicious anaemia. Brit. J. Haemat. 9: 1-12.

Weigle, W. O. 1965. Induction of autoimmunity in rabbits following injection of heterologous or altered homologous thyroglobulin. J. Exp. Med. 121: 289-307.

Weiner, L. B. and K. E. Osserman. 1966. Studies in myasthenia gravis: Demonstration of presence of immunofluorescence in serums correlated with clinical findings. Ann. N. Y. Acad. Sci. 135: 644-655.

Weiner, W., D. A. Battey, T. E. Cleghorn, F. G. W. Marson and M. J. Meynell. 1953. Serological findings in a case of haemolytic anaemia. Brit. Med. J. 2: 125-128.

—, N. K. Shinton and I. R. Gray. 1960. Antibody of blood group specificity in simple ("cold") haemolytic anaemias. J. Clin. Path. 13: 232-236.

—, and G. H. Vos. 1963. Serology of acquired hemolytic anemias. Blood 22: 606-613.

Whitaker, J., B. H. Landing, V. M. Esselborn and R. R. Williams. 1956. The syndrome of familial juvenile hypoadrenocorticism, hypoparathyroidism and superficial moniliasis. J. Clin. Endocr. 16: 1374-1387.

White, R. G. and A. H. E. Marshall. 1962. The autoimmune response in myasthenia gravis. Lancet 2: 120-123.

Widal, F., P. Abrami and M. Brulé. 1908. Les ictères d''origine hémolytique sera. Mal. Coeur 1:193-231.

Wiener, A. S., E. B. Gordon and C. Gallop. 1953. Studies on autoantibodies in human sera. J. Immun. 71: 58-65.

—, L. J. Unger, L. Cohen and J. Feldman. 1956. Type-specific cold auto-antibodies as a cause of acquired hemolytic anemia and hemolytic transfusion reactions: biologic test with bovine red cells. Ann. Intern. Med. 44: 221-240.

Wilkinson, J. F. 1949. Megalocytic anaemias. Lancet 1: 249-255, 291-296.

Williams, E. D., E. Engel and A. P. Forbes. 1964. Thyroiditis and gonadal dysgenesis. N. Eng. J. Med. 270: 805-810.

—, —, P. D. Taft and A. P. Forbes. 1966. Gonadal dysgenesis and ulcerative colitis. A case report with clinical, cytogenetic, and postmortem studies. J. Med. Genet. 3: 51-55.

Winter, J., W. R. Eberlein and A. M. Bongiovanni. 1966. The relationship of juvenile hypothyroidism to chronic lymphocytic thyroiditis. J. Pediat. 69: 709-718.

Witebsky, E. and F. Milgrom. 1962. Immunological studies of adrenal glands. II. Immunization with adrenals of the same species. Immunology 5: 67-68.

—, and N. R. Rose. 1956. Studies on organ specificity. IV. Production of rabbit thyroid antibodies in the rabbit. J. Immun. 76: 408-416.

—, —, K. Terplan, J. R. Paine and R. W. Egan. 1957. Chronic thyroiditis and autoimmunization. J. A. M. A. 164: 1439-1447.

Wren, P. J., D. A. P. Evans, J. M. Vetters and A. Chew. 1967. Autoimmune antibodies in Mongol families. Lancet 2: 186-188.

Wright, R. and J. H. Kerr. 1967. Autoimmunity in myasthenia gravis: A family study. Clin. Exp. Immun. 2: 93-101.

—, R. Whitehead, A. G. Wangel, S. N. Salem and K. F. R. Schiller. 1966. Autoantibodies and microscopic appearance of gastric mucosa. Lancet 1: 618-621.

Wuepper, K. D., and H. H. Fudenberg. 1966. Family studies of "idiopathic" Addison's disease: an immunologic survey. Third Int. Congr. Hum. Genet. abstract no. 351, p. 106.

—, and —. 1967. Moniliasis, "autoimmune" polyendocrinopathy, and immunologic family study. Clin. Exp. Immun. 1: 71-82.

CHAPTER 5

# The Application of Bacterial Genetics to the Study of Human Genetic Abnormalities

**Jean-Claude Dreyfus**

Institut de Pathologie Moléculaire
Hôpital d Cochin, Paris

JACOB AND MONOD (1961) proposed a coherent theory to codify the laws of bacterial genetics. This theory emphasized the role of regulatory genes which control the level of production of proteins, in addition to genes which determine the structure of proteins.

Although the discoveries leading to such conclusions were derived from the study of bacterial mutations, Monod and Jacob (1961) suggested that the same rules should apply to all living matter, even if they were eventually masked by the complexity of cellular interrelationships in higher organisms. Indeed, many investigators have reported finding diseases due to lesions of controller genes. We think that these attempts have not been as successful as reported and that the laws of bacterial genetics have been applied without sufficient criticism to pathology.

In the first part of this paper we shall present a critical review of the present data. In the second part we shall propose a mechanism, not yet demonstrated, but which could serve as a working hypothesis for a new approach to the application of the theory .

## APPLICATION OF THE DATA OF BACTERIAL GENETICS TO PATHOLOGY

### *Review of the Theory*

The classical theory of Jacob and Monod is summarized in Fig. 1. We

shall recall only the features relevant to our discussion. Hereditary traits are ruled by 2 major types of genes.

*Structural genes,* through the medium of messenger ribonucleic acid, direct the synthesis of protein molecules, most of which are enzymes. A mutation in a structural gene leads to a qualitative alteration of the protein, which often modifies its biological properties, while in principle its rate of synthesis is not changed. In fact, this last idea is no longer valid (Ames and Martin, 1964) since several mechanisms have been described by which mutations would alter the rate of synthesis, among which are the following:

1) A "nonsense" mutation (giving a nucleotide triplet which codes for no amino acid) or a "frameshift" mutation (addition or deletion of one nucleotide, which changes entirely the nature of all the triplets "beyond" the mutation) end in the making of no protein at all.

2) An alteration can result in a protein which can no longer be recognized, because it has lost its biological activity, and sometimes also its immunological properties.

3) The protein may have become unstable and may be present at a low level while being made at a normal rate.

4) Finally, some mutations may give rise to codons which require the presence of rare transfer nucleic acids and slow down the synthesis (Stent, 1964).

These mechanisms are not exhaustive (Cline and Bock, 1966; Ralph and Baguley, 1966). The only positive criterion, however, of a mutation of a structural gene remains the demonstration of a qualitative alteration of its product, and this in most cases is a very difficult task. Nevertheless, numerous examples of structural gene mutations are known.

*Regulatory, or controller genes,* govern the amount of protein to be synthesized, through a mechanism involving a repressor. When the regulator gene is functioning the protein is not made by its specific structural gene. The

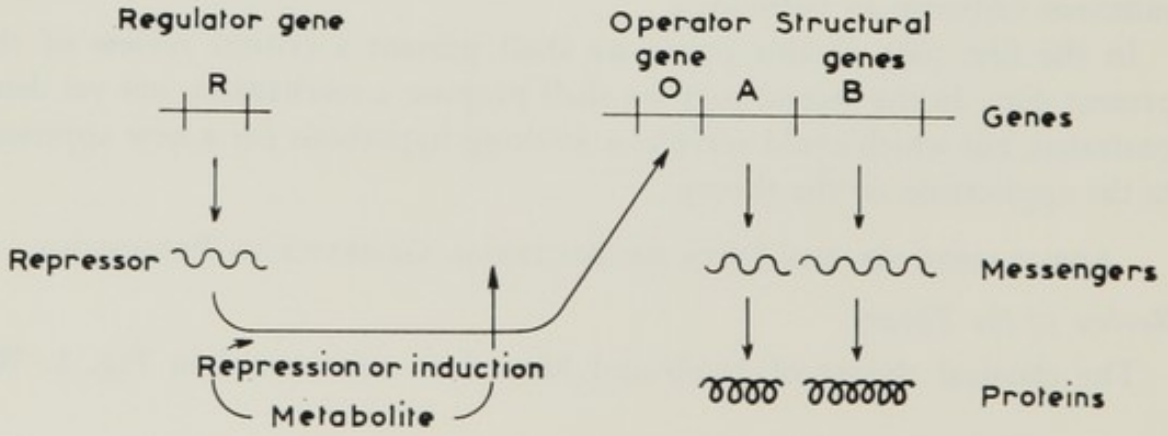



FIG. 1—Model of regulation of protein synthesis. (From Jacob and Monod)

latter becomes active when the regulator is "switched off" as the result of combining the repressor with an inducer. When several structural genes are under the control of one rgeulator gene, the system composed of the operator gene and the several structural genes is called an *operon.* Quite a few examples of operons are known in bacteria. In animals the existence of operons does not seem to be the rule. From pathological data, it seems that a few systems may be ordered in operons in the human, and we shall deal with them below. Regulator genes, in any case, act on the initial part of the operon or of an isolated structural gene; this part is called the *operator.*

A regulator gene is not necessarily close to the structural genes under its command, since its product is diffusible. Its action, therefore, in a diploid cell, is not limited to alleles in the "cis" position, but can be exerted on alleles in the "trans" position. The properties of regulator genes have consequences which must be known if one wants to apply this notion to pathology (Table 1). Two types of mutations of regulator genes are possible.

1) The most frequent is the non-functioning of the gene. The repressor is no longer synthesized, or is altered in such a way that it is no longer recognized by the operator. Such a mutation is called *regulator negative.* It results in a gain of function in a haploid organism, since the structural gene is no longer repressed and protein is synthesized continuously. Such de-repressed bacteria are called *constitutive mutants.* In a diploid organism this mutation is recessive, since the repressor made by the homologous allele acts in "trans" and suffices to repress the synthesis on both chromosomes. An alteration in a regulator gene, therefore, results in a recessive gain of function, e.g. the

TABLE 1. — *Theoretical Effects of Controller Genes or Operator Mutations*

| Mutation | Type | Function | Expression | Effect in "trans." |
|---|---|---|---|---|
| Regulator gene | Regulator negative | Gain | Recessive | — |
| | Super-repressor | Loss | Dominant | + |
| Operator | Operator negative | Loss | Recessive | — |
| | Operator constitutive | Gain | Dominant | — |

appearance of a new protein (or increase in production) in the homozygote, but no effect in the heterozygote.

2) A rarer mutation is the "super-repressor" type; the regulator gene manufactures a repressor modified in such a way that it can no longer be switched off by combining with an inducer, while being still able to react with the operator. The protein therefore is not synthesized at all. Since the repressor acts in "trans" the mutation is dominant. A mutation of the super-repressor type ends in a dominant loss of function. In pathology such a mutation would lead to the disappearance of a biological activity in the heterozygous state.

One remark may be made about the meaning of the words "dominance" and "recessivity." The use of these words has been criticized, because most characters are neither dominant nor recessive: when the difference between 2 proteins comes from a mutation of a structural gene, the presence of both can be demonstrated at the biological level; the best example is that of abnormal hemoglobins. In the case of a mutation of a regulator gene, the words "dominance" and "recessivity" recover their original meaning: the heterozygous trait should be either completely dominant or recessive to the normal allele.

3) A third type of mutation described in bacterial genetics is less frequent: it bears on the *operator* zone, which we called the "operator gene" above. Two types are possible. The first is the "operator negative" mutation: the operator is absent or altered; it prevents any synthesis of the message and the protein is not made. This loss of function is recessive since in diploid the protein is still synthesized by the homologous chromosome. In contrast, the second type is a "constitutive operator mutation": the operator has become insensitive to its repressor and this mutation is dominant. However, its effects are localized to the chromosome located in "cis." The notion of operator itself is presently under discussion (Cline and Bock, 1966; Ralph and Baguley, 1966). Even if it is accepted in its original sense, the mutations of the operator gene must be rarer than the others, since they are confined to a small segment of DNA, while mutations of structural or controller genes may lie in many points of a larger segment of DNA.

*Diseases Due to Alterations of Structural or Regulator Genes*

*The general case.* In 1963, Parker and Bearn made a thorough survey of genetic diseases, dividing them into two groups. In the first group the product of the gene is qualitatively altered, and the disease is due to an abnormality of a structural gene. In the second group, only the amount of product is changed and the product itself is not qualitatively altered. Excluding a future demonstration of an alteration, such cases could be due to an abnor-

mality of a regulator gene. In the authors' classification the second group by far outnumbers the first, which comprises little more than the hemoglobinopathies (Ingram, 1963) and some variants of glucose-6-phosphate dehydrogenase (McCurdy et al., 1966) and acetylcholinesterase (Kalow, 1962).

In the last edition of their authoritative book, Stanbury, Wyngaarden and Fredrickson (1966) extend Parker and Bearn's list and use the same approach.

Against such an affirmation we can raise two objections.

The first objection is methodological and evident: in most cases the enzymatic deficiency is such that no qualitative study proves feasible, and the anomalies have to be temporarily classified as quantitative until a check becomes possible.

The second objection is theoretical and much more compelling. We have seen that a controller gene mutation should give rise to a recessive gain of function in the usual case of a negative regulator mutation, or to a dominant loss in the rarer type of super-repressor. Now in the vast majority of biochemical lesions we observe a recessive loss of function. The homozygote is deprived of the enzymological activity and eventually displays clinical symptoms; the heterozygote is in general clinically healthy and possesses a certain amount of enzyme. These observations could be explained if the control of protein synthesis were positive: synthesis would be under direct control of an inducer, whose presence in the heterozygous state would be enough to trigger it. The control being negative proves incompatible with the facts.

In order to overcome this difficulty, Parker and Bearn have used a more complex scheme proposed by Monod and Jacob (1961), which is shown in Fig. 2.

On an operon 2 there is a regulator gene, $RG_2$, which is able to repress operon 1 and to prevent the expression of structural genes $SG_1$ and $SG_2$. But operon 1 also synthesizes a regulator gene, $RG_1$, whose unique effect is to react with $O_2$: it therefore represses $RG_2$ allowing $SG_1$ and $SG_2$ to function. If $RG_1$ does not work, then $RG_2$ becomes active and the synthesis of $SG_1$ and $SG_2$ products is repressed. In such a way one can conceive that a

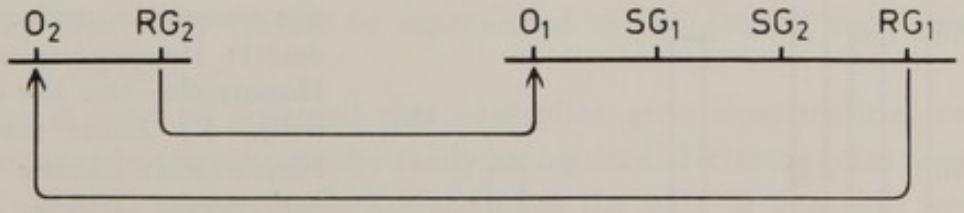



FIG. 2—Reciprocal model of controller gene action. Operon 1 is repressed by $RG_2$. On operon 1 is a regulator $RG_1$ which represses $O_2$. Inactivation of $RG_1$ allows $RG_2$ to function and to block the functioning of operon 1. (This is model 5 of Monod and Jacob, *C. S. H. Symposium*, 1961, pg. 399.)

disappearance of the regulator gene gives rise to a loss of function. But here again two objections oppose this conclusion. Given the number of quantitative alterations which are described in pathology, it is unlikely that such a complex mechanism could be at work each time, while that of a simple regulator negative mutation would rarely come into play. A more serious difficulty is the following: a regulator negative mutation is completely recessive in bacteria. In zygotes, bearing 2 chromosomes, and obtained by conjugation of a constitutive (regulator negative) and of a normal wild strain, the rate of enzyme synthesis is the same as in the normal controls. Now we shall see that this is not the case in pathology, and that the heterozygotes do not possess a normal level of enzyme.

The above model could only, in the best case, apply to genes present in a haploid set, and Parker and Bearn (1963) took the precaution to describe it for the case of glucose-6-phosphate dehydrogenase: this enzyme, borne by the X chromosome, is present in a haploid state at least in the male and is probably functionally haploid in the female according to the mosaic hypothesis (Lyon, 1961; Beutler et al., 1962; Kaplan, Dreyfus and Bessis, 1965). But one cannot generalize from such a model.

Precise determination of the amount of enzyme produced in the tissues of individuals heterozygous for an enzymatic deficiency shows it to be decreased by about half. (Fig. 3) Moreover, the standard deviation is not higher in heterozygotes than in controls, with the exception of glucose-6-phosphate dehydrogenase. One observes, therefore, the remarkable fact that when 1 gene out of 2 is functioning, the tissues produce about half of what is produced by a double gene dose (Sutton, 1965). This demonstrates that the gene

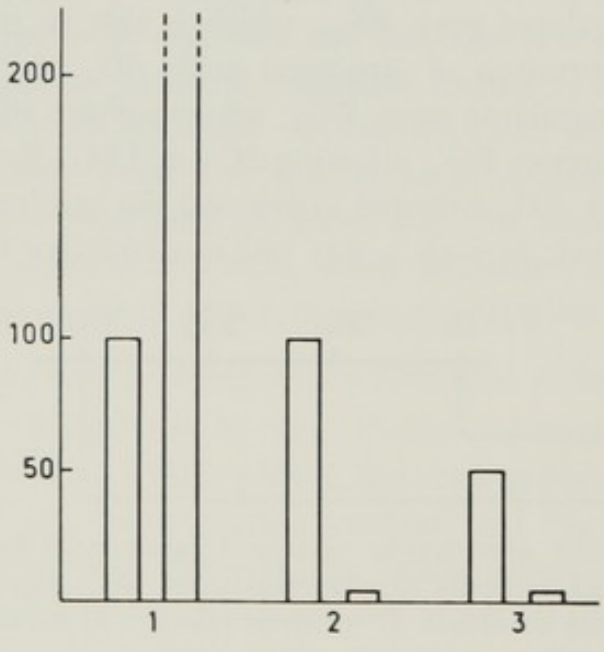



FIG. 3—Enzyme activity in hetero- and homozygotes according to various genetic hypotheses. 1.) Straightforward prediction of bacterial genetics for controller gene deficiency: heterozygote—no effect. Homozygote—increased activity. 2.) prediction of a controller gene model with a positive control (i.e. products and inducer): Heterozygote—no effect. Homozygote—very low or no activity. 3.) Actual observations. Heterozygote: decrease to about half normal activity. Homozygote: very low or no activity. (100 = normal activity.) Left column: heterozygote. Right column: homozygote.

inherited from the healthy parent is neither inhibited nor stimulated by its partner. This relationship holds true, whether there is a complete loss of synthesis of the enzyme, or if there is a modified inactive protein, whose presence is attested by immunological reactions (cross-reacting material) (Robbins, 1960; Hodgkin et al., 1965). Each gene then functions by itself regardless of the action of its allele. The unavoidable conclusion is that in pathology, when a mutated gene synthesizes an inactive product or no product at all, no effect is exerted on the active allele to increase its production. The controller gene, if it exists, remains a passive witness in the presence of a mutation. Such a remark has already been made by Harris (1964).

When a protein deficiency is observed in plasma the results are less clear-cut. In the only known case of atransferrinemia, both parents had a level diminished by half (Heilmeyer et al., 1961). By contrast, in the case of analbuminemia (Bennhold et al., 1954) the parents' serum-albumin was normal. The same is true in agammaglobulinemia (Good et al., 1962). Until recently it was impossible to detect a decrease in plasma factor VIII in women with hemophilia trait (Rapaport et al., 1960) but recent advances now allow the detection of most hemophilia carriers. At any rate, results obtained with plasma appear less convincing than those gained by direct determination of enzyme activity in the producing tissue. Regulation of plasma proteins, which are made in the organs but poured out immediately into the blood stream, probably obeys laws which are far from being only genetic. They are likely to involve feed-back signals, among which their concentration in plasma may be the major one.

In conclusion, from the above there stands out a fundamental fact: nearly all single hereditary biochemical lesions display a loss of function. This loss is complete or nearly complete in the homozygous state, partial and generally remarkably near 50 per cent in the heterozygous state. This well-known observation is not compatible with an abnormality of a regulator gene, as described for bacterial genetics. It would be compatible with mutations of the type "operator negative," which would actually provoke a loss of function—complete in the homozygote, partial in the heterozygote. But the operator zone forms a very small part of the genome. While operator negative mutations may exist, they cannot be represented as the general case, neither in man nor in bacteria.

Finally, it must be observed that controller gene abnormalities could be more frequent but would not be easily recognized. Heterozygotes would display no anomaly whatever. In homozygotes the gain of function would result in an increase in one enzymatic activity. If this enzyme is already normally not rate-limiting, chances are that this modification remains undetected.

*Operons in Higher Animals and Man*

No clear-cut demonstration of the existence of operons in higher animals and in man has been presented. Most tissues show well-defined enzyme patterns. Some enzymes, notably of the glycolytic pathway, are present in constant proportions, and the hypothesis of a common operon, with a polycistronic messenger, has been put forward for these enzyme groups (Pette et al., 1962; Mier and Cotton, 1966). The operon hypothesis is only one of the possible explanations of physiological regulation, and no decisive argument can yet be given for or against it.

Two pathological cases, rare but interesting, suggest the intervention of an operon.

*Sucrose intolerance.* Sucrose intolerance is due to a lack of intestinal sucrase, whose absence causes severe digestive troubles because of bacterial fermentation. But there is also a deficiency in maltase ($\alpha$ 1 $\rightarrow$ 4 glucosidase) and isomaltase ($\alpha$ 1 $\rightarrow$ 6 glucosidase) (Auricchio et al., 1961; Rey and Frezal, 1967). According to Dahlquist et al. (1963) these activities are not borne by the same enzymes, although no clear-cut chromatographic separation has yet been possible. In heterozygous parents a partial deficiency of the 3 activities can be shown in intestinal biopsies.

*Oroticaciduria.* In 1959 Huguley et al. described a refractory megaloblastic anemia with abundant urinary excretion of orotic acid. Four such cases are now known (Smith et al., 1966; Neimann et al., 1965). The interest in making the diagnosis is that the anemia can be alleviated by giving cytidylic and uridylic acids. The disease is due to a defect of pyrimidine biosynthesis which hits 2 successive enzymes catalyzing steps of the synthesis, e.g. orotidylic acid pyrophosphorylase and decarboxylase. The level of both enzymes is decreased in the erythrocytes of the parents and other relatives of the patients.

The 2 cases which we just quoted may be interpreted as evidence for operons. The "switching off" of the operon would be evidenced by a deficiency of its constituent enzymes (3 for sucrose intolerance, 2 for oroticaciduria). We still must insist on the fact that if an operon is at hand, it does not follow that a regulator gene mutation is involved, since here again we observe a recessive loss of function. Two interpretations can be offered: an operator negative mutation, or, more likely, a polar mutation: this would be, for example, a nonsense mutation of the first structural gene of the operon, which may elicit a complete interruption of messenger translation of the rest of the operon.

However, the operon interpretation is not the only one possible. These enzymes with very close activities may be made of several subunits, one of

which could be common and would be affected by the mutation. A direct approach to the problem through the analysis of pure enzymes will be necessary to settle the discussion.

Very recently Pinsky and Krooth (1967) have shown that in tissue cultures of leukocytes from a case of oroticaciduria the missing enzymes are synthesized in the presence of azauridine. The mechanism of this activation is not clear, but a mutation of a structural gene seems to be excluded by this observation.

*Possible Cases of Regulator Gene Intervention*

In a small number of cases, the regulator gene concept can withstand the criticisms raised above. A few of them merit discussion.

1) Dominant diseases could be due to a mutation of the super-repressor type. Most of them display a complex clinical picture with multiple symptoms; the initial lesion cannot be recognized. One notable exception could be von Willebrand's disease. We shall discuss this disease in detail in the last section of this paper.

2) Presently the diseases for which the controller gene concept fits best are the *porphyrias*: they appear under 2 genetically and clinically different types, erythrocytic and hepatic porphyrias.

a) There are 2 forms of erythrocytic porphyrias. The first one is the erythropoietic congenital porphyria. It is transmitted as an autosomal recessive trait, and is marked by an overproduction of type I porphyrins. The synthesis of type III porphyrins and of hemoglobin is not impaired. The hypothesis of an excess activity of porphobilinogen deaminase therefore seems logical. It has been supported by the work of Watson et al. (1964): the bone marrow of a porphyric beef synthesizes an increased amount of type I and III porphyrins in the presence of porphobilinogen.

The second form is as exceptional as the first one: erythropoietic protoporphyria is generally accepted as a dominant abnormality (1966), but its genetic transmission is still unclear. It results in an over-production of free protoporphyrin. Porter (1963) has studied 2 brothers with probably recessive inheritance. He showed that their bone marrow makes an excessive amount of protoporphyrin when incubated with glycine and α ketoglutaric acid. The exact biochemical lesion has not been demonstrated. However, it is apparent that a recessive increase of an enzymatic activity exists, in agreement with the production of a regulator negative mutation.

The lesion is probably different in the 2 forms of the disease. That it may be the same has not been definitely excluded, however. In some patients the isomerase which transforms type I into type III porphyrins would be active enough to cope with the increase due to excess activity of the deaminase,

leading to protoporphyrin accumulation (Fig. 4, III). In others the isomerase would be less active and type I porphyrins would accumulate (Fig. 4, II). Such a situation would result from a polymorphism of isomerase, or from a simultaneous derepression of both enzymes.

Even here a divergence exists from the straightforward predictions of bacterial genetics: in human heterozygous patients and in the one heterozygous bone marrow studied by Watson (1964) increased production of type I porphyrins can be demonstrated. One intact regulator gene therefore would not be sufficient in man to prevent excess production of porphobilinogen deaminase.

*b)* Among *hepatic porphyrias* the best known is *acute porphyria.* It is inherited as an autosomal dominant trait, appears in most cases in the young adult and shows bursts of activity. The patients excrete high levels of δ aminolevulinic acid and porphobilinogen in their urine. A remarkable phenomenon is that the syndrome is elicited, in man as well as experimentally, by a variety

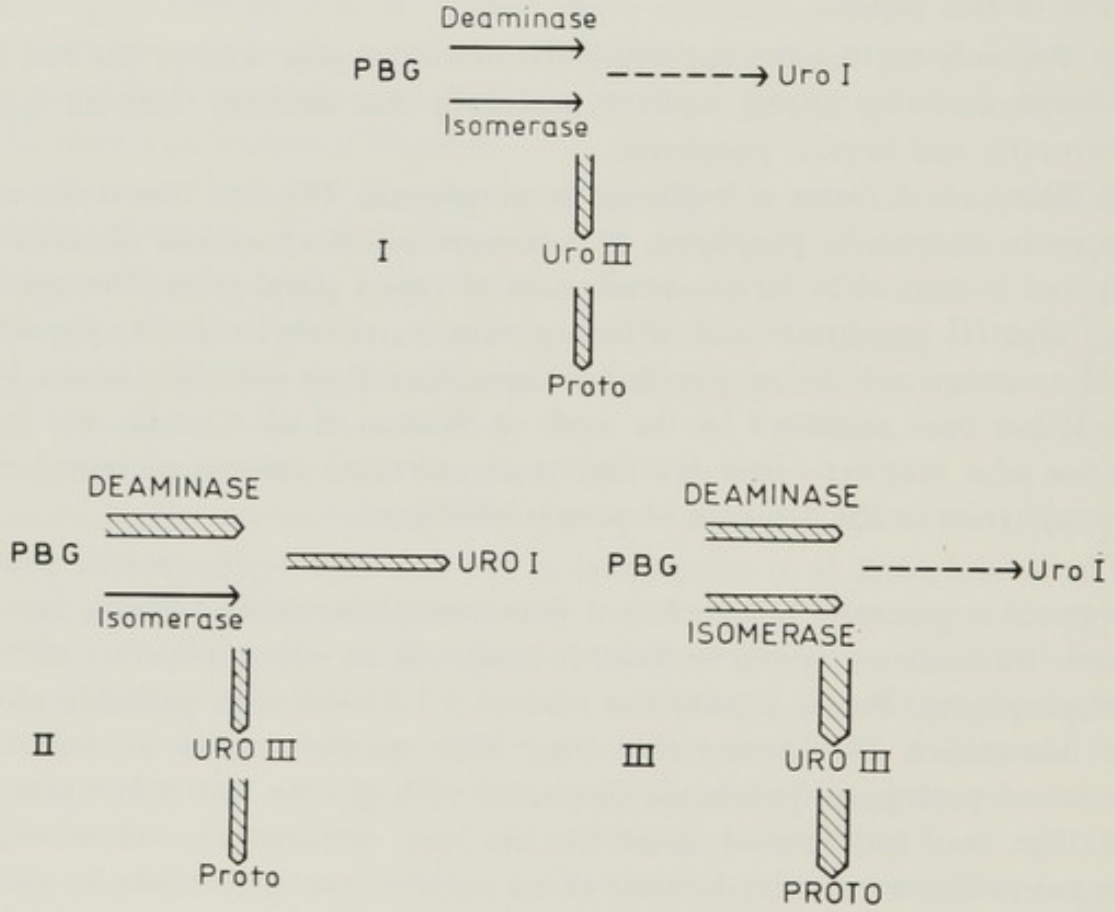



FIG. 4—Erythropoietic porphyrias. I: Normal pathway: simultaneous action of deaminase and isomerase results in bulk production of isomer III with only traces of isomer I. II: Erythropoietic porphyria: excess of deaminase with normal activity of isomerase results in excess production of porphyrin, normal amounts of isomer III and increase in isomer I. III: protoporphyria: excess of both deaminase and isomerase results in excess production of isomer III.

of drugs, particularly allylisopropylacetamide. The biochemical lesion was demonstrated in the experimental animal by Granick (1965) and in the porphyric man by Tschudy et al. (1965); there is a conspicuous increase of the first enzyme of heme synthesis, δ aminolevulinate synthetase. In this dominant disease, a gain of function in the heterozygous state cannot be explained by a control gene mutation. The only interpretation compatible with bacterial genetics is an operator constitutive mutation: the operator, insensitive to the action of its normal repressor, functions permanently and produces an excessive amount of enzyme. While plausible, the idea of a mutation of the small operator zone must be regarded with caution: so much more so, because hyperproduction must be very moderate in early life, since the disease does not, as a rule, manifest itself in childhood. In experimental porphyrias the mechanism must be different and is quite unclear; one would have to assume that the various drugs capable of inducing porphyria would combine with the repressor to inactivate it.

*3) Resistance to Antivitamins K*

A family was described in 1965 (O'Reilly and Aggeler, 1965) in which very high doses of warfarin were necessary to increase the prothrombin time. By contrast, the sensitivity to antidotal action of vitamin K was increased. In addition, in the absence of any treatment, moderate decreases of various clotting factors whose synthesis is dependent on vitamin K were observed in 6 out of 8 members of the family. The mode of transmission was dominant. The authors interpreted their observations as possibly due to an alteration of a controller gene. The anomaly would be of the super-repressor type, since in heterozygous people normal doses of warfarin have no effect at all. However, we are dealing here with a very complex situation: the decreased clotting factors were not the same in different members of the family. In addition, the mechanism of action of vitamin K is not yet known. This very interesting case must, therefore, be considered with caution.

*Gout*

Gout can be discussed in this connection (Wyngaarden, 1966). Many gouty patients synthesize an excess of uric acid, as shown by the increased incorporation of labeled glycine into uric acid (Benedict et al., 1952). This increase may be due to several causes, including a relative block in the normal synthetic pathway of nucleotides, provoking a short-circuit towards free purines. If a true increase takes place, the following possibilities have been suggested:

a) Increased activity of one of the enzymes of the pathway, (glutamine, phosphoribosyl pyrophosphate amidotransferase) (Pardee and Wilson, 1963).

b) Modifications in the feedback mechanism which may control the rate of purine synthesis (Caskey et al., 1964; Nierlich and Magasanik, 1965).

Neither of the above hypotheses has been substantiated. Their interpretation requires an exact knowledge of the heredity of gout, which is still controversial (Smyth, 1957). If, according to classical data, gout is inherited as an autosomal dominant trait, a controller gene disease can be rejected: a dominant gain of function is not a consequence of abnormalities of regulator genes. Actually, it has now been demonstrated that some cases of overproduction of uric acid are due to the deficiency of an enzyme, hypoxanthine-guanine phosphoribosyl transferase (Kelley et al., 1967).

*Hemoglobin Diseases*

The problem of genetics of hemoglobin diseases is extremely complex and would require an article by itself. Many papers have attempted to interpret it (Neel, 1961; Motulsky, 1962; Zuckerkandl, 1964) without being able to account for all the contradictory facts.

Hemoglobin is made of 4 chains of 2 types. One type of chain, the $\alpha$ chain, is present in all human hemoglobins. It combines with non-$\alpha$ chains which may be:

*(a)* $\beta$ chains, giving normal adult hemoglobin Hb A. Its formula is $\alpha_2\beta_2$, it makes up to 98 per cent of the hemoglobin in the adult.

*(b)* $\gamma$ chains, giving foetal hemoglobin Hb F. Its formula is $\alpha_2\gamma_2$; it is the major hemoglobin of the fetus and the newborn.

*(c)* $\delta$ chains, giving with a chains hemoglobin A2. Its formula is $\alpha_2\ \delta_2$; it amounts to 2 per cent of the hemoglobin in the adult.

*(d)* $\varepsilon$ chains, normally only present in the embryo.

After birth fetal hemoglobin disappears within 6 months, being progressively replaced by Hb A. The synthesis of $\beta$ and $\delta$ chains, repressed during fetal life, develops after birth while that of $\gamma$ chains stops. In some diseases $\gamma$ chains continue to be made.

The diseases of hemoglobin are of 2 types: the first one is that of abnormal hemoglobins. These abnormalities are due to a mutation of a structural gene and will not be discussed here. The second one deals with quantitative modifications of production of one or more chains, without apparent change in the nature of the chains. Since we cannot hope to cover the whole field, we shall limit the discussion to the following points:

$\beta$ thalassemia
$\delta\beta$ thalassemia
High fetal gene
Hemoglobin Lepore

We shall consider only the major characteristics of these forms, which are summarized in Table 2.

*a)* β thalassemia is defined as a deficiency of the production of β chains. The homozygous state shows the picture of thalassemia major, with little or no Hb A, a considerable increase in Hb F, and variable, but generally normal, amounts of Hb $A_2$. In the heterozygous state (thalassemia minor or minima), there is only a small increase or no increase in the Hb F, and the most marked biochemical symptom is an increase in Hb $A_2$ concentrations.

β thalassemia therefore results in a decrease of production of β chains with a maintenance or even an increase of production of α chains. The β thalassemia gene acts only in "cis." This can be seen in double heterozygotes for β thalassemia and a structural anomaly of β chain, like sickle cell trait; no decrease is observed in the "trans" allele and the production of Hb S is high (Silvestroni and Bianco, 1946; Singer et al., 1955).

While β thalassemia indeed presents itself as an imbalance of production of the various chains of hemoglobin (Bank and Marks, 1966; Heywood et al, 1964), it is quite possible that other mechanisms come into play: a *nonsense* mutation, suppressing production of β chains; defective messenger blocking the ribosomes, leading to lack of functional protein (Ingram, 1964); or mutation giving rise to another codon which would fit to a rare transfer RNA and would, therefore, slow down translation according to Stent's model (Stent, 1964).

TABLE 2. — *Comparative Effects of Current* β *Thalassemia,* δβ *Thalassemia, High F Gene (Negro type), and Lepore Hemoglobin*

| | β thal | δβ thal | High F gene | Lepore |
|---|---|---|---|---|
| β | diminished or absent | absent | absent | absent |
| δ | increased | absent | absent | absent |
| γ | increased | increased | increased | increased |
| *Percentage in red cells:* | unequal | unequal in hetero-zygotes | equal in hetero-zygotes | |
| *Effect in trans:* | none | none | none | none |
| *Clinical symptoms:* | + | + | none | + |

*b)* Besides this usual form of β thalassemia there exists a rarer form, called δβ thalassemia. In this case the decrease of production affects equally β and δ. In the homozygote, as described by Brancati and Baglioni (1966) there exists only Hb F, no Hb A nor Hb $A_2$ being produced. The heterozygote possesses a certain amount of Hb F (10 per cent on an average) and a normal level of Hb $A_2$. Here again, as shown in double heterozygotes for δβ thalassemia and sickle cell disease (Comings and Motulsky, 1966) the suppression of β chain synthesis takes place only in "cis" position.

*c)* Persistence of fetal hemoglobin (high F gene) is observed in some American and African Negroes (Edington and Lehmann, 1955; Herman and Conley, 1960). These people are free of any clinical or hematological trouble. Their hemoglobin contains 20 to 30 per cent of Hb F with a decrease of $A_2$.

The only known homozygote shows only minor hematological symptoms. His hemoglobin is entirely of the Hb F type, without any trace of A or $A_2$ (Wheeler and Krevans, 1961).

*d*) At first sight the pictures of thalassemia (especially in its δβ form) and high fetal gene are very similar. However, 2 major differences separate them:

> Thalassemia is a severe hematological disease, while the carrier of high fetal gene is not sick.
>
> All red cells of the heterozygote for high fetal gene carry an approximately equal percentage of Hb F. By contrast the percentage of Hb F varies considerably in red cells of thalassemic subjects, as is the case in the newborn (Betke and Kleihauer, 1958). Red cells are therefore genetically homogeneous in the high fetal gene trait, heterogeneous in thalassemia.

Genes which govern the synthesis of β and δ chains are genetically linked (Boyer et al., 1963; Horton and Huisman, 1963). They are very close to each other on the same chromosome, and even, according to some authors, belong to the same operon (Neel, 1961; Motulsky, 1962). The latter hypothesis has to meet several objections, the main difficulty being the opposite effects on the amount of β and δ chain production in the usual β thalassemia.

If now we limit the discussion to a comparison between δβ thalassemia and high fetal gene, 2 major hypotheses can be presented: a deletion of the β δ segment of the genome, or an abnormality of the control system of the β and δ genes, and allowing the γ gene to function, is possible. A similar mechanism is at play in the Lepore hemoglobin, to be discussed below. Comings and Motulsky (1966) favor the idea that a deletion is the cause of δβ thalassemia.

But if this is really the case, then the same mechanism cannot apply to the different picture of the high fetal gene. One is led to assume the interven-

tion of a controller gene: the repression of β and δ synthesis would go on after birth. This repression is complete in the African type of high fetal gene, since only Hb F is made by the homozygote. It is only partial in the form described in Greece by Fessas and Stamatoyannopoulos (1964), since the production of β and δ chains is not completely suppressed.

However, the controller gene hypothesis encounters several difficulties. A regulator negative mutation affecting β and δ does not seem possible: it would result not in a block, but in an increased synthesis. A super-repressor mutation should be dominant and act in "trans." A last problem stems from the fact that synthesis of both β and δ is repressed: in the presence of a co-ordinated repression one is led again to the operon hypothesis, but this is confronted with the difficulties outlined above.

*e)* Hemoglobin Lepore (Gerald and Diamond, 1958) is an unusual protein: its complete analysis has shown it to comprise 146 amino acids like β and δ chains. Its N terminal part has the sequence of the δ chain, its C terminal part has the sequence of the β chain (Baglioni, 1962; Labie et al., 1966). Its most probable mechanism of appearance is that of an unequal cross-over between δ and β genes. As in δβ thalassemia or high fetal gene, there is a loss of production of normal β and δ chains, and the homozygote has only F and Lepore, and no A or $A_2$ hemoglobin. The Lepore syndrome shows that a partial deletion (loss of carboxyl part of δ and amino part of β) is able to give a δβ-like thalassemia syndrome, and reinforces, in this case, the deletion theory.

In addition, Lepore hemoglobin permits one more consideration (Fig. 5). The postulates of bacterial genetics allow the prediction of the rate of syn-

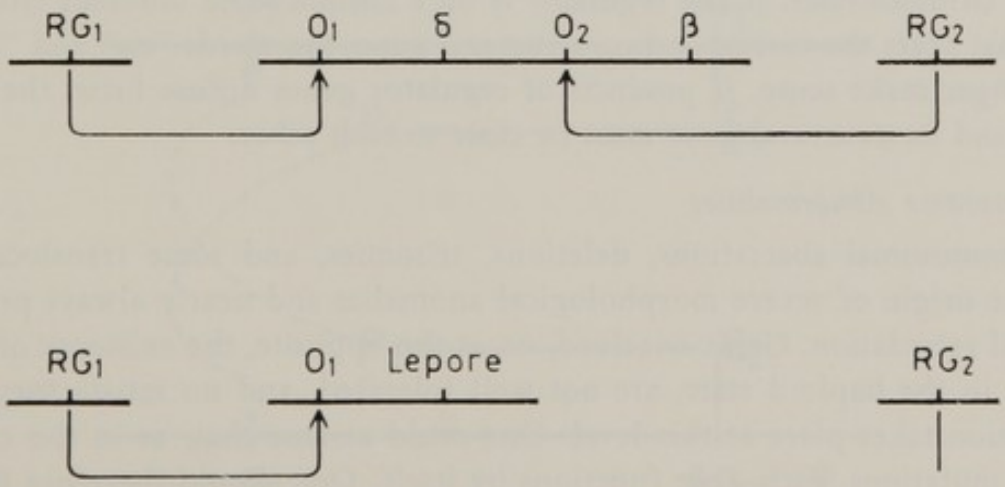



FIG. 5—Hypothetic control of δ and β chain synthesis in normal men and patients with hemoglobin Lepore. *Above:* Normal situation. $RG_1$ and $RG_2$ control δ and β chain production. Rate of synthesis of δ is only 2% of that of β. *Below:* Hemoglobin Lepore: Initial part of β, including operator, has disappeared. Control is maintained only on δ operator. Rate of synthesis should be that of δ.

thesis of Hb Lepore if the regulation takes place at the transcription level. Normally β and δ chains are synthesized in a ratio of 50 to 1. In Hb Lepore a deletion has eliminated the end of δ and the beginning of β chain. The operator zone of β has also necessarily disappeared, since the sequence of Lepore is only made of normal sequences of δ and β chains. No regulation, therefore, can be exercised on the synthesis of β, and only the regulator gene for δ and the δ operator remain. Under such conditions the rate of synthesis should be that of δ; in fact, it is 5 times faster. The rate of synthesis of Hb Lepore apparently reflects a compromise between those of β and δ, and cannot be explained by the interplay of factors deduced from bacterial genetics. (In the foregoing discussion we have assumed the existence of 2 distinct regulators and 2 operators for β and δ. The reasoning would still be valid if β and δ were part of the same operon).

*f)* In conclusion, the mechanisms of thalassemia and high F gene are far from being well understood. It is quite evident that they involve regulatory disturbances. But the latter are not readily explained by the interplay of regulators and operators as described by bacterial genetics. At any rate, one fundamental postulate would have to be revised: thalassemia, as well as the high fetal gene, only acts in "cis" position. If a regulator gene acts via a repressor, one must accept the conclusion that in the human, repressor products of regulatory genes are not diffusible and cannot reach the "trans" allele, as they readily do in bacteria.

This would have far-reaching consequences: in a bacterium there is only one chromosome. A regulator gene, even if distant from its operon, is on the same chromosome and therefore in "cis" position. In mammals there are many chromosomes: if the regulator is on a chromosome different from the one that bears the corresponding structural gene, the words "cis" and "trans" no longer make sense. If products of regulator genes diffuse little, the regulator and its structural gene must be close to each other.

*Chromosome Abnormalities*

Chromosomal aberrations, deletions, trisomies, and some translocations, are the origin of severe morphological anomalies and nearly always provoke mental retardation. Genic overload, or, at the opposite, the existence of some DNA in the haploid state, are not well tolerated, and no satisfactory compensation takes place at this level. One could assume that, as in the case of point mutations, each gene functions by itself. One should therefore find in the tissues some activities increased by 50 per cent in trisomies, or diminished by half in deletions (Rucknagel, 1964; Lejeune, 1963). Such imbalance of a number of proteins could, of course, give rise to the observed disturbances. It must be admitted that no unequivocal data exist to substantiate this

view, despite numerous attempts, made blindly indeed since no genetic map exists in higher animals. The first observations made in mongolism (increase in alkaline phosphatase) (Trubowitz et al., 1962) or in galactose-1-phosphate uridyltransferase (Brandt et al., 1963) are difficult to interpret (Mellman et al., 1964; Shih et al., 1965). Perhaps the increase in phosphofructokinase (Baikie et al., 1965) in these patients is more valid, but this has still to be confirmed. Preliminary studies made in our laboratory by Dr. Fanny Schapira do not seem to support it.

If the search for enzymatic disturbances in cases of chromosomal aberrations remains negative, one could be led to conclude that they are compensated in the adult. Such a compensation must be late and certainly does not exist in the embryonic stage when the lesions are developing. Even the X chromosome, which, according to the Lyon hypothesis, has only one active copy in each female cell, does not behave this way during ontogenesis: otherwise a person with a 45X formula would be a normal woman instead of one with a Turner syndrome (Ford et al., 1959); a person with 47XXY formula would be a normal man instead of showing symptoms of Klinefelter's syndrome (Jacobs and Strong, 1959). It will require a more thorough enzymological study of chromosomal abnormalities during development to solve questions which remain presently unanswered.

## MODEL OF CELL DIFFERENTIATION AND GENETIC DISEASES

At the end of their Cold Spring Harbor Symposium paper (1961) and chiefly in the book *Cytodifferentiation and Macromolecular Synthesis* (1963), Monod and Jacob have attempted to extend their concepts on the control of

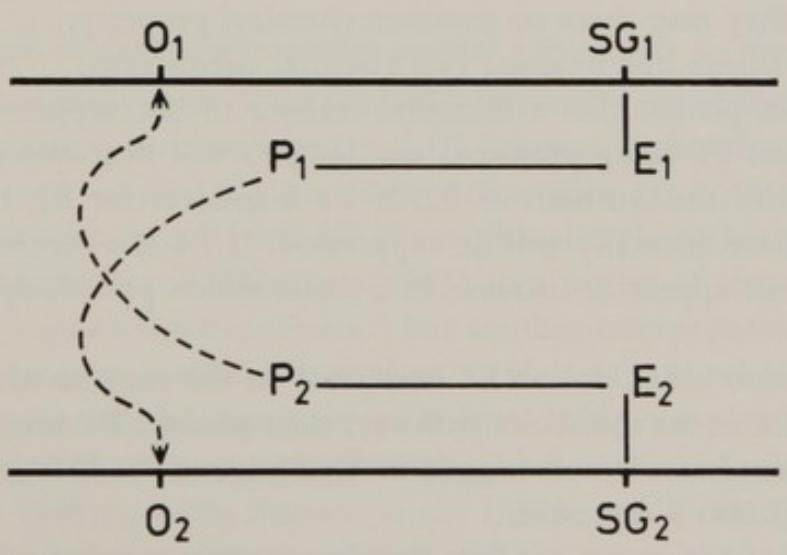



FIG. 6—Model of gene interaction. Two genes $SG_1$ (making enzyme $E_1$) and $SG_2$ (making enzyme $E_2$) are located on different operons. Product $P_1$ of enzyme $E_1$ acts as an inducer or a repressor on $O_2$. Product $P_2$ of enzyme $E_2$ acts as an inducer or a repressor on $O_1$.

protein biosynthesis to multicellular organisms. They described several models of interactions between the products of various genes or operons, from which distinct patterns of synthesis could derive. These models were given as possible examples, and their meaning and implications have not been further investigated. However, they are liable to at least theoretical checks, since they imply predictions which can be compared with what actually exists.

Many models could be reviewed. For convenience, we shall only discuss one of the models of Monod and Jacob (Fig. 6): it involves 2 independent structural genes, SG1 and SG2. They govern the synthesis of the 2 enzymes E1 and E2, which catalyze the production of the products P1 and P2 from their precursors. Product P1 acts on the synthesis of E2, product P2 acts on the synthesis of E1. The authors describe 3 variants to this interaction:

1) P1 induces the synthesis of E2, and P2 that of E1: both enzymes being made simultaneously, or being simultaneously absent.

2) P1 represses the synthesis of E2, and P2 that of E1: only one of the two enzymes can be made, since its very functioning prevents the synthesis of the other.

3) P1 induces the synthesis of E2 but P2 represses that of E1. E2 will be permanently functional and E1 never, except if E2 happens not to be made for some physiological or pathological reason.

In addition to this "reciprocal" model it would be possible to build other types, e.g. in "cascade," in which P1 would induce (or repress) the synthesis of E2, while P2 would act on the synthesis of a third enzyme E3. The reasoning which follows may fit to any type of model. The essential point is that the products P1 and P2 do not necessarily belong to the same metabolic pathway and that they may share no common chemical property.

The model allows one to make two essential predictions:

First, we can predict that a mutation leading to the suppression of synthesis of E1 (no P1 being produced any longer) will bear as a consequence a modification of the synthesis of E2. If P1 is inducer for E2, the synthesis which takes place normally will be suppressed. If P1 is a repressor the synthesis of E2 will appear or increase in a tissue which previously made none or little of it.

If the mutation bears, not on E1 itself, but on the enzyme which immediately follows E1 in the metabolic pathway, then product P1 which lies below the block accumulates: it will trigger the synthesis of E2 if it is an inducer, or prevent it if it is a repressor.

In conclusion: the model predicts that in a certain number of cases a mutation on an enzyme should not remain isolated, but should carry with it a modification, plus or minus, of one or several other enzymes.

The second prediction of the model is that those secondary abnormalities

may bear or not bear any apparent metabolic relationship with the primary event: one must expect that in some cases no rational biochemical explanation will be available.

The question, therefore, is to know if such associations actually exist in pathology. They would be a powerful argument in favor of Monod and Jacob's model of cell differentiation. At first sight these associations do not appear to be frequent. But two reasons why we may not observe them must be taken into account: if these associations are too active, acting in cascade, the resulting damage may be incompatible with survival, or the picture too complex to be analyzed. On the other hand, when an observation is made which seems to be unrelated to the primary lesion and to be inexplicable it is often disregarded. It may be that some pleiotropic dominant lesions fit this frame.

However, it is possible to find in the literature a number of examples of combined abnormalities, whose present interpretations are either unconvincing or even have been disproved. In some of the examples given below the two lesions are metabolically related. In some they are apparently not.

*A Number of Associations Which Have Been Observed in the Course of Genetic Diseases*

1) In several diseases of carbohydrate metabolism one observes an increase in liver glucose-6-phosphatase activity: it is found in galactosemia, fructose intolerance and even in diabetes. We do not insist on the increase which has little specificity. Microsomal enzymes can be activated by a variety of drugs (Reynold and Cahill, 1966; Conney et al., 1957; Langdon and Weakley, 1955).

2) Glycogen storage diseases offer several approaches to the problem.

*a)* In a good many cases 2 simultaneous deficiencies are observed. The most frequent association involves type I and III [glucose-6-phosphatase (Cori and Cori, 1952) and amylo-1-6-glucosidase deficiencies (Cori, 1957)], but an association between type III and VI (liver phosphorylase) has been noted at least twice. Auerbach and diGeorge (1965) in a review of these associations, propose the "copy-choice hypothesis." But another interpretation can be proposed on 2 pieces of evidence. According to Hers (1959) who described the type VI glycogenosis, the deficiency is far from being complete (Hers, 1964) as it is in the primary muscle phosphorylase deficiency (Mc Ardle disease) (Schmid and Mahler, 1959; Pearson et al., 1959); and it is suspected that it might be secondary to some other lesion. On the other hand, Moses (1966) has recently treated a case of type I + type III association with triamcinolone and observed an increase in glucose-6-phosphatase activity. This indicates that glucose-6-phosphatase deficiency was indeed secondary in this case.

*b)* Normally the activity of glucose-6-phosphatase and fructose diphosphatase is high in the liver and very low in the muscle, so that the synthesis of glucose and glycogen from pyruvate is minimal in muscle. According to Mahler (1966), in type I glycogenosis (lack of liver glucose-6-phosphatase) muscle fructose diphosphatase is greatly increased, and glycogen synthesis from pyruvate becomes noticeable.

*c)* Finally, several authors have demonstrated an increase in plasma uric acid level in type I glycogenesis (Jeune et al., 1957). The currently accepted explanation is that hyperlactacidemia, which is constant in this disease, lowers the renal clearance of uric acid (Jeandet and Lestradet, 1961; Howell et al., 1962). However, incorporation of glycine I-$^{14}$C into uric acid has been found to be excessive (Alepa et al., 1962) and the hypothesis of an overproduction of phosphoribosyl-pyrophosphate appears plausible.

3) Complete deficiency in red cell glucose-6-phosphate dehydrogenase as observed in Caucasians (as opposed to partial deficiency in Negroes) is accompanied by a decrease of red cell acid phosphatase activity (Oski et al, 1963).

4) In sickle cell anemia Henstell et al. (1965) described a coagulation defect, bearing probably on thrombin, which provokes a faulty thromboplastin generation test.

5) In thalassemia major the biosynthesis of red cell stroma is modified: incorporation of glucosamine proceeds 5 to 10 times too fast, giving evidence of a disorder in the building of membrane (Eylar and Matioli, 1965).

6) In hemophilia A, Blömback et al. (1965) described a greater heterogeneity in molecular size of fibrinogen.

7) Injection of a mixture of l-tyrosine and gluconate, or better, the compound d-gluconyl-l-tyrosine, initiates fibrinolysis *in vivo* within a few minutes. Tyrosine cannot be replaced with another amino acid, nor gluconate with glycuronate. The compound does not lyse fibrin *in vitro*. Kopper (1966) believes that the active compound acts as the prosthetic group of an apo-enzyme. It may equally as well act as an inducer for one of the factors of fibrinolysis.

8) Finally, it seems that 2 well-known diseases deserve to be investigated along these lines:

*a)* In *pernicious anemia* one observes on the one hand an anemia, on the other hand a very special neurological syndrome. Lack of vitamin $B_{12}$ has an effect on the formation of a type of cell (erythroblasts) that is among those showing the highest rate of mitosis. It also strikes nervous tissue, where mitoses are minimal or absent. Insufficient knowledge of the exact mechanism of action of vitamin $B_{12}$ (Wagner, 1966) prevents a more thorough discussion, but it may well be that the integrity of the nervous system is dependent on

some metabolite produced under the action of vitamin $B_{12}$ (Cox and White, 1962).

*b) Wilson's disease,* or hepatolenticular degeneration, displays 2 major metabolic disturbances, each of which has been advocated as the primary event: one is a disturbance of copper metabolism, consisting of a deficiency in a copper-rich plasma protein, ceruloplasmin (Scheinberg and Gitlin, 1952), and an increase in intestinal absorption of copper, resulting in a copper overload of many organs, especially the liver and the central nervous system. The other is a generalized aminoaciduria stemming from a lack of tubular reabsorption of amino acids (Iber et al., 1957). Many authors have attempted to connect these 2 types of deficiency, but the evidence is not conclusive (Bearn, 1966).

Examples which we have just given are not all convincing and some may prove explainable in other ways. They are few, but they certainly do not exhaust the list of published associations. In addition one may remark that most of them have been described during the last 2 years. It is therefore likely that we shall find more of them in the future.

*A Review of Two Diseases Which Have Been Studied in More Detail*

*Von Willebrand's disease.* Von Willebrand's disease presents itself with a variable clinical and biological picture and its limits are still under discussion. However, it is agreed that it includes 2 associated hematological symptoms (Alexander and Goldstein, 1953; Larrieu and Soulier, 1953; Quick and Hussey, 1953):

The first is a vascular disturbance, which is the origin of the prolonged bleeding time, accompanied by a disorder of platelet adhesivity. The second is a disturbance of coagulation affecting factor VIII, the same that is deficient in hemophilia. This identity is accepted because plasmas of Willebrand and hemophilia do not correct each other's coagulation defect *in vitro.*

Two major differences distinguish the two diseases:

First, the deficiency in factor VIII is variable in Willebrand patients, as a rule milder, and appears as a relatively secondary symptom.

Second, genetic transmission is different: hemophilia is inherited as a sex-linked recessive trait, von Willebrand's disease as an autosomal trait, generally regarded as dominant. The gene for hemophilia appears to control the factor VIII deficiency directly: it is therefore improbable, if the factor is really the same, that the deficiency of factor VIII in Willebrand cases is directly dependent on a gene borne by the X chromosome.

A remarkable observation has been made by Nilsson et al. (1957) and by Cornu et al, (1963): transfusion of plasma from normal donors, or from hemophiliac (the action would even be stronger with the latter) to a patient

with von Willebrand's disease, immediately corrects the vascular trouble, which proves that the infused plasma contains the lacking factor. By contrast, the coagulation defect is not immediately corrected. But this correction appears within a few hours, reaches a maximum after 5 to 10 hours and decreases slowly during the following days.

Everything happens as if the injected plasma (which if the donor is an hemophiliac contains no factor VIII) had induced the synthesis of factor VIII in the recipient. The latter then would have the genetic equipment necessary for this synthesis, but the lack of a vascular factor would have as a consequence the suppression of factor VIII production. We think that this interpretation is as likely as that which invokes the presence of a cofactor of factor VIII in the donor's blood. In that case we have to postulate the presence in the donor's plasma of two independent agents, one capable of curing the bleeding tendency, and the cofactor of antihemophiliac globulin. In our concept, on the contrary, the vascular factor itself plays, directly or indirectly, the role of inducer of factor VIII (Fig. 7).

A similar interpretation was given by Graham (1963). However, in a more recent work based on what he thinks to be homozygous patients (Barrow et al., 1965), Graham concluded that this hypothesis was unlikely because these patients did not respond to the infusion of plasma from normal or hemophiliac donors. On the other hand, the effect of therapy on the bleeding time is often very difficult to evaluate, and it is likely that platelet adhesiveness will lend itself much better to correct estimation. In any case, much work is still needed before any hypothesis can be definitely accepted.

*Phenylketonuria.* Phenylketonuria is a disease transmitted as an autosomal recessive trait and which brings about severe mental retardation. The primary lesion is the absence of phenylalanine hydroxylase in the liver (Jervis, 1953). Phenylalanine can no longer be oxidized into tyrosine and it accumulates, along with some of its metabolites, which appear or increase in the urine (Folling, 1934): phenylpyruvic and phenyllactic acids, phenylacetylglutamine (Woolf, 1951) and others. In addition, some oxidation *in ortho* takes place and o-tyrosine and its derivatives become detectable. Excess of phenylalanine and

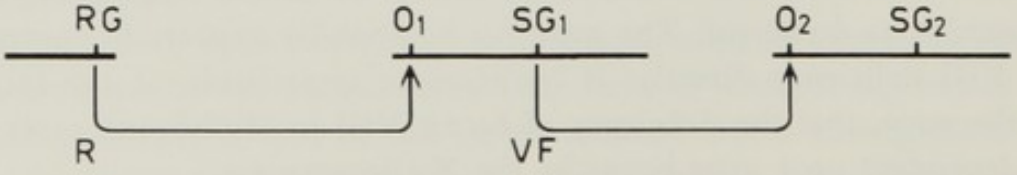



FIG. 7—Hypothetical mechanism of von Willebrand's disease. Normal situation: $SG_1$ makes a vascular factor (or platelet adhesivity factor) which induces $O_2$ and $SG_2$ (antihemophilic factor). Willebrand: RG is modified as a superrepressor: its product R permanently blocks $O_1$.

its metabolites is certainly the cause of mental troubles, as shown by the effects of the phenylalanine-restricted diet (Bickel et al., 1954).

In these patients secondary disturbances exist, among which two can be individualized. Tyrosine oxidation to melanins is *impeded*: this is clinically evidenced by the fair color of the face and hair of many patients. These sometimes darken under the effect of treatment (Armstrong and Tyler, 1955). The other trouble bears on the metabolism of tryptophan: there is a urinary increase of some of its metabolites, like indole-acetic and lactic acids (Armstrong and Robinson, 1954) and indican (Bessman and Tada, 1960). By contrast, serotonin (5-hydroxytryptamine) is low in plasma (Pare et al., 1959), and the urinary output of its deamination product, 5-hydroxyindoleacetic acid, is decreased (Armstrong and Robinson, 1954; Ferrari et al., 1955).

Classical interpretations bring into play the mechanisms of competitive inhibition: phenylalanine or its derivatives would inhibit tyrosinase (Boylen and Quastel, 1961) and prevent transformation into melanin. They would also inhibit tryptophan oxidation, decarboxylation of 5-hydroxytryptophan (Davison and Sandler, 1958) or passage of the latter through the blood-brain barrier (McKean et al., 1962).

The actual concentrations of phenylalanine or phenylpyruvic acid in plasma or tissues appear to account at least partially for the inhibition of tyrosinase (Boylen and Quastel, 1961). They are much too low to inhibit tryptophan oxidation or decarboxylation, and other explanations must be sought for (Davison and Sandler, 1958).

Experimental production is difficult but has been attempted, in rats, using phenylalanine overload (Auerbach et al., 1958). It results notably in a decrease of serotonine concentration (Yuwiler and Loutit, 1961) and an increase of tyrosine and tryptophan transaminases. The production of tyrosine transaminase can also be increased in the rat by a load in tryptophan (Kenney and Flora, 1961) and methyltryptophan or hydroxytryptophan (Rosen and Milholland, 1963).

However, these results become less impressive if, according to Jacoby and La Du (1962), the same transaminase acts on phenylalanine, tyrosine, and tryptophan.

The most interesting experimental results are those of Yuwiler et al. (1965). In rats overloaded with phenylalanine, there is considerable decrease in tryptophan hydroxylase liver activity, while phenylalanine hydroxylase is unchanged. From the actual data this decrease seems to result not from an inhibition but from a true diminution. Excess of phenylalanine, therefore, would repress tryptophan hydroxylase. Similar observations could perhaps be made more often if they were looked for systematically. With regard to phenylketonuria, the discovery of the "dilute lethal gene" strain of mice,

which shows greatly diminished liver phenylalanine hydroxylase activity, looks promising (Coleman, 1960).

The above discussion has been centered on aromatic amino-acid metabolism, and it may be argued that the second prediction of our model has been neglected. An additional observation is therefore of interest. The brain damage in phenylketonuria has been attributed to a competitive inhibition of amino acid penetration from the gut and through the blood-brain barrier (Chirigos et al., 1960; Linneweh and Ehrlich, 1960) leading to a defective incorporation into brain proteins. However, the search for a biochemical lesion in brain in autopsy cases has shown a deficient myelination (Poser and van Bogaert, 1959; Knox, 1966; Gerstl et al., 1967) and more specifically a low content in cerebrogalactosides, more marked in young subjects (Crome et al., 1962). Amino acid imbalance, therefore, results in a probable disturbance in the synthesis of glycolipids.

In the second part of this article we have discussed a possible mechanism (Jacob and Monod, 1963) of enzymatic interaction which may play a part in cell differentiation, and tried to support it with examples taken from human pathology. This mechanism does not exclude others which have been recently proposed to regulate protein synthesis: feed-back mechanisms like end-product repression, which probably act less simply in animals than in bacteria, as shown by Eagle (1965) in tissue cultures; allosteric transitions, which can influence both activity and biosynthesis of enzyme (Monod et al., 1959); control of rate of synthesis through peptides or prosthetic groups (Cline and Bock, 1966; Ralph and Baguley, 1966; Grayzel et al., 1966), and hormonal interactions (Weber et al., 1965).

## General Conclusions

The foregoing study demonstrates the difficulty of applying the idea of regulator genes, as defined by bacterial genetics, to human diseases. It appears very likely that the vast majority of hereditary molecular abnormalities, usually transmitted as autosomal recessive traits, are due to mutations of structural genes, but this for technical reasons cannot yet be proved. If alterations of controller genes are indeed involved, then their mechanism of regulation is different from that predicted by the bacterial model. At least the repressor would be less diffusible or not diffusible and would only act on the gene located in "cis" position. We have found only 2 diseases which can be attributed with likelihood to a controller gene mutation: the exceptional erythrocytic porphyrias for a regulator negative mutation, and von Willebrand's disease for a super-repressor type mutation. The existence of regulator genes itself has not been demonstrated in animals. Analogous conclusions have been

drawn in genetic studies on regulation of protein synthesis in Drosophila (Glassman, 1965).

It is possible, starting from the work of Monod and Jacob, to propose an evolutive scheme which takes account both of the lack of intervention of regulator genes in most cases, and of their apparent intervention in a few cases.

Bacteria function with structural genes, often grouped in operons, and regulated through controller genes. Higher organisms are much more complex and possess a much larger number of genes. In the course of evolution some genes are duplicated, while still initially submitted to the action of the same regulator. Later there takes place independent mutations on both duplication products, as well as translocations to other chromosomes. As a result, one of the 2 duplicated genes acquires a new function. The same process is renewed a certain number of times. The new genes, modified in their function, escape from their initial regulator gene and acquire a different type of regulation. It is therefore possible that in the present stage of evolution 2 types of regulation coexist. Some structural genes have not been changed significantly since the bacterial ancestor: these genes have probably preserved their regulator genes. Other genes, after having undergone modification, have escaped from this control and are now submitted to other types of regulation. The respective proportion of genes which would have kept or lost their initial type of control is at present impossible to estimate.

## ACKNOWLEDGEMENTS

We thank L'Institut Nationale de la Santé et de la Recherche Médicale; Le Centre National de la Recherche Scientifique; La Délégation Générale à la Recherche Scientifique et Technique et ses Comités Scientifiques (Fonds de Dévelopment); The National Cancer Institute, Public Health Service (Grant No. CA 08900) for support given to this work.

## REFERENCES

Alepa, F. P., R. R. Howell and J. E. Seegmiller. 1962. The occurrence of glycogen storage disease with tophaceous gout. Arthritis Rheum. 5: 634-635, abstract.

Alexander, B., and R. Goldstein. 1953. Dual hemostatic defect in pseudohemophilia. J. Clin. Invest. 32: 551, abstract.

Ames, B. N., and R. G. Martin. 1964. Biochemical aspects of genetics: the operon. Ann. Rev. Biochem. 33: 235-258.

Armstrong, M. D., and F. H. Tyler. 1955. Studies on phenylketonuria. I. Restricted phenylalanine intake in phenylketonuria. J. Clin. Invest. 34: 565-580.

Armstrong, M. D., and K. S. Robinson. 1954. On the excretion of indole derivatives in phenylketonuria. Arch. Biochem. Biophys. 52: 287-288.

Auerbach, V. H., and A. M. DiGeorge. 1965. Genetic mechanisms producing multiple enzyme defects. A review of unexplained cases and a new hypothesis. Amer. J. Med. Sci. 249: 718-747.

—, H. A. Waisman and L. B. Wyckoff. 1958. Phenylketonuria in the rat associated with decreased temporal discrimination learning. Nature 182: 871-872.

Auricchio, S., A. Prader, G. Mürset, and G. Witt. 1961. Sacchärinoseintoleranz. Durchfall infolge hereditâren Mangels an intestinaler Saccharaseaktivitat. Helv Paediat. Acta 16: 483-505.

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: 1880-1886.

Baikie, A. G., P. B. Loder, G. C. de Gruchy and D. B. Pitt. 1965. Phosphohexokinase activity of erythrocytes in mongolism. Another possible marker for chromosome 21. Lancet 1: 412-414.

Banks A., and P. A. Marks. 1966. Protein synthesis in a cell free human reticulocyte system: ribosome function in thalassemia. J. Clin. Invest. 45: 330-336.

Barrow, E. M., C. C. Heindel, H. R. Roberts and J. B. Graham. 1965. Heterozygosity and homozgosity in von Willebrand's disease. Proc. Soc. Exp. Biol. Med. 118: 684-687.

Bearn, A. G. 1966. Wilson's disease. *In* Stanbury, J. B., Wyngaarden, J. B. and Fredrickson, D. S. (Eds.): The Metabolic Basis of Inherited Disease. New York: McGraw-Hill. 2nd Edition. pp. 761-779.

Benedict, J. D., H. Roche, T. F. Yü, E. J. Bien, A. B. Gutman and D. Stetten, Jr. 1952. Incorporation of glycine nitrogen into uric acid in normal and gouty man. Metabolism 1: 3-12.

Bennhold, H., H. Peters and E. Roth. 1954. Uber einem Fall von kompletter Analbuminaemie ohne wesentliche klinische Krankheitszeichen. Ver. Deutsch. Ges. Inn. Med. 60: 630-634.

Bessman, S. P., and K. Tada. 1960. Indicanuria in phenylketonuria. Metabolism 9: 377-385.

Betke, K., and E. Kleihauer. 1958. Fetaler und bleibender Blutfarbstoff in Erythrozyten und Erythroblasten von menschlichen Feten und Neugeborenen. Blut 4: 241-249.

Beutler, E. M. Yeh and V. F. Fairbanks. 1962. The normal human female as a mosaic of X-chromosome activity: studies using the gene for G-6-PD-deficiency as a marker. Proc. Nat. Sci. Acad. USA. 48: 9-16.

Bickel, H., J. Gerrard, and E. M. Hickmans. 1954. The influence of phenylalanine intake on the chemistry and behavior of a phenylketonuric child. Acta Pediat. 43: 64-77.

Blombäck, B., M. Blombäck, T. C. Laurent and H. Persson. 1965. Anomalous behavior of haemophilia A fibrinogen during ultracentrifugation. Biochim. Biophys. Acta 97: 171-173.

Boyer, S. H., D. L. Rucknagel, D. J. Weatherall and E. J. Watson-Williams. 1963. Further evidence for linkage between the β and δ loci governing human hemoglobin and the population dynamics of linked genes. Amer. J. Hum. Genet. 15: 438-448.

Boylen, J. B., and J. H. Quasel. 1961. Effects of L-phenylalanine and sodium phenylpyruvate on the formation of adrenaline from L-tyrosine in adrenal medulla *in vitro*. Biochem. J. 80: 644-648.

Brancati, C., and C. Baglioni. 1966. Homozygous βδ-thalassaemia (βδ-microcythaemia).

Brandt, N. J., A. Frøland, M. Mikkelsen, A. Nielsen and N. Tolstrup. 1963. Galactosaemia locus and the Down's syndrome chromosome. Lancet 2: 700-703.

Caskey, C. T., D. M. Ashton and J. B. Wyngaarden. 1964. The enzymology of feedback inhibition of glutamine phosphoribosylpyrophosphate amidotransferase by purine ribonucleotides. J. Biol. Chem. 239: 2570-2579.

Chirigos, M. A., P. Greengard and S. Udenfriend. 1960. Uptake of tyrosine by rat brain *in vivo*. J. Biol. Chem. 235: 2075-2079.

Cline, A. L., and R. M. Bock. 1966. Translational control of gene expression. Cold Spring Harbor Symp. Quant. Biol. 31: 321-333.

Coleman, D. L. 1960. Phenylalanine hydroxylase activity in dilute and nondilute strains in mice. Arch. Biochem. Biophys. 91: 300-306.

Comings, D. E., and A. G. Motulsky. 1966. Absence of cis delta chain synthesis in ($\delta\beta$) thalassemia (F-thalassemia). Blood 28: 54-69.

Conney, A. H., E. C. Miller and J. A. Miller. 1957. Substrate-induced synthesis and other properties of benzpyrene hydroxylase in rat liver. J. Biol. Chem. 228: 753-766.

Cori, G. T. 1957. Biochemical aspects of glycogen deposition disease. Bibl. Paediat. 66: 344-358.

—, and C. F. Cori. 1952. Glucose-6-phosphatase of the liver in glycogen storage disease. J. Biol. Chem. 199: 661-667.

Cornu, P., M. J. Larrieu, J. Caen and J. Bernard. 1963. Transfusion studies in von Willebrand's disease: effect on bleeding time and factor VIII. Brit. J. Haemat. 9: 189-202.

Cox, E. V., and A. M. White. 1962. Methylmalonic acid excretion: an index of vitamin-$B_{12}$ deficiency. Lancet 2: 853-856.

Crome, L., V. Tymms and L. I. Woolf. 1962. A chemical investigation of the defects of myelination in phenylketonuria. Neurol. Neurosurg. Psychiat. 25: 143-148.

Dahlqvist, A., S. Auricchio, G. Semenza and A. Prader. 1963. Human intestinal disaccharidases and hereditary disaccharide intolerance. The hydrolysis of sucrose, isomaltose, palatinose (isomaltulose), and 1,6-$\alpha$-oligosaccharide (isomalto-oligasaccharide) preparation. J. Clin. Invest. 42. 556-562.

Davidson, R. G., H. M. Nitowsky and B. Childs. 1963. Demonstrations of two populations of cells in the human female heterozygous for glucose-6-phosphate dehydrogenase variants. Proc. Nat. Acad. Sci. USA. 50: 481-485.

Davidson, A. N., and M. Sandler. 1958. Inhibition of 5-hydroxytryptophan decarboxylase by phenylalanine metabolites. Nature 181: 186-187.

Dreyfus, J.-C., M. Bessis and J.-C. Kaplan. 1964. L'existence de deux populations de globules rouges ches les femmes hétérozygotes pour le déficit en glucose-6-phosphate déshydrogénase. Démonstration par spectrophotométrie sur globules rouges examinés individuellement. C. R. Acad. Sci. (Paris) 258: 3791-3793.

Eagle, H. 1965. Metabolic controls in cultured mammalian cells. Science 148: 42-51.

Edington, G. M., and H. Lehmann. 1955. Expression of the sickle-cell gene in Africa. Brit. Med. J. 1: 1308-1311.

Eylar, E. H., and G. T. Matioli. 1965. Glycoprotein biosynthesis in human reticulocytes: a lesion in thalassemia. Science 147: 869-870.

Fessas, P., and G. Stamatoyannopoulos. 1964. Heriditary persistence of fetal hemoglobin in Greece. A study and a comparison. Blood 24: 223-240.

Fölling, A. 1934. Uber Ausscheidung von Phenylbrenztraubensäure in den Harn als Stoffewechselanomalie in Verbindung mit Imbezillität. Z. Physiol. Chem. 227: 169-176.

Ford, C. E., K. W. Jones, P. E. Polani, J. C. de Almeida and J. H. Briggs. 1959. A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). Lancet 1: 711-713.

Fudenberg, H. H., and K. Hirschhorn. 1964. Agammaglobulinemia: the fundamental defect. Science 145: 611-612.

Gerald, P. S., and L. K. Diamond. 1958. A new hereditary hemoglobinopathy (The Lepore trait) and its interaction with thalassemia trait. Blood 13: 835-844.

Gerstl, B., N. Malamud, L. F. Eng and R. B. Hayman. 1967. Lipid alterations in human brains in phenylketonuria. Neurology 17: 51-57.

Glassman, E. 1965. Genetic regulation of xanthine dehydrogenase in *Drosophila melanogaster*. Fed. Proc. 25: 1243-1251.

Good, R. A., W. D. Kelly, J. Rotstein and R. L. Varco. 1962. Immunological deficiency diseases: Agammaglobulinemia, hypogammaglobulinemia, Hodgkin's disease and sarcoidosis. Progr. Allerg. 6: 187-319.

Graham, J. B. 1963. Biochemical genetic speculations provoked by considering the enigma of von Willebrand's disease. Thromb. Diath. Haemorrh. 9, suppl. 2: 119-

Granick, S. 1965. Hepatic porphyria and drug-induced or chemical porphyria. Ann. NY. Acad. Sci. 123: 188-197.

Grayzel, A. I., P. Hörchner and I. M. London. 1966. The stimulation of globin synthesis by heme. Proc. Nat. Acad. Sci. USA. 55: 650-655.

Harris, H. 1964. The genetic control of enzyme formation in man. *In* Proc. Second Inter. Conf. on Congenital Malformations, New York, 1963. New York: International Medical Congress, pp. 135-144.

Heilmeyer, L., R. Clotten, L. Kerp, H. Merker, Cr. A. Parra and H. P. Wetzel. 1963. Porphyria erythropoetica congenita Günther: Bericht über zwei Familien mit Erfassung der Merkmalsträger. Deutsch Med. Wschr. 88: 2449-2456.

—, W. Keller, D. Vivell, W. Keiderling, K. Betke, F. Wöhler and H. E. Schultze. 1961. Kongenitale Atransferrinämie bie einem sieben Jahre alten Kind. Deutsch. Med. Wschr. 86: 1745-1751.

Henstell, H. H., M. Kligerman, and L. E. Irwin. 1965. Sickle cell disease. A serum defect in the thromboplastin generation test. Blood 25: 907-920.

Herman, E. C., and C. L. Conley. 1960. Hereditary persistence of fetal hemoglobin: a family study. Amer. J. Med. 29: 9-17.

Hers, H. G. 1959 Études enzymatiques sur fragments hépatiques; application à la classification des glycogénoses. Rev. Int. Hépat. 9: 35-55.

—. 1964. Glycogen storage disease. Advances Metab. Dis. 1: 1-44.

Heywood, J. D., M. Karon and S. Weissman. 1964. Amino acids: incorporation into α-and β-chains of hemoglobin by normal and thalassemic reticulocytes. Science 146: 530-531.

Hodgkin, W. E., E. R. Giblett, H. Levine, W. Bauer and A. G. Motulsky. 1965. Complete pseudocholinesterase deficiency: genetic and immunologic characterization. J. Clin. Invest. 44: 486-493.

Horton, B. F., and T. H. J. Huisman. 1963. Linkage of the β-chain and the δ-chain structural genes of human hemoglobins. Amer. J. Hum. Genet. 15: 394-397.

Howell, R. R., D. M. Ashton and J. B. Wyngaarden. 1962. Glucose-6-phosphatase deficiency glycogen storage disease. Studies on the interrelationships of carbohydrate, lipid, and purine abnormalities. Pediatrics 29: 553-565.

Huguley, C. M., Jr., J. A. Bain, S. L. Rivers and R. B. Scroggins. 1959. Refractory megaloblastic anemia associated with excretion of orotic acid. Blood 14: 615-634.

Iber, F. L., T. C. Chalmers, and L. L. Uzman. 1957. Studies of protein metabolism in hepatolenticular degeneration. Metabolism 6: 388-396.

Ingram, V. M. 1963. The Hemoglobins in Genetics and Evolution. New York: Columbia University Press.

—. 1964. A molecular model for thalassemia. Ann. N. Y. Acad. Sci. 119: 485-495.

Jacob, F., and J. Monod. 1961. Genetic regulatory mechanisms in the synthesis of proteins. J. Molec. Biol. 3: 318-356.

— and —. 1963. Genetic repression, allosteric inhibition, and cellular differentiation. *In* Locke, W. (Ed.): Cytodifferentiation and Macromolecular Synthesis. New York: Academic Press, pp. 30-64.

Jacobs, P. A., and J. A. Strong. 1959. A case of human intersexuality having a possible XXY sex-determining mechanism. Nature 193: 302-303.

Jacoby, G. A., and B. N. La Du. 1962. Nonspecificity of tyrosine transaminase: An explanation for the simultaneous induction of tyrosine, phenylalanine, and tryptophan transaminase activities in rat liver. Biochem. Biophys. Res. Commun. 8: 352-356.

Jeandet, J., and H. Lestradet. 1961. L'hyperlactacidémie, cause probable de l'hyperuricémie dans la glycogénose hépatique. Rev. Franc. Etud. Clin. Biol. 6: 71-72.

Jervis, G. A. 1953. Phenylpyruvic oligopherenia deficiency of phenylalnnine-oxidizing system. Proc. Soc. Exp. Biol. Med. 82: 514-515.

Jeune, M., A. Charrat and J. Bertrand. 1957. Polycorie hépatique, hyperuricémie et goutte. Arch. Franç. Pédiat. 14: 897-909.

Kalow, W. 1962. Pharmacogenetics; Heredity and the Response to Drugs. Philadelphia: Saunders.

Kaplan, J. C., J. C. Dreyfus and M. Bessis. 1965. La structure en mosaïque du chromosome X chez la femme. Nouv. Rev. Franç. Hémat. 5: 835-849.

Kelley, W. N., F. M. Rosenbloom and J. E. Seegmiller. 1967. The effects of azathioprine (Imuran) on purine synthesis in clinical disorders of purine metabolism. J. Clin. Invest. 46: 1518-1529.

Kenney, F. F., and R. M. Flora. 1961. Induction of tyrosine-α-ketoglutarate transaminase in rat liver. I. Hormonal nature. J. Biol. Chem. 236: 2699-2702.

Knox, W. E. 1966. Phenylketonuria. *In* Stanbury, J. B., Wyngaarden, J. B. and Frederickson, D. S. (Eds.): The Metabolic Basis of Inherited Disease. New York: McGraw-Hill. 2nd Edition. pp. 258-294.

Kopper, P. H. 1966. Rapid induction of *in vivo* fibrinolysis with D-gluconyl-L-tyrosine. Nature 211: 1417.

Labie, D., W. A. Schroeder, and T. H. J. Huisman. 1966. The amino acid sequence of the δ-β chains of hemoglobin Lepore Augusta—Lepore Washington. Biochim. Biophys. Acta. 127: 428-437.

Langdon, R. G., and D. R. Weakley. 1955. The influence of hormonal factors and of diet upon hepatic glucose-6-phosphatase activity. J. Biol. Chem. 214: 167-174.

Larrieu, M. J., and J. P. Soulier 1953. Déficit en facteur antihémophilique A chez une fille associé à un trouble du saignement Rev. Hémat. 8: 361-370.

LeJeune, J. 1963. Autosomal disorders. Pediatrics 32: 326-337.

Linneweh, F., and M. Ehrlich. 1960 Die renalen und prärenalen Störungen des Aminosäuren-Stoffwechsels bei Phenylalaninarmer Ernährung. Klin. Wschr. 38: 904-910.

Lyon, M. F. 1961. Gene action in the X-chromosome of the mouse. Nature 190: 372-373.

McCurdy, P. R., H. N. Kirkman, J. L. Naiman, R. T. S. Jim and B. M. Pickard. 1966. A Chinese variant of glucose-6-phosphate dehydrogenase. J. Lab. Clin. Med. 67: 374-385.

McKean, C. M., S. M. Schanberg, and N. J. Giarman. 1962. A mechanism of the indole defect in experimental phenylketonuria. Science 137: 604-605.

Mahler, R. 1966. Glycogen synthesis from pyruvate in muscle. Nature 209: 616.

Mellman, W. J., F. A. Oski, T. A. Tedesco, A. Maciera-Coelho and H. Harris. 1964. Leucocyte enzymes in Down's syndrome. Lancet 2: 674-675.

Mier, P. D., and D. W. K. Cotton. 1966. Operon hypothesis: new evidence from the 'constant proportion' group of the Embden-Meyerhof pathway. Nature 209: 1022-1023.

Monod, J., and F. Jacob. 1961. Teleonomic mechanisms in cellular metabolism, growth, and differentiation. Cold Spring Harbor Symp. Quant. Biol. 26: 389-401.

—, J. Wyman and J.-P. Changeux. 1965. On the nature of allosteric transitions: a plausible model. J. Molec. Biol. 12: 88-118.

Moses, S. W., S. Levin, R. Chayoth and K. Steinitz. 1966. Enzyme induction in a case of glycogen storage disease. Pediatrics 38: 111-121.

Motulsky, A G. 1962. Controller genes in synthesis of human haemoglobin. Nature 194: 607-609.

Neel, J. V. 1961. The hemoglobin genes: a remarkable example of the clustering of related genetic functions on a single mammalian chromosome. Blood 18: 769-777.

Neimann, N., Y. Najean, C. Scialom, M. Boulard, M. Pierson and J. Bernard. 1965. Etude d'un cas d'anémie mégaloblastique de l'enfant avec excrétion anormale d'acide orotique. Nouv. Rev. Franç. Hémat. 5: 445-457.

Nierlich, D. P., and B. Magasanik. 1965. Regulation of purine ribonucleotide synthesis by end product inhibition. J. Biol. Chem. 240: 358-365.

Nilsson, I. M., M. Blombäck, E. Jorpes, B. Blombäck, and S.-A. Johansson. 1957. Von Willebrand's disease and its correction with human plasma fraction 1-0. Acta Med. Scand. 159: 179-188.

O'Reilly, R. A., and P. M. Aggeler. 1965. Coumarin anticoagulant drugs: hereditary resistance in man. Fed. Proc. 24: 1266-1273.

Oski, F. A., N. T. Shahidi and L. L. Diamond. 1963. Erythrocyte acid phosphomonoesterase and glucose-6-phosphate dehydrogenase deficiency in Caucasians. Science 139: 409-411.

Pardee, A. B., and A. C. Wilson. 1963. Control of enzyme activity in higher animals. Cancer Res. 23: 1483-1490.

Pare, C. M. B., M. Sandler, R. S. Stacey. 1959. The relationship between decreased 5-hydroxyindole metabolism and mental defect in phenylketonuria. Arch. Dis. Child. 34: 422-425.

Parker, W. C., and A. G. Bearn. 1963. Application of genetic regulatory mechanisms to human genetics. Amer. J. Med. 34: 680-691.

Pearson, C. M., D. G. Rimer and W. F. N. M. Mommaerts. 1959. Defect in muscle phosphorylase: a newly defined human disease. Clin. Res. 7: 298.

Pette, D., W. Luh and Th. Bücher. 1962. A constant-proportion group in the enzyme activity pattern of the Embden-Meyerhof chain. Biochem. Biophys. Res. Commun. 7: 419-424.

Pinsky, L., and R. S. Krooth. 1967. Studies on the control of pyrimidine biosynthesis in human diploid cell strains. I. Effect of 6-azauridine on cellular phenotype. Proc. Nat. Acad. Sci. USA. 57: 925-932.

Porter, F. S. 1963. Congenital erythropoietic protoporphyria. II. An experimental study. Blood 22: 532-544.

Poser, C. M., and L. van Bogaert. 1959. Neuropathologic observations in phenylketonuria. Brain 82: 1-9.

Quick, A. J., and C. V. Hussey. 1953. Hemophilic condition in the female. J. Lab. Clin. Med. 42: 929-930, abstract.

Ralph, R. K., and B. C. Baguley. 1966. Regulation of protein biosynthesis. J. Theor. Biol. 13: 164-186.

Rapaport, S. I., M. J. Patch and F. J. Moore. 1960. Anti-hemophilic globulin levels in carriers of hemophilia A. J. Clin. Invest. 39: 1619-1625.

Renold, A. E., and G. F. Cahill, Jr. 1966. Diabetes mellitus. *In* Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S. (Eds.): The Metabolic Basis of Inherited Disease, 2nd ed. New York: McGraw-Hill, pp. 69-108.

Rey, J., and J. Frézal, 1967. Les anomalies des disaccharidases. Arch Franç. Pédiat. 24: 65-101.

Robbins, P. W. 1960. Immunological study of human muscle lacking phosphorylase. Fed. Proc. 19: 193, abstract.

Rosen, F., and R. J. Milholland. 1963. Glucocorticoids and transaminase activity. VII. Studies on the nature and specificity of substrate induction of tyrosine-α-ketoglutarate transaminase and tryptophan pyrrolase. J. Biol. Chem. 238: 3730-3735.

Rucknagel, D. L. 1964. Current concepts of the genetics of thalassemia. Ann. NY. Acad. Sci. 119: 436-449.

Scheinberg, I. H., and D. Gitlin. 1952. Deficiency of ceruloplasmin in patients with hepatolenticular degeneration (Wilson's disease). Science 116: 484-485.

Schmid, R. 1966. The porphyrias. *In* Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S. (Eds.): The Metabolic Basis of Inherited Disease, 2nd ed. New York: McGraw-Hill. pp. 813-970.

—, and R. Mahler. 1959. Chronic progressive myopathy with myoglobinuria: demonstration of glycogenolytic defect in the muscle. J. Clin. Invest. 38: 2044-2058.

Shih, L.-Y., P. Wong, T. Inouye, M. Makler and D. Y.-Y. Hsia. 1965. Enzymes in Down's syndrome. Lancet 2: 746-747.

Silvestroni, E., and I. Bianco. 1946. Singolare associazone di "anemia microcitica costituzionale" con "drepanocito-anemia" in soggetto di razza bianca. Policlinico [Prat.] 53: 265-287.

Singer, K., L. Singer and S. R. Goldberg. 1955. Studies on abnormal hemoglobins. XI. Sickle cell-thalassemia disease in the Negro. The significance of the S + A + F and S + A patterns obtained by hemoglobin analysis. Blood 10: 405-415.

Smith, L. H., Jr., C. M. Huguley, Jr. and J. A. Bain. 1966. Hereditary orotic aciduria. *In* Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S. (Eds.): The Metabolic Basis of Inherited Disease, 2nd ed. New York: McGraw-Hill. pp. 739-758.

Smyth, C. J. 1957. Hereditary factors in gout. A review of recent literature. Metabolism 6. 218-229.

Stanbury, J. B., J. B. Wyngaarden and D. S. Fredrickson (Eds.). 1966. The Metabolic Basis of Inherited Disease, 2nd Ed. New York: McGraw-Hill.

Stent, G. S. 1964. The operon: on its third anniversary. Science 144: 816-820.

Sutton, H. E. 1965. Biochemical genetics and man: accomplishments and problems. Science 150: 858-862.

Trubowitz, S., D. Kirman and B. Masek. 1962. The leucocyte alkaline phosphatase in mongolism. Lancet 2: 486-487.

Tschudy, D. P., M. G. Perlroth, H. S. Marver, A. Collins, G. Hunter, Jr. and M. Rechcigl, Jr. 1965. Acute intermittent porphyria: the first "overproduction disease" localized to a specific enzyme. Proc. Nat. Acad. Sci. USA. 53: 841-847.

Wagner, F. 1966. Vitamin $B_{12}$ and related compounds. Ann. Rev. Biochem. 35: 405-434.

Watson, C. J., W. Runge, L. Taddeini, I. Bossenmaier and R. Cardinal. 1964. A suggested control gene mechanism for the excessive production of types I and III porphyrins in congenital erythropoietic porphyria. Proc. Nat. Acad. Sci. USA. 52: 478-485.

Weber, G., R. L. Singhal, and S. K. Srivastava. 1965. Action of glycocorticoid as inducer and insulin as suppressor of biosynthesis of hepatic gluconeogenic enzymes. Advances Enzym. Regulat. 3: 43-75.

Wheeler, J. T., and J. R. Krevans. 1961. The homozygous state of persistent fetal hemoglobin and the interaction of persistent fetal hemoglobin with thalassemia. Bull. Hopkins Hosp. 109: 217-233.

Woolf, L. I. 1951. Excretion of conjugated phenylacetic acid in phenylketonuria. Biochem. J. 49: ix-x.

Wyngaarden, J. B. 1966. Gout. *In* Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S. (Eds.): The Metabolic Basis of Inherited Disease, 2nd Ed. New York: McGraw-Hill. pp. 667-728.

Yuwiler, A., E. Geller and G. G. Slater. 1965. On the mechanism of the brain serotonin depletion in experimental phenylketonuria. J. Biol. Chem. 240: 1170-1174.

—, and R. T. Louttit. 1961. Effects of phenylalanine diet on brain serotonin in the rat. Science 134: 831-832.

Zuckerkandl, E. 1964. Controller-gene diseases: the operon model as applied to β-thalassemia, familial fetal hemoglobinemia and the normal switch from the production of fetal hemoglobin to that of adult hemoglobin. J. Molec. Biol. 8:128-147.

CHAPTER 6

# Histocompatibility in Man — Genetic and Practical Considerations

**Fritz H. Bach**

Departments of Medical Genetics and Medicine
University of Wisconsin
Madison, Wisconsin

PROGRESS MADE in human kidney homotransplantation since the first transplant between individuals other than identical twins can be largely attributed to advances in 2 areas of research: immunosuppression, in which various drugs are used to suppress the body's reaction to the transplanted tissue, and histocompatibility testing, in which the attempt is made to pair donors and recipients who have antigenically similar tissues. It is the purpose of this review

Supported in part by NIH grant GM 15422 and ONR grant H 00014-67-A-0128-0003. Paper 1194 from the Laboratory of Genetics, University of Wisconsin.

* I will use the term "pairing" for the general problem of choosing an appropriate donor for a given recipient. "Matching" will refer to matching tests as discussed and "typing" to serological procedures used to identify histocompatibility antigens. "System" will refer to a unit of genetic information which cannot readily be separated into subunits at the level of resolution allowed by formal human genetics (W.H.O. 1965). In cases where a system associated with a given phenotype is separable by extensive recombinational studies, I will refer to the separate parts of the system as "sub-systems." "Allele" will be used to refer to the genetic material of a system or of a sub-system, i.e. alleles of the system or alleles of the sub-system. These terms should in no way prejudice our conceptions of the nature of the genetic organizations within the system. "Allogeneic" refers to 2 non-identical members of a single species. Isoimmune antisera are those prepared in one member of a species against antigens of another member of that same species.

to discuss the progress that has been made in the second of these 2 areas, histocompatibility testing, considering both how histocompatibility pairing* contributes to the success of transplantation and what these tests tell us of the genetic basis of transplantation rejection in man.

We owe our present understanding of the role of transplantation antigens mainly to work in inbred animal species, such as the mouse. The concept that a tissue is rejected because that tissue carries antigens which are foreign to the recipient, i.e. not possessed by the recipient, can be illustrated by grafting experiments between 2 inbred parental strains and their F-1 hybrid offspring (see Figure 1). In such a situation the F-1 animal carries antigens which are foreign to either parent—given that the two inbred parents differ in their histocompatibility genotypes. Thus, either parent will reject a graft from the F-1 animal, but the F-1 will not reject grafts from either parental strain because there will be no antigens foreign to the F-1 on the graft.

The response of a recipient to a graft is specific to the antigens carried by that graft. For instance, skin transplanted for the first time from one member of a species to an unrelated member of that species will usually be rejected in 8 to 12 days (a first set reaction). A second graft between the same individuals will be rejected more quickly, in an "accelerated" or "second-set" reaction, because the recipient animal has been "sensitized" to the for-

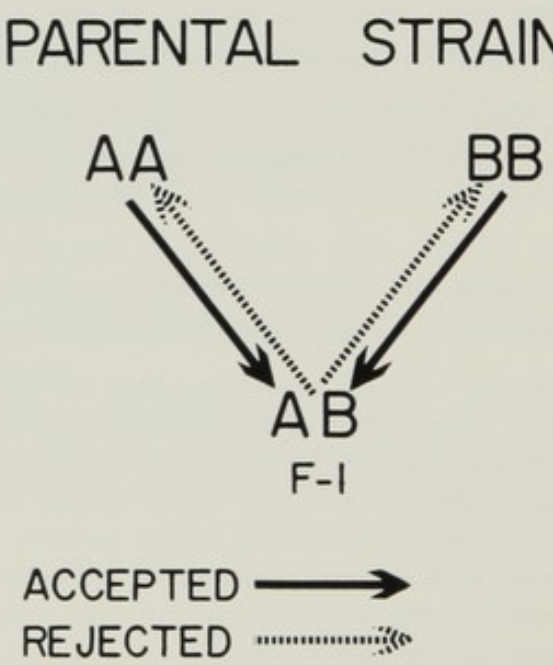



FIG. 1—Mating experiment between inbred strains of mice. Letters A and B represent different genes (alleles) at a locus coding for histocompatibility antigens. Thus, the two types of parental mice are identical except that they have different histocompatibility antigens. A graft from the parental mouse to the F-1 will not be rejected since the F-1 mouse does not encounter any foreign antigens on the graft. However, a graft from the F-1 animal to either parent will be rejected since cells of that parent will encounter foreign antigens, the genes for which were inherited by the F-1 from the other parent.

eign antigens of the first graft. The accelerated second-set rejection is specific, since a second graft carrying none of the antigens of the first graft will be rejected in a first-set manner, i.e. in 8 to 12 days.

Little is known about the mechanisms by which a recipient rejects a skin graft or a homograft of other tissue. Is graft rejection mediated by lymphoid cells, by circulating antibody, or both? Most people will agree that the second of these alternatives is not correct. However, it is difficult to determine which of the other two possibilities applies in various situations. For our purposes in this paper, it is enough to stress that there is evidence from many different kinds of experiments that small lymphocytes are involved in graft rejection.

Methods for pairing donor and recipient for transplantation can be divided into 2 categories. One approach ("typing") identifies specific histocompatibility antigens against which a recipient could react if he does not himself possess these antigens. This permits one to pick donors who have antigens identical, or nearly so, to a given recipient. The other approach ("matching") uses the "physiological response" which the cells of a recipient show to the allogeneic tissue of the donor. The 2 most commonly used matching tests assess incompatibility by measuring *in vivo* or *in vitro* the response of recipient lymphocytes against foreign tissue antigens of prospective donors. We will come back to this in the discussion on histocompatibility testing in man.

In 1963, Amos reviewed the status of our knowledge of histocompatability antigens in several species including man. Investigators have, in the last 4 years, expended great effort developing techniques for "typing" histocompatiblity antigens and for improving "matching" tests for pairing individuals. Knowledge gained in this period suggests that human histocompatibility is probably very similar to that of the mouse, a species in which there is considerable information on the genetics of histocompatibility.

## HISTOCOMPATIBILITY STUDIES IN THE MOUSE

Understanding of the genetics of histocompatibility in the mouse has been greatly facilitated by the use of coisogenic strains of animals. The history of the development of these strains has been reviewed (Snell, 1958; Strong, 1942). By appropriate breeding, 2 mouse strains can be produced which differ for all practical purposes by only one gene (coisogenic strains). Whereas an animal of an inbred strain will not reject skin from another animal of the same strain, a member of one coisogenic strain will reject a skin graft from a member of another coisogenic strain differing by only one histocompatibility gene. Rejection is again associated with the recognition by the host of foreign (non-self) histocompatibility antigens on the allogeneic skin graft.

Many histocompatibility systems have been discovered in the mouse

TABLE *1 – The Known Alleles and Isoantigenic Specificities of the H-2 System and the Inbred Strains that Carry Them.*

*Proposed Numerical Designations for Isoantigenic Specificities and their Relation to the Letters of the Old System†*

| H-2 alleles | A | $D^b$ | C | D | E | F | G | H | I | J | K | M | N | P | Q | S | V | Y | $A^1$ | $B^1$ | $C^1$ | $D^1$ | $E^d$ | $D^k$ | $K^b$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| a | 1 | – | 3 | 4 | 5 | 6 | – | 8 | – | 10 | 11 | 13 | 14 | – | – | – | – | 25 | 27 | 28 | 29 | – | – | – | – |
| b | – | 2 | – | – | 5 | 6 | – | – | – | – | – | – | 14 | – | – | – | 22 | – | 27 | 28 | 29 | – | – | – | 33 |
| c | * | – | 3 | 4 | 5 | * | – | 8 | – | * | – | 13 | * | – | – | – | * | * | 27 | 28 | 29 | – | * | * | * |
| d | – | – | 3 | 4 | – | 6 | – | 8 | – | 10 | – | 13 | 14 | – | – | – | – | – | 27 | 28 | 29 | – | 31 | – | – |
| e | – | – | 3 | – | 5 | 6 | – | – | – | – | – | 13 | * | * | * | – | * | 25 | 27 | 28 | 29 | 30 | * | * | * |
| f | – | – | – | – | – | ? | 7 | 8 | 9 | * | – | – | * | – | – | – | * | – | 27 | – | – | – | * | * | * |
| g | – | 2 | – | – | – | 6 | * | ? | * | * | – | – | 14 | * | – | – | 22 | * | * | * | * | * | 31 | – | – |
| h | 1 | 2 | 3 | – | 5 | 6 | * | 8 | * | * | 11 | – | ? | * | – | – | – | * | * | * | * | * | – | – | – |
| i | * | – | 3 | 4 | 5 | 6 | * | * | * | * | 11 | 13 | * | * | – | – | 22 | * | * | * | * | * | – | – | 33 |
| j | – | – | – | – | – | 6 | ? | – | * | – | – | – | – | * | – | – | 22 | ? | ? | * | * | * | * | * | * |
| k | 1 | – | 3 | – | 5 | – | – | 8 | – | – | 11 | – | – | – | – | – | – | 25 | – | – | – | – | – | 32 | * |
| l | – | – | – | – | – | 6 | ? | – | * | 10 | – | – | – | * | – | – | 22 | – | ? | ? | * | * | * | * | * |
| m | * | – | 3 | – | 5 | – | – | 8 | – | * | 11 | 13 | * | – | – | – | * | * | 27 | 28 | 29 | 30 | * | * | * |
| n | 1 | – | – | – | 5 | 6 | * | 8 | * | 10 | – | – | 14 | * | – | – | – | * | ? | ? | * | * | * | * | * |
| p | * | – | 3 | – | 5 | 6 | – | – | – | * | – | – | * | 16 | – | – | * | * | – | – | – | – | * | * | * |
| q | – | – | 3 | – | 5 | 6 | – | – | – | – | 11 | 13 | – | – | 17 | – | – | * | 27 | 28 | 29 | 30 | * | * | * |
| r | * | – | 3 | – | 5 | – | – | 8 | – | * | 11 | – | * | – | – | – | * | 25 | – | – | – | – | * | * | * |
| s | * | – | 3 | – | 5 | 6 | 7 | – | – | * | – | – | * | – | – | 19 | * | – | – | 28 | – | – | – | – | – |

†From Snell et al, 1964. Published with permission of Dr. Snell and the publisher.

(named H-1 through H-13, H-Y and H-X), incompatibility at any one of which will lead to graft rejection. One of these, the H-2 system, seems to be of greater importance than any of the other systems and has been termed the "major" histocompatibility system in the mouse. Similarly with the rat (Palm, 1964) and the chicken (Shierman and Nordskog, 1964) a single major histocompatibility system has been identified. A detailed description of the phenotypic expression of the H-2 system and its genetic control is given to help our later understanding of studies in man.

The development of antisera which can specifically define the antigens associated with the H-2 system in the mouse led to our knowledge of the complexity of this immunogenetic system. More than 20 different alleles of the system (designated H-$2^a$, H-$2^b$, etc.) have been identified. (From Snell, Holkes, Amos and Stimpfling, 1964). As seen in Table 1, each allele is responsible for the presence of multiple antigenic specificities. Further, there is extensive overlapping in the specificities in that 2 different alleles (e.g. alleles *c* and *d*) may determine many antigens in common and only a very few different ones, or 2 alleles (e.g. alleles *b* and *k*) may determine very few antigens in common but many different. Likewise, some of the antigenic specificities are associated with many of the alleles (e.g. antigens 3, 5 and 6), while some are unique to a single allele (e.g. antigen 19). Any unique combination of antigenic specificities would constitute the phenotypic expression of a new allele.

The first examples of genetic recombination in the H-2 system were described by Amos, Gorer and Mikulska (1955) and Allen (1955). The observations of crossing-over and recombination within the H-2 region have allowed subdivision of the system into "sub-systems" (Gorer and Mikulska, 1959; Shreffler, Amos and Mark, 1966; Stimpfling and Richardson, 1965). Twenty-two cases of recombination have been described; 14 of these have been ex-

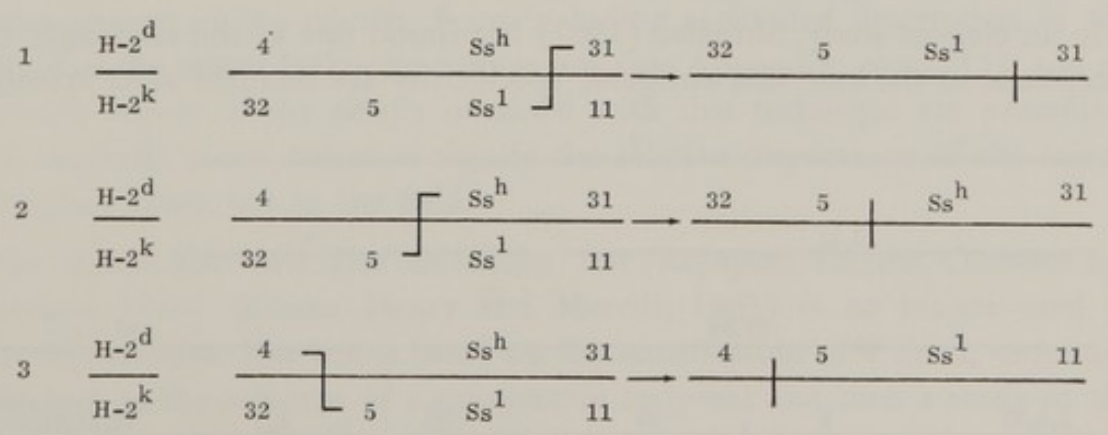



FIG. 2—Recombination map of the H-2 region of the mouse. The numbers represent determinants which cannot be assigned to a single subdivision but which lie within the portion of the region encompassed by the line.

tensively analyzed. In the great majority of the 14 cases, antigens associated with the parental alleles are present without change in the animal carrying the recombinant allele. I shall use 3 examples of crossing-over (obtained by Shreffler) to demonstrate the linear organization proposed for the H-2 region. The numbers in Figure 2 refer to antigenic specificities, the $Ss^h$ and $Ss^1$ symbols to alternate forms of a gene controlling the quantitative level of a serum protein first described by Shreffler and Owen (1963).

Study of homozygous parental mice allows one to list the antigens associated with the parental alleles. Thus, allele $H\text{-}2^d$ has associated with it antigens 4, 6, 13, 31 and the $Ss^h$ allele. Allele $H\text{-}2^k$ has associated with it antigens 1, 5, 11, 32 and the $Ss^1$ allele. Therefore, mice heterozygous for the $H\text{-}2^d$ and $H\text{-}2^k$ alleles carry antigens 1, 4, 5, 6, 11, 13, 31 and 32. In each of the 3 cases of crossing-over shown in Figure 2, one of the parent mice was heterozygous for the $H\text{-}2^d$ and $H\text{-}2^k$ alleles and in each case one of the progeny had an allele which had associated with it a new combination of antigens. Thus, in example 1, Figure 2, a new allele determining antigens 5, 31 and 32 and carrying the allele for $Ss^1$ appeared. Assuming that a single cross-over occurred with equal pairing of the homologous H-2 alleles, one can group the allele(s) for antigens 5 and 32 and the allele for $Ss^1$ together and place them to one side (arbitrarily the left) of the allele for antigen 31.

In example 2, Figure 2, it is possible to place the allele(s) for antigens 5 and 32 to the left of the Ss allele. We have already learned from example 1 that the Ss allele is to the left of the allele for antigen 31, so that we can now say that the allele(s) for antigens 5 and 32 are to the left of the allele for the Ss protein, which in turn is to the left of the allele for antigen 31. From the cross-over in example 3 it is possible to define still further a linear order for the alleles controlling the various antigenic specificities. Similar analyses of other recombinants have permitted derivation of a linear structure of the H-2 region depicted in Figure 3.

In an elegant study, Shreffler (1967) has found one of the seemingly rare exceptions to the rule that antigenic specificities are retained after crossing-

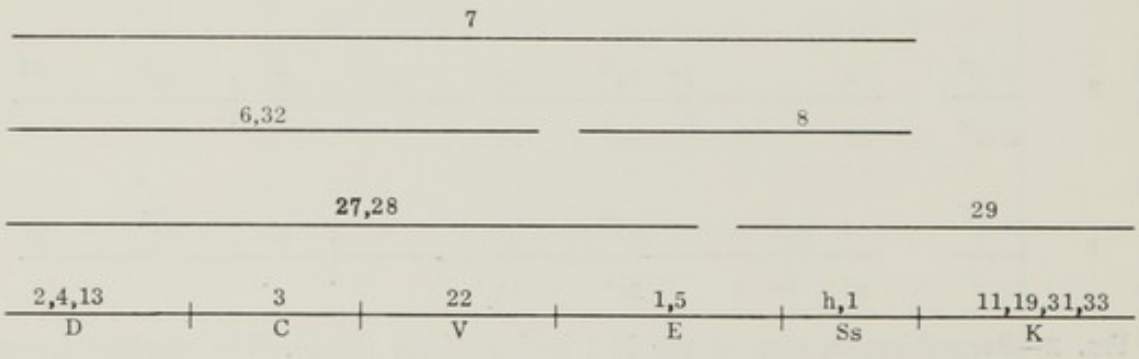



FIG. 3—Recombination map of the H-2 region of the mouse. The different subdivisions are identified by capital letters, as indicated.

over. In recombinants producing the H-2° allele, he found an antigenic specificity (No. 3—shown in Figure 3) which is identical when associated with either of the 2 parental alleles (H-$2^d$ and H-$2^k$) but which appears slightly altered when analyzed in the recombinant compared to either of the parental strains.

## HISTOCOMPATIBILITY STUDIES IN MAN

The eventual goal of histocompatibility testing is to identify important histocompatibility antigens and to pair individuals on this basis. For such pairing, we must know: 1) that the antigens we are measurng are histocompatibility antigens; 2) that all of the important antigens can be recognized; and 3) the relative strengths of the antigens, and whether "strengths" vary depending on the genotype of the recipient. A differential "incompatibility strength" for certain ABO antigens dependent on the ABO genotype of the recipient has already been described (Ceppellini, Curtoni, Mattuiz, Leigheb, Visetti and Columbi, 1966). Despite great progress in the field of leukocyte typing in man, it is almost certain that not all the antigens are yet defined, and that at present we have little information on the "strength" of various antigens, or the dependence of "strength" on the genotype of the recipient.

Histocompatibility studies have evolved along 2 lines, as previously mentioned: "matching" by *in vivo* and *in vitro* tests and "typing" for specific leukocyte antigens which are thought to be transplantation antigens.

### *Matching Tests*

The 3 major matching tests that have been used are the *"third man" test,* the *normal lymphocyte transfer test,* and the *mixed leukocyte culture test.* Russell, Nelson and Johnson (1966) reviewed the status of these tests in 1966. Although these tests tell us much less about the nature of the genes controlling the antigenic specificities than do typing procedures, they are perhaps more directly related to the physiological events underlying homograft rejection. At the request of the editors, I am including a detailed description of the method of the mixed leukocyte culture test (see Appendix, page 235), and an extensive report of the results obtained with this technique are presented. This emphasis is not meant to signify the relative importance of the mixed leukocyte culture test in the field.

*The "Third Man" test.* The "third man" test (Rapaport, Thomas, Converse and Lawrence, 1960; Wilson, Henry and Merrill, 1963) is no longer used in many laboratories. The test is based on the sensitization of a third, unrelated individual to the antigens of a prospective recipient and then a study of the rejection times of grafts from a series of potential donors onto the sensitized third individual. If a recipient and a donor share many antigens, the "third man" will be sensitized to the shared antigens by the graft from the poten-

tial recipient and he will then reject the graft of the donor in a second-set fashion. However, as many authors have pointed out, the fact that 2 individuals have antigens in common does not necessarily mean that they do not have antigens which are different and it is, of course, the difference that is important. Further, the genotype of the third "man" can materially influence the test. I have previously discussed specific examples which could be very misleading (Bach, 1966). Nevertheless, Mathe, Méry, Bréziu, Sekiguchi, Vaubel and Amiel (1965) reported some success using the third man test to predict skin graft rejection. Their degree of success would suggest that there are not a great number of important factors playing a role in skin graft rejection; otherwise, an estimate of similarity would seldom rule out differences.

*The normal lymphocyte transfer test.* The normal lymphocyte transfer (NLT) test was the first histocompatibility matching test which made use of the ability of peripheral blood lymphocytes to "recognize" foreign transplantation antigens and respond to these antigens. Brent and Medawar (1963) observed that the intradermal injection of lymphocytes from one guinea pig into an unrelated guinea pig resulted in 2 separate reactions which were maximal at 24 to 48 hours and at 7 days, respectively. When parental peripheral blood lymphocytes are injected into F-1 hybrid animals, only the 48-hour reaction is observed, which is presumed to be a graft-versus-host reaction; no reaction is seen at 7 days. The 7-day reaction is presumed to be the host-versus-graft response. Applying this test to predict graft survival, Brent and Medawar injected cells of an intended recipient into a series of potential donors and recorded the 48-hour response as 0-4+. This response should indicate how cells of the recipient will react to donor tissue. They demonstrated a very convincing correlation between the intensity of the response and the rapidity of skin graft rejection, i.e., the most severe reaction as recorded at 48 hours correlated with the most rapid rejection of the skin graft.

The NLT test was applied to man by several investigators (Amos, Nicks, Peacocke and Sieker, 1965; Bridges, Nelson and McGeown, 1964; Ceppellini, Curtoni, Leigheb, Mattuiz, Miggiano, Visetti, 1965; Gray and Russell, 1963, 1965). A strong response can be elicited at 48 hours in man as in guinea pigs. A useful modification of the test, using multiple concentrations of test cells that are injected into the skin of the potential donors, has been suggested by Ceppellini et al, (1965). Using this modification of the technique, Ceppellini obtained a correlation between NLT results and skin graft survival. Amos and his colleagues (Amos et al., 1965; Hattler and Amos, 1965) and Aisenberg (1965) suggested on 2 grounds that the reaction at 48 hours could not be interpreted as strictly a graft-versus-host reaction. First, peripheral blood cells taken from an individual sensitized to the recipient's

antigens did not give an increased reaction when injected into the skin of the recipient. Second, cells from normal individuals injected into the skin of patients with varying degrees of immunological deficiency gave weaker reactions than normal cells injected into normal recipients. They argued on these latter grounds that the reaction determined at 48 hours in man must involve to some extent the reaction of the host against the graft.

Apart from the theoretical difficulties of the NLT test, there are potential dangers to the subjects. It is evident that any histocompatibility matching test which involves transfer of human cellular material from individual to individual is undesirable because of the attendant dangers of such a procedure, such as the transfer of viral illness and the possibility of sensitization.

*The mixed leukocyte culture test.* The mixed leukocyte culture (MLC) test (Bain, Vas and Lowenstein, 1964; Bach and Hirschhorn, 1964) is an *in vitro* model of the normal lymphocyte transfer test in which the responding cells are peripheral blood lymphocytes, and the cells to which they respond,

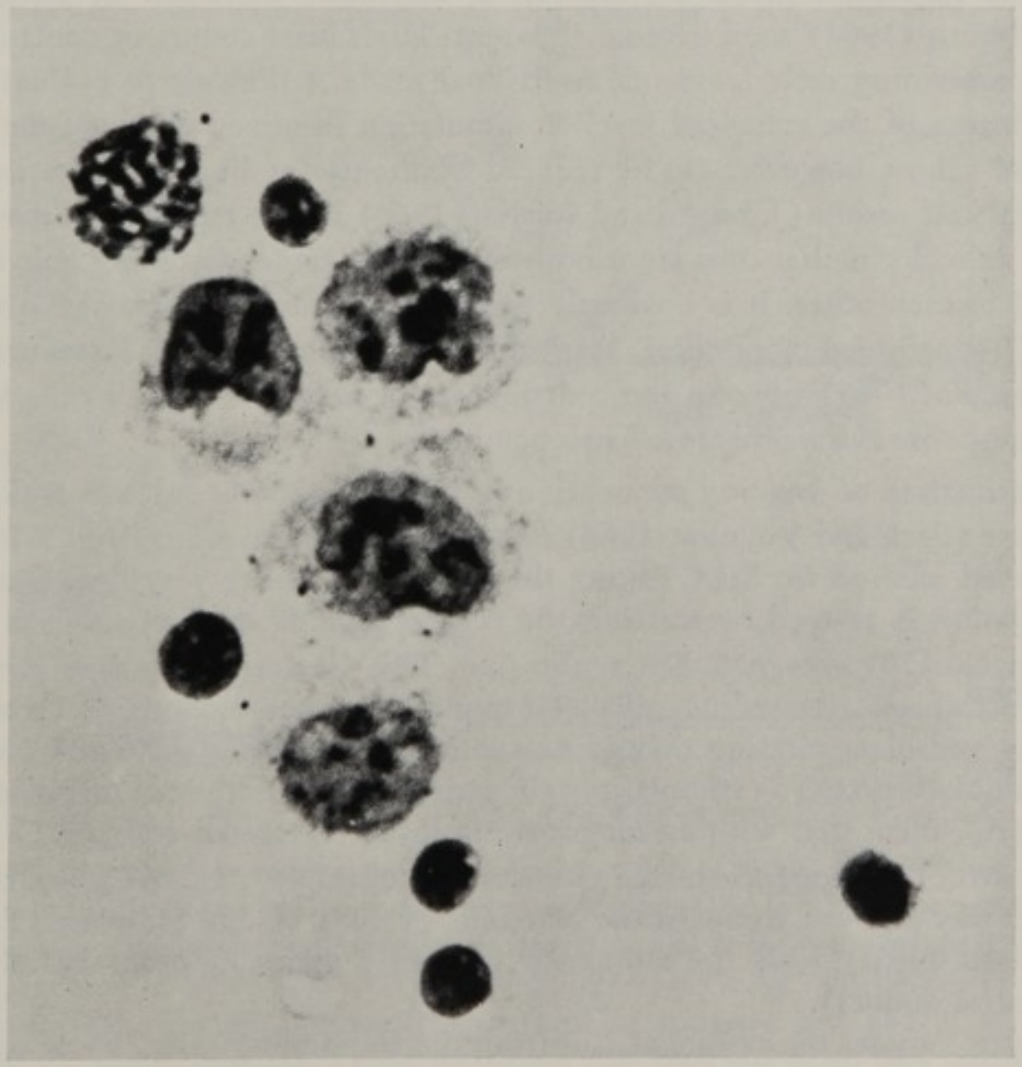


FIG. 4—Stained preparation of cells from a mixed lymphocyte culture harvested at 7 days. Five small cells, 4 enlarged cells, and one mitosis are seen.

i.e., the tissue carrying the stimulating antigens, are peripheral blood lymphocytes of the potential donor rather than skin cells and other migratory cells.

The original observation on which the test is based was that mixtures of leukocytes from 2 genetically unrelated individuals resulted in mutual stimulation, evidenced by enlargement and mitosis of lymphocytes in culture (Figure 4). The method seemed to have potential as a measure of histocompatibility because: 1) mixtures of cells from identical twins did not result in stimulation (Bain et al., 1964; Bach and Hirschhorn, 1964); 2) mixtures of cells from related individuals resulted in less stimulation, on the average, than mixtures of cells from unrelated individuals (Bain and Lowenstein, 1964), and 3) the histocompatibility status of one set of 3 individuals based on MLC determinations correlated with their compatibilities determined by the third-man test (Bach and Hirschhorn, 1964). Other reported data (Dausset, Ivanyi and Ivanyi, 1965; Russell et al., 1966) suggested that the test does provide some measure of histocompatibility in man.

Some shortcomings in the method as originally described by Bach and Hirschhorn (1964) soon became apparent. High blast counts in control cultures containing cells from one individual made it difficult to evaluate the significance of the enhanced level of stimulation in mixed cultures; the high control values, however, can be reduced by the use of human serum instead of fetal calf serum (Johnson and Russell, 1965) in the medium. A more serious difficulty is that cells from both individuals under test were able to respond to each other. It is obviously preferable to measure one-way stimulation (Ceppellini, Franceschini, Maggiano and Tridente, 1965; Kasakura and Lowenstein, 1965) wherein the contribution of each individual's cells to the total response can be measured independently.

The method of one-way stimulation in mixed leukocyte cultures which we now use (Bach and Voynow, 1966) removes most of the difficulties. With the improved method for MLC testing the control values are very low. One-way stimulation is assayed by treating the cells of one of the test subjects with mitomycin C to prevent DNA replication. This treatment prevents the cells from acting as "responding cells" (responding to homologous tissue by enlarging and incorporating thymidine) without preventing them from acting as "stimulating cells" (stimulating allogeneic cells to respond). Responding cells (A), incubated with autologous mitomycin C-treated cells ($A_m$) serve as a control for allogeneic mixed cultures (for example, $AB_m$, $AC_m$ and such). In this way one can compare the reactions of cells of one individual (a potential recipient) with the allogeneic cells of a series of other individuals (potential donors).

Figure 5 shows the results of culturing the responding cells (F) of one individual with his own mitomycin C-treated cells ($F_m$) and with those from another individual ($G_m$). Radioactive thymidine was added after 160 hours

and triplicate cultures were harvested at the times shown. Four slopes representing rates of thymidine incorporation were obtained. While the rate of incorporation in the culture $FG_m$ is markedly positive, the rates of the cultures FF, $FF_m$ and $F_mG_m$ are negligible. The virtual identity of the last 3 rates—assayed far beyond the usual time—indicates that cells treated with mitomycin C are rendered incapable of acting as responding cells in MLC, while they retain ability to stimulate ($FG_m$ and $GF_m$ — not shown); and that addition of autologous treated cells ($F_m$) to cells able to respond (F) does not cause them to respond ($FF_m$).

The results of using the standard method with cells of 2 unrelated individuals, H and J, appear in Figure 6. Incorporation of thymidine is close to a linear function of time from 1.5 to 5.5 hours. In both $HJ_m$ and $JH_m$ (reciprocal one-way cultures) positive stimulation is present. Reciprocal one-way studies with 2 sets of siblings whose cells did not stimulate each other are demonstrated in Figures 7 and 8. Figure 7 shows results with cells from N and O; the very close identity of the controls ($OO_m$ and $NN_m$) with

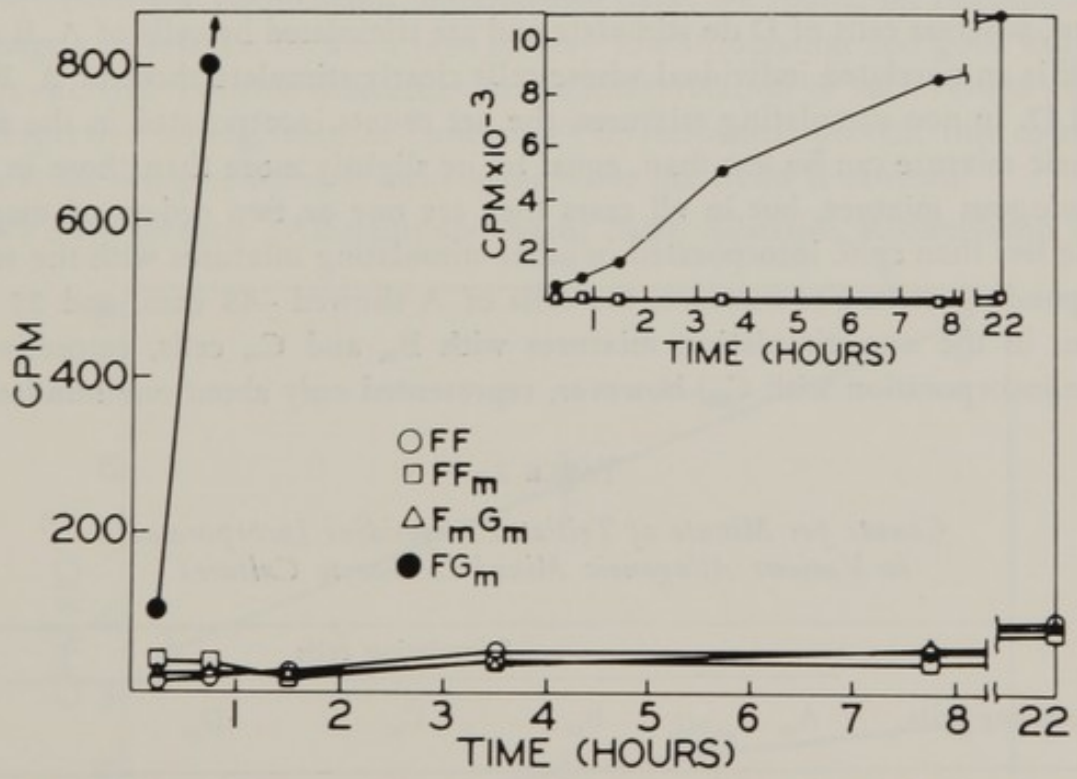



FIG. 5—$H^3$ thymidine incorporation into acid precipitable material in different mixed leukocyte cultures. Mixed leukocyte cultures were incubated for 160 hours, at which time $H^3$ thymidine was added. Replicate cultures were harvested at the times indicated following the addition of label. This is a long term experiment using cells of two unrelated individuals. $FG_m$ and $FF_m$ represent the response of untreated cells (F) of one individual to the treated cells ($G_m$) from the second individual and to his own treated cells ($F_m$). FF and $F_mG_m$ are other mixtures. The inset is on a different scale to permit all the data to be plotted.

their respective allogeneic mixtures ($ON_m$ and $NO_m$) is striking, despite the slightly positive slope of $NN_m$. Figure 8 shows the results with cells from S and U; these cultures also show no stimulation as judged by comparison of rates of incorporation (cpm. incorporated per hour of labeling) of the control and mixed cultures. On the other hand, simple comparison of values of thymidine incorporation in the $US_m$ and $UU_m$ or $SU_m$ and $SS_m$ cultures at any single time might lead to a different conclusion. Clearly rate measurements should be used in some circumstances. Usually, however, it is satisfactory to study the incorporation of radioactive thymidine for a single 5 or 5.5 hour period on the seventh day.

All MLC test results can be grouped as either showing or not showing stimulation, as illustrated in Table 2, wherein MLC results from one of the 2 families used to correlate one-way MLC tests with skin graft survival are given. Net values of stimulation are given in counts per minute (cpm.) of tritiated thymidine incorporated in the allogeneic mixture minus cpm. of tritiated thymidine incorporated in the autologous mxture given in parentheses. Cells of A, B and C do not stimulate each other in any paired combinations, whereas cells of D do stimulate and are stimulated by cells of A, B and C. E is an unrelated individual whose cells clearly stimulate those of A, B, C and D. In non-stimulating mixtures, the net counts incorporated in the allogeneic mixture can be less than, equal to, or slightly more than those in the autologous mixture, but in all cases they are one or two orders of magnitude less than cpm. incorporated in other stimulating mixtures with the same responding cells. For instance, the cells of A showed –48 cpm. and 23 net cpm. in the non-stimulating mixtures with $B_m$ and $C_m$ cells, respectively. The incorporation with $C_m$, however, represented only about one hundredth

TABLE 2.—

*Counts per Minute of Tritiated Thymidine Incorporated in Various Allogeneic Mixed Leukocyte Cultures*

| Responding cells | Stimulating cells | | | | |
|---|---|---|---|---|---|
| | $A_m$ | $B_m$ | $C_m$ | $D_m$ | $E_m$ |
| A | (120) | –48 | 23 | 2126 | 3412 |
| B | 99 | (74) | 68 | 3031 | 3978 |
| C | 185 | 111 | (190) | 4641 | 13529 |
| D | 7732 | 7948 | 7892 | (407) | 5504 |

The values in parentheses are the control values. These have been subtracted from the other values in the table. Thus the value of –48 ($B_m$—A) corresponds to a reading of 72 from which 120 was subtracted.

of the cpm. present in the stimulating mixture $AD_m$ and even less in comparison to $AB_m$, the mixture with the unrelated individual's cells. In the reciprocal tests, C's cells show incorporation of 185 and 111 cpm. in the $CA_m$ and the $CB_m$ mixtures, respectively. Again, however, the stimulating mixtures $CD_m$ and $CE_m$ gave 4,641 cpm. and 13,529 cpm. Siblings can thus be classified as *MLC compatible siblings* when their cells do not stimulate in culture, and *MLC incompatible* when stimulation does occur.

Skin grafts were performed between MLC identical (compatible) and MLC nonidentical (incompatible) siblings in each of 2 families. The mean survival time for 4 grafts between incompatible pairs was 12.25 days (range 11 to 13 days) and for 8 grafts between compatible pairs, 26 days (range 15-36 days). This difference is biologically and statistically significant, as previously discussed (Bach and Kisken, 1967) and supports the predictive value of MLC tests for skin graft survival.

At a recent workshop in Denver, where all living kidney recipients and their donors were typed and matched by Amos, van Rood, Terasaki and Bach, (Bach and Klostemann, unpublished data) we found, on retrospective testing, a correlation between zero or very low stimulation and good kidney graft survival. Possible difficulties in the interpretation of such results using retrospective testing, such as "tolerance" in recipients, prevent this correlation from being completely convincing—but the trend is obvious.

We have recently (Albertini and Bach; Bach, Albertini, Klostermann and

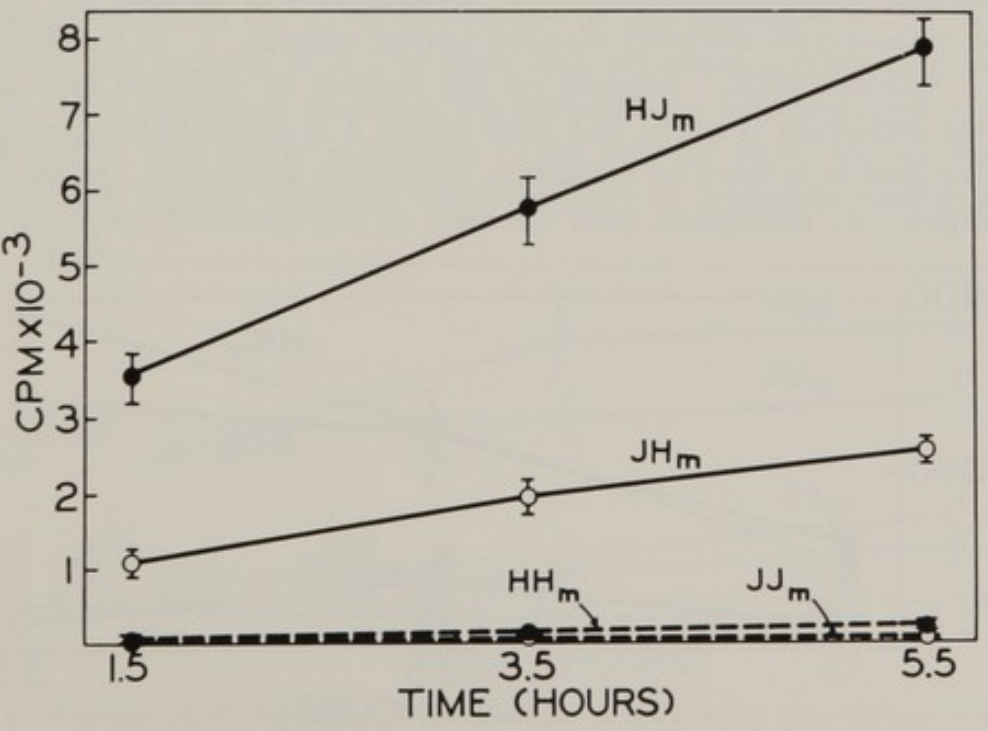



FIG. 6—Protocol as in Figure 5. Cells of two unrelated individuals tested in reciprocal ($JM_m$ and $HJ_m$) mixed cultures with their respective controls $JJ_m$ and $HH_m$).

Day, 1968) attempted to quantitate different degrees of stimulation in MLC tests. Results of these experiments will be presented after we have discussed leukocyte typing and correlations between matching and typing tests.

*Leukocyte Antigens*

Antisera used to define human leukocyte antigens have been obtained from multiparous females, whose sera contained antibodies against antigens of the fetus inherited from the father and not possessed by the mother. Other antisera have been obtained from persons deliberately immunized with leukocytes or skin grafts. In either case, if the antigenic complexity in man is as great as that in the mouse, one would expect that (except for occasional cases of carefully planned immunizations) any such serum would probably contain antibodies against several antigens—a multispecific antiserum. Operationally monospecific sera, defining" single" antigens, are usually more desirable and have been sought in most of the laboratories doing leukocyte typing in man. The difficulties which can arise with multispecific sera can be illustrated in a hypothetical example. A multispecific serum tested with cells of 2 individauals, A and B, could react positively with both A and B, and yet be reacting with different antigens in each of the 2 individuals.

The method of obtaining monospecific sera can be illustrated by our example above. Suppose that individual A has only a single antigen, "1," that individual B has two antigens, "1" and "2," and that the antiserum contains antibodies against only these two antigens. In this case, cells of A added to the antiserum will absorb the antibody directed against antigen "1," leaving only the monospecific antibody directed against antigen "2" of B. The definition of "monospecificity" depends on the number of different cells against which the antiserum is tested and with which it is absorbed. In operational terms, the goal is to have an antiserum containing antibodies against only a unit antigen, i.e., absorption with the cells of any positively reacting mem-

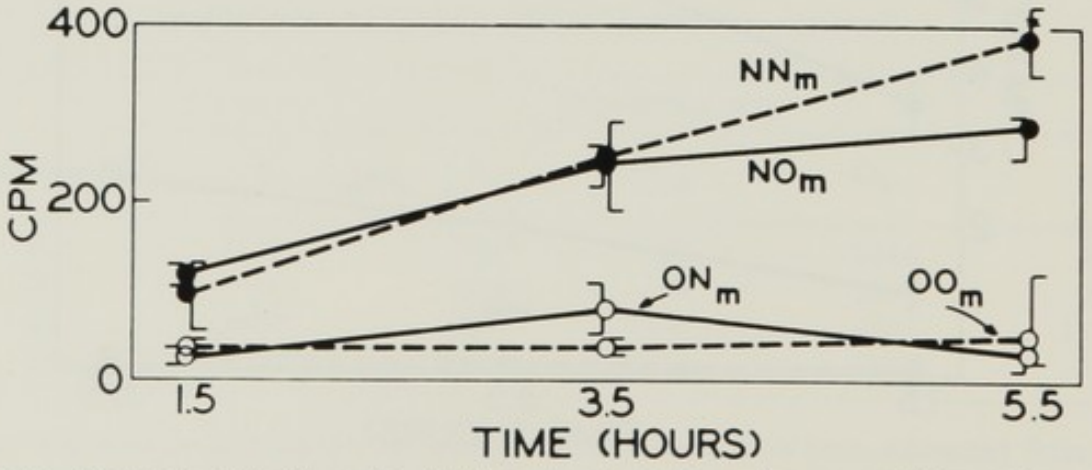



FIG. 7—Protocol as in Figure 5. Reciprocal rate studies using two siblings whose cells did not stimulate by either rate criteria or by single point measurements.

ber of the population will completely inactivate the serum. The operational definition of monospecificity has been discussed by Walford, Shanbrom, Troup, Zeller and Ackerman, 1967 and by Hirschfeld, 1965).

For antileukocyte sera to be useful in assessing histocompatibility, a correlation must be established between leukocyte and transplantation antigens. The work of Medawar (1946) showed that the intradermal injection of peripheral blood leukocytes induced homograft sensitivity. Further work by Amos (1953) and Gorer (1955) also indicated that the peripheral blood leukocytes must carry the transplantation antigens. In 1954, 2 groups (Dausset, 1954; Miescher and Fanconnet, 1954) showed that leukocyte agglutinins were isoimmune antibodies, and not autoimmune as had been suspected. The evidence defining the first leukocyte isoantigen, "Mac," was presented by Dausset (1958).

Payne and Rolfs (1958), and van Rood, van Leeuwen, and Eernisse (1959) described the presence of leukocyte agglutinins in the serum of females presumably immunized during pregnancy. This observation contributed greatly to the development of leukocyte typing since these antisera were much easier to use. van Rood and van Leeuwen (1963) introduced the use of computers to study results obtained with different sera. 2 x 2 association analyses of results obtained with different sera on the same panel of individuals demonstrate that it is possible to group those sera which recognize the "same" antigen, (a significant positive association) and those sera which possibly measure antigens determined by allelic genes (a significant negative association). One of the great advantages of this approach is that it allows one to test cell samples with 2 or more sera presumably measuring the same antigen. This procedure decreases the probabilities of undetected erroneous typing results. The reader is referred to the original paper by van Rood and van Leeuwen (1963) applying these methods to leukocyte typing and the

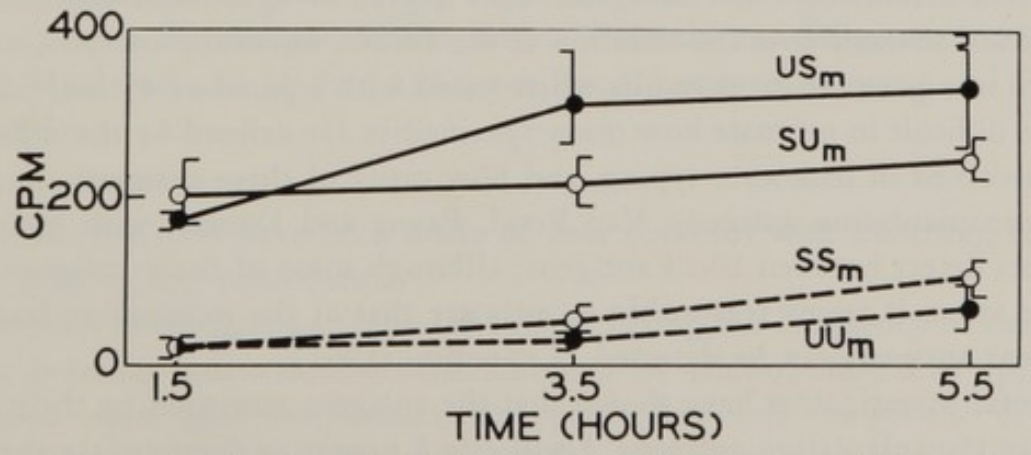



FIG. 8—Protocol as in Figure 5. Reciprocal rate studies using two siblings whose cells do not stimulate by rate criteria but in which single point comarisons could be otherwise interpreted.

contributions of Payne, Tripp, Weigle, Bodmer and Bodmer (1964) for further discussion of these techniques.

I will restrict this review to a discussion of the present status of leukocyte antigens, and refer the reader to the detailed papers of various workers in the field for more extensive discussion of the problem in the 3 volumes of *Histocompatibility Testing* (1964, 1965, 1967).

As in any developing immunogenetic field, individual nomenclatures evolved as different laboratories discovered new antigens. However, it is obvious from the above discussion of methods of preparing monospecific antisera that the final "single" antibody left in the serum is defined operationally and will depend on the panel of individuals used to absorb the multispecific serum and the panel against which the absorbed serum is tested. Only after it is clear that a substantial number of the sera used in different laboratories react similarly when tested against a given large panel of individuals is it reasonable to adopt a uniform nomenclature. While such a uniform nomenclature has not yet been adopted,* data presented at the third Histocompatibility Workshop in Torino, Italy, make it likely that such antigens are now being defined in many different laboratories, using different antisera and different techniques, and that the genes controlling many of these antigens seem to behave as alleles of a single system as indicated by transmissional analysis. Thus, for instance, an antiserum used by Dausset to measure 'Mac," the antigen first described by him, reacted positively with the cells of 22 individuals in a panel and did not react with the cells of the remaining 19. Sera used by 4 other investigators reacted identically with the same panel of 41. Many other sera, from different laboratories, reacted positively with most of the 22 cells reacting positively with the serum of Dausset—but some also gave positive reactions with some of the 19 samples with which the serum of Dausset had not reacted. Comparable patterns of agreement were obtained with sera measuring other antigens. This degree of agreement represents a significant advance over the situation at the Leiden workshop in 1965, where no two sera gave identical results when tested with a panel of 45 individuals.

It is difficult to estimate how many specificities are defined by the different sera involved in leukocyte typing, and how many of these antigens are actually transplantation antigens. Van Rood, Payne and Dausset with their coworkers detect between 10-14 antigens; although some of these antigens may be the same, it seems reasonable to estimate that at the moment at least 15 different antigens can be detected (Ceppellini, 1967).

Several investigators have shown that the antigens measured by their antisera are transplantation antigens. I will cite 3 papers to demonstrate the different methods used to show this correlation of the antigens with histocom-

* It has now been adopted for some antigens.

patibility. One method, first tried by van Rood (van Rood, van Leeuwen, Schippers, Vooys, Fredericks, Balner, and Eernisse, 1965), is to immunize individuals with cells presumed to carry only 1 or 2 antigens which the recipients themselves do not possess, and then a short time afterward to transplant skin from individuals who carry and do not carry the relevant antigen. The prediction would be that skin from individuals carrying the antigen would be rejected more rapidly (since the recipient is sensitized to that antigen) than skin not carrying the antigens. Both van Rood et al., (1965) and Dausset, Rapaport, Ivanyi and Colombani (1965) used this method to demonstrate that certain of the leukocyte antigens were indeed transplantation antigens. Van Rood has pointed out that such studies may be less than optimal since the recipients are pre-immunized—which may unduly weight the importance of these antigens. Van Rood, van Leeuwen, Schippers, Ceppellini, Mattiuz and Curtoni (1966) attempted to predict skin graft rejection times based simply on leukocyte typing data. Although they found a significant correlation in siblings, they were unable to find a correlation in parent-child grafts, and had only a suggestive correlation in unrelated individuals. Amos, Hutchin, Hattler, McClowskey and Zmijewski have also found a good correlation in siblings—comparing siblings who typed identically, or nearly so, with those in whom there were many differences. If there is one major system which exerts strong control of skin graft survival in man as there is in the mouse and the rat, then the situation would be expected to be much simpler genetically in siblings. This may well explain the success in siblings referred to above. Success is not, however, confined to siblings. Walford, Martin, Troup, and Goodwin (1965) were able to select unrelated individuals who typed similarly and in whom skin graft survival was prolonged. There is also good evidence that compatibility for leukocyte antigens correlates with improved homograft survival in kidney transplant patients (Dausset, Rapaport, and Legrand, 1967; Payne, Perkins, and Najarian, 1967; Rapaport, Dausset, Hamburger, Hume, Kano, Williams, and Milgrom, 1967; Terasaki, Vredevee, and Mickey, 1967; van Rood, Leeuwen, Bruning and Porter, in press).

*Genetic Analysis*

Genetic analyses have been made of data obtained with matching tests as well as by typing procedures.

Newth (1961) devised formulae for predicting the frequency of compatibility between individuals of different genetic relationships. His approach assumed equal gene frequencies, full penetrance and no genetic or gene product interaction, and is based on the following argument: If a single locus is important in compatibility, and there is only one allele in the population, then 100 per cent of individuals will be homozygous for that allele and in

100 per cent of matches between unrelated individuals, parent-child combinations or sibling-sibling combinations, there will be compatibility, i.e., genetic identity. However, if there are an infinite number of alleles at such a locus, all unrelated individuals will, at that locus, be heterozygous for different alleles, and the frequency of compatibility between unrelated individuals will approach zero—as will that for parent-child matches. Siblings, however, will still be compatible in 25 per cent of matches, since the two parents can possess only 4 different alleles at one locus.

Simonsen (1965, 1966) used this approach to estimate the number of strong transplantation antigens in man. He based his analysis on the percentage of surviving kidney transplant recipients at one year who had received transplants from donors of different genetic relationships. He suggested from

TABLE 3. — *Expected Percentages of Nonreacting Mixtures When One-way Stimulation was Used*

| Alleles (No.) | Genetic relationship | |
|---|---|---|
| | Sib-sib | Unrelated |
| | *One locus* | |
| 1 | 100 | 100 |
| 2 | 78 | 63 |
| 3 | 61 | 33 |
| 10 | 35 | 3.7 |
| 15 | 32 | 1.7 |
| 20 | 30 | 0.96 |
| 30 | 28 | 0.43 |
| ∞ | 25 | 0.00 |
| | *Two loci* | |
| 2 | 61 | 39 |
| 3 | 37 | 11 |
| 4 | 27 | 4 |
| 5 | 21 | 1.8 |
| 6 | 18 | 0.94 |
| | *Three loci* | |
| 2 | 47 | 24 |
| 3 | 22 | 3.6 |
| 4 | 14 | 0.8 |
| | *Four loci* | |
| 2 | 37 | 15 |
| 3 | 14 | 1.2 |

these data that one locus with 2 to 4 strong alleles controls homograft rejection in man. Simonsen should be credited with using this form of analysis in its application to transplantation problems. While it is a useful method, Simonsen's detailed conclusions must be questioned for several reasons: 1) The percentage survival figures are based on survival for arbitrarily chosen periods of time and the choice of other times of survival could give different solutions. 2) The same antigen could well be "strong" in one donor-recipient combination and weak in another. This is true in the ABO system in which the A antigen has different incompatibility strengths depending on the ABO genotype of the recipient (Ceppellini et al., 1966). 3) Any such estimate would demand data in which the 3 groups (unrelated donors, parent donors and sibling donors) can be validly compared. It is likely that immunosuppressive therapy and other factors are quite different in the 3 groups —thus making the percentage survival figures not directly comparable. 4) With the probable antigenic complexity of the locus and with the multiple antigens associated with each allele, Simonsen's figures probably do not give us an estimate of the number of *strong antigens* in the system.

Ceppellini et al., (1965) have done a similar analysis of the results of MLC tests performed by them. Their criterion of activation was an increase in the counts incorporated of at least 2 standard deviations over the mean number of counts present in their control cultures. If a mixed culture resulted in fewer counts incorporated than this threshold, that mixed culture was considered "compatible," i.e., not to have stimulated. On this basis they found that 75 per cent of sibling mixtures did not stimulate and 12 per cent of unrelated mixtures did not stimulate. The authors suggested that one locus with 3 to 4 alleles of equal frequency is compatible with the data. These authors have also used an arbitrary level of "stimulation" below which compatibility is assumed, and for this reason some of the arguments presented above also apply here. In particular, the "genetic solution" will, of course, vary with the level chosen for compatibility.

The approach we have used to analyze results of MLC tests (Bach 1966-a,b; Bach et al., 1968) also involves dividing MLC results into non-stimulating mixtures and stimulating mixtures. However, in our work these 2 categories are clearly and unambiguously separable, as previously discussed and illustrated in Table 2. No arbitrary limits for compatibility need be imposed.

In Table 3, the expected values for the proportion of non-stimulators (for different genetic relationships) are given for varying numbers of loci and alleles, assuming equal allelic frequencies, full penetrance, and no genetic or gene-product interactions. The total Madison series includes 209 sibling pairs in 29 families. Of these, the cells of 61 pairs (29.2 per cent) have not stimulated. Of 282 mixtures of cells from unrelated pairs, all have stimulated. The

95 per cent confidence limits for these percentages are 23 to 35 per cent for the siblings and 0 to 1.4 per cent for the unrelated individuals. These combined data only fit the expectation for one locus with 15 or more alleles. Although the data on siblings also fit the possibility that there are 2 loci each with 4 alleles, the data for unrelated tests are not compatible with this solution. Results of two-way MLC tests in more than 500 cell mixtures from unrelated subjects—all of which have stimulated—further support the interpretation in terms of multiple alleles at a single locus. As already stated, this analysis requires no imposition of the extent of compatibility, since nonstimulators are clearly separated from stimulators; nor is it sensitive to deviations from the assumption of equal allelic frequencies—since with a large number of alleles, any deviation from equality will closely approximate a simple decrease in the number of effective alleles (Crow and Kimura, 1964). In other words, assuming equal allelic frequencies gives a low estimate of the number of alleles.

The question of whether there are alleles at this locus controlling antigens (perhaps "weak" antigens) which will not cause stimulation in MLC tests cannot be answered. Therefore, zero stimulation cannot be equated to genetic identity. However, our results with unrelated matches indicate that if there are such alleles, their frequency is very low. Because of this uncertainty, I will consider MLC-identical individuals as "effectively identical" for genetic factors controlling reactivity in MLC tests.

Using 2 x 2 association analysis, Payne and Bodmer have defined a series of antigenic specificities which they have called LA-1, LA-2, LA-3, and LA-4, each seemingly associated with an allele of a single system. This concept was strengthened by transmission analysis: first, an individual had only 2 of these antigens—never 3—i.e., one antigen determined by each allele; second, an individual heterozygous for LA-1 and LA-2 transmitted only either LA-1 or LA-2 to one of his offspring—never both. In similar fashion, van Rood has described a series of other "allelic" systems, some of which are less clearly defined. He described a "4" system with antigens 4a, 4b, etc., as well as other systems which he designated "5" through "9."

In 1965 van Rood suggested from 2 x 2 analysis as well as transmissional data that several of the "allelic" systems he had described seemed to be linked, and that "this would show the existence in man of a leukocyte group system that in many aspects resembles the H-2 locus in mice" (van Rood et al., 1965). Dausset, Ivanyi and Ivanyi (1965) at the same conference suggested that 10 of their antigens, some of which are also detected by van Rood, "belong to one complex system of tissue alloantigens."

Data obtained from the Third International Histocompatibility Workshop in Torino fully support these suggestions. Transmissional information for

TABLE 4.

| Antisera | Father | Mother | Siblings 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PAY | +++ | 0 | 0 | 0 | 0 | 0 | +++ | +++ | +++ | +++ | +++ |
| RA | +++ | 0 | ++ | +++ | ++ | ++ | 0 | 0 | 0 | 0 | 0 |
| ROY | 0 | +++ | ++ | +++ | +++ | ++ | ++ | +++ | 0 | 0 | ++ |
| BH | +++ | +++ | +++ | ++++ | ++++ | ++++ | 0 | 0 | ++ | ++ | 0 |

antigens can best be obtained when one parent is heterozygous for the gene controlling the antigen in question and the other parent does not have the antigen. This situation is phenotypically represented when one parent is positive for a given antigen, the other parent negative and only some of the children are positive. Typing results obtained in one of our families studied by Amos are given in Table 4.

Only 4 antisera are listed, all of which demonstrate the polymorphism in this family. Let us assume that each of these antisera is monospecific. Thus, when the father reacts positively with a given antiserum and the mother does not react with that antiserum, we can assume that the father is heterozygous for that antigen, since some of the children do not inherit the antigen. If we assign that antigen to one of the father's alleles at the locus in question, we can then say that all positively reacting children have received that allele from the father and all negatively reacting children have received the other allele. This can be verified if we have another antigen, associated with the father's second allele. All children negative for the first antigen should be positive for this second antigen and those positive for the first should now be negative (contrasting distributions). This is exactly the case in the family listed in Table 4. If we assign the antigen measured by antiserum PAY to allele *a* of the father and the antigen measured by antiserum RA to allele *b* of the father, we can say that siblings 5, 6, 7, 8 and 9 receive allele *a* from the father and siblings 1, 2, 3 and 4, allele *b*. We can do a similar analysis for the 2 alleles, *c* and *d* of the mother. Allele *c* is given to children 1, 2, 3, 4, 5, 6 and 9, and allele *d*, presumably, to children 7 and 8. Although we have no antigen we can clearly associate with allele *d* of the mother, we can assign allele *d* by the argument of contrasting distributions. There is one additional antiserum, BH, which is informative. Although both parents are heterozygous, the data fits if we assume that siblings 1, 2, 3 and 4 are positive because they have inherited allele *b* from the father and siblings 7 and 8 are positive because they have inherited allele *d* of the mother.

Analysis of data obtained at the Third Histocompatibility Workshop clearly indicates, in family studies, that alleles controlling most of the antigens being measured by the many teams at the Workshop segregate as though they belonged to a single genetic system (Ceppellini, 1967). However, it is interesting to note that some antigens, such as 5a and 5b of van Rood, are determined by alleles which segregate independently of alleles of the major system.

*Correlation Between Matching Tests and Typing Procedures*

So far I have considered the evidence that incompatibility in matching tests or for some of the leukocyte antigens correlates with homograft rejec-

tion; and conversely that skin graft survival and in some cases kidney graft survival correlates with compatibility in matching tests and for leukocyte antigens. If both of these test procedures do in fact provide some measure of histocompatibility, then there should also be some correlation between them.

Van Rood et al. (1965) attempted to correlate results of leukocyte typing with the absence or presence of a reaction in NLT tests. Because of some of the difficulties with the NLT test previously discussed, they were "not surprised" by the weak correlation they obtained. Some of the individuals who were leukocyte group incompatible still showed no NLT test reaction despite the relatively large numbers of cells injected ($3 \times 10^6$) for the NLT test. One could explain NLT reactivity without detected leukocyte antigen incompatibility by hypothesizing that there are antigenic incompatibilities which were not detected by the available antisera; but the reverse finding is more difficult to explain. The authors suggest as one possibility that the outcome of the NLT test may depend only on "strong" leukocyte antigens. Nelson et al. (1965) also failed to find a significant correlation between the NLT test and leukocyte antigens.

Two types of correlation have been obtained between the mixed leukocyte culture (MLC) test and leukocyte antigens. Dausset et al. (1965) and Ivanyi, Rychlikova, Sasportes, Ivanyi and Dausset (1967) found a correlation between the degree of stimulation in a two-way stimulation assay and antigenic incompatibility for at least 1 of 4 leukocyte antigens (antigens 1, 2, 3 and 7 of Dausset). As a criterion for quantitation, they suggested that if more than 7 per cent of cells were enlarged at the end of culture, stimulation was considered positive, between 4 per cent and 7 per cent slightly positive, and less than 4 per cent negative. Twenty-nine out of 32 positive reactions were correlated with antigenic incompatibility. In the slightly positive mixtures 7 out of 15 were incompatible for at least one antigen, and in the mixtures showing less than 4 per cent large cells, 0 of 14 showed such an incompatibility.

The approach I have used in collaboration with Dr. Bernard Amos (Bach and Amos, 1967) is to use siblings who seemed "effectively identical" for genetic factors controlling reactivity in MLC tests. Such individuals, one would expect, may also be identical with respect to their leukocyte antigens.

In our experiments, siblings from 7 large families were studied in all combinations of 2 within each family using the one-way MLC tests. The cells were also typed with 31 selected cytotoxic antisera. Results of the 2 methods are compared. All studies were done in Madison, Wisconsin, on cells from freshly drawn blood.

Two or more siblings whose cells are mutually non-stimulatory in reciprocal one-way MLC tests are referred to as "MLC-identical siblings." Table 5

TABLE 5. — *Stimulating Cells*

| *Responding cells* | $A_m$ | $B_m$ | $C_m$ | $D_m$ | $E_m$ | $F_m$ | $G_m$ |
|---|---|---|---|---|---|---|---|
| A | (52) | 16 | 1 | 29 | 2415 | 1507 | 2974 |
| B | -46 | (70) | -55 | -35 | 784 | 734 | 451 |
| C | 6 | 5 | (13) | 12 | 186 | 221 | 598 |
| D | -6 | -36 | -41 | (79) | 2055 | 2033 | 3184 |
| D | 2099 | 1906 | 1241 | 2517 | (61) | -13 | 2100 |
| F | 3190 | 2015 | 1491 | 2581 | 30 | (254) | 4412 |
| G | 812 | 538 | 1082 | 1325 | 837 | 1134 | (43) |

Results of MLC tests on siblings tested in one-way culture in all combinations. Results represent the net number of counts per minute incorporated in allogeneic mixed cultures. Control cultured (treated and untreated cells from the same donor) are listed in parentheses. Radioactivity of the controls was subtracted from that incorporated in allogeneic mixtures to obtain the next results listed.

TABLE 6 — *Results of Cell Typing on Siblings and Parents*

| *E-Family:* *Antisera* | *Father* | *Mother* | *A* | *B* | *C* | *D* | *E* | *F* | *G* |
|---|---|---|---|---|---|---|---|---|---|
| Pay | +++ | 0 | 0 | 0 | 0 | 0 | +++ | +++ | +++ |
| Ra | +++ | 0 | ++ | +++ | ++ | ++ | 0 | 0 | 0 |
| Bc | ++++ | 0 | ++++ | ++++ | +++ | ++++ | 0 | 0 | 0 |
| Lbu | 0 | +++ | ++ | +++ | +++ | ++ | +++ | +++ | 0 |
| Roy | 0 | +++ | ++ | +++ | +++ | ++ | ++ | +++ | 0 |
| Kw | 0 | ++++ | +++ | ++++ | ++++ | +++ | +++ | +++ | 0 |
| Sd | 0 | +++ | ++ | +++ | +++ | +++ | ++ | ++ | 0 |
| Kh | 0 | ++ | ++ | ++ | ++ | + | + | ++ | 0 |
| Fs | 0 | +++ | ++ | +++ | +++ | +++ | ++ | ++ | 0 |
| Em | 0 | +++ | +++ | ++++ | ++++ | ++++ | +++ | ++++ | 0 |

Pluses refer to the percentage of cells killed in the assay. Any rank from + to 4+ is considered positive.

shows MLC results on 7 siblings from one of the families studied. The numbers in the table represent the number of counts per minute of tritiated thymidine incorporated by untreated cells during a 5-hour period of labeling on the 7th day of culture (for example, $AB_m$) less the number of counts per minute in control cultures (for example, $AA_m$), whose values are given in parentheses. Non-stimulation can, as stated, readily be distinguished from positive stimulation—the maximum ratio between an allogeneic mixture and the control in non-stimulating combinations being less than 1, whereas the comparable ratio for the positively stimulating cultures varies between 6 and 57. Four siblings (A, B, C and D) form one group, 2 others (E and F) form a second, and the remaining sibling (G) is unique.

Although typing sera are most suitable for genetic study when they are monospecific, sera containing more than one antibody can be used if the antibodies react against antigens of the same genetic system. While such antisera do not give the maximum resolution possible, observed differences are still valid. Previous tests with the antisera used in this study demonstrated that no 2 of the sera contain exactly the same antibodies, and that where more than one antibody is present the components appear to react against antigens controlled by the same system. The reactions of some of the sera against the same siblings and their parents are summarized in Table 6. Only those sera capable of detecting the polymorphism for any given antigen in this particular family are included, i.e., the table omits data for sera which reacted with all the cells or failed to react with any.

The results of MLC tests correlate well with the results of leukocyte typing. In the family presented, there are two MLC-identical groups—each tested with 10 antisera. For each group we can compare patterns of reaction to each antiserum. For instance, the reaction patterns of A, B, C and D with antiserum Pay are considered a single comparison, those with antiserum Ra are a second comparison. Similarly, the reactions of E and F with these same 2 antisera counted as 2 further comparisons. Each of these 4 comparisons fits the prediction that MLC-identical siblings are identical in their reactions to the antisera. Of the 20 comparisons, all fit this prediction. In seven families studied, 11 MLC-identical sibling groups were detected. Individuals in these groups were compared for identity in reaction with from 10 to 21 antisera, depending on the family—a total of 185 comparisons. In 177 cases, all the members of a MLC-identical group typed identically. In 8 cases MLC-identical siblings did not type identically. Two antisera were associated with 5 of the 8 cases of noncorrelation.

The random probability of 2 siblings typing identically depends on the genotypes of the parents. We included only those parental genotypes which resulted in both positive and negative offspring. Such offspring occur either

when both parents are heterozygous for the alleles controlling a given antigen or when one parent is heterozygous and the other does not possess the allele for that antigen. The random probability that 2 siblings will react identically, whether positive or negative, with respect to a given antiserum will be either ½ or ⅝*. Thus, if the locus controlling MLC reactivity segregates independently of a locus controlling the antigens, the cells of a maximum of 5 out of 8 of MLC-identical siblings will react identically with the antisera. The probability of obtaining the correlation we have found (177/-185) by chance alone is vanishingly small.

In the last section, I presented some of the data which suggest that most of the leukocyte antigens as well as responsiveness in the MLC test are controlled by a single genetic system. It would seem reasonable to suggest that this is the same system in both histocompatibility tests. In MLC tests cells may sometimes respond when there are no detectable antigenic disparities. This suggests that some of the antigens determined by HLA are not detected by presently available antisera. Antibodies for these specificities might be prepared by reciprocal immunization of siblings whose leukocytes type alike but whose cells mutually stimulate.

*Preliminary Attempts to Quantitate MLC Stimulation*

Thus far, we have considered stimulation in MLC to be either present or absent, without attempting to measure different degrees of positive stimulation. To demonstrate that MLC tests can give meaningfully quantitative results, we need to show not only that the response of cells of a prospective recipient to the stimulus provided by cells of a series of potential donors is quantitatively reproducible, but also that these different degrees of stimulation are related to antigenic disparity.

A maximum of 4 MLC identical groups should be identifiable in a sibship. Thus, if both parents are heterozygous for different alleles and the father has

* If one parent is positive, the other negative and some children negative, then we know that the parent having the allele determining the antigen in question must be heterozygous for that allele. The probability that 2 siblings will both receive that allele from the one parent is $\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$. Thus the probability that they will both be positive is ¼. Similarly, the probability that they will both be negative is ¼. The combined probability that they will both react identically with the antiserum in question is, therefore, $\frac{1}{4} + \frac{1}{4} = \frac{1}{2}$. If both parents are heterozygous for an allele determining a given antigen, the probability that any sibling inherits the allele determining the antigen will be ¾ or the probability that 2 siblings both inherit this allele will be $\frac{3}{4} \times \frac{3}{4} = 9/16$. The probability that a given sibling inherits only alleles which do not determine this antigen is only ¼. Therefore, the probability that 2 siblings will both not possess the antigen is $\frac{1}{4} \times \frac{1}{4} = 1/16$. The combined probability that 2 siblings in this situation would both react similarly with the antiserum in question is, therefore $9/16 + 1/16 = \frac{5}{8}$.

alleles *a* and *b (ab)* and the mother is also heterozygous with alleles *c* and *d (cd)*, then the 4 groups of siblings would be respectively characterized by allele pairs: *ac, ad, bc* or *bd*. With any group as reference (e.g. *ac*), 2 groups *(ad* and *bc)* share one allele, while the remaining group *(bd)* has no common allelic inheritance, thus differing by 2 alleles. Within such a sibship, a sibling differing by both alleles from the prospective recipient would necessarily show the maximum antigenic disparity within that sibship (assuming no "null alleles"). Depending on which sibling is chosen as responder, all other siblings in the family differ by either "no allele," "1 allele" or "2 alleles."

Five families in which determination of the genotype was possible (unequivocally in three families, and in terms of the more likely of two possible

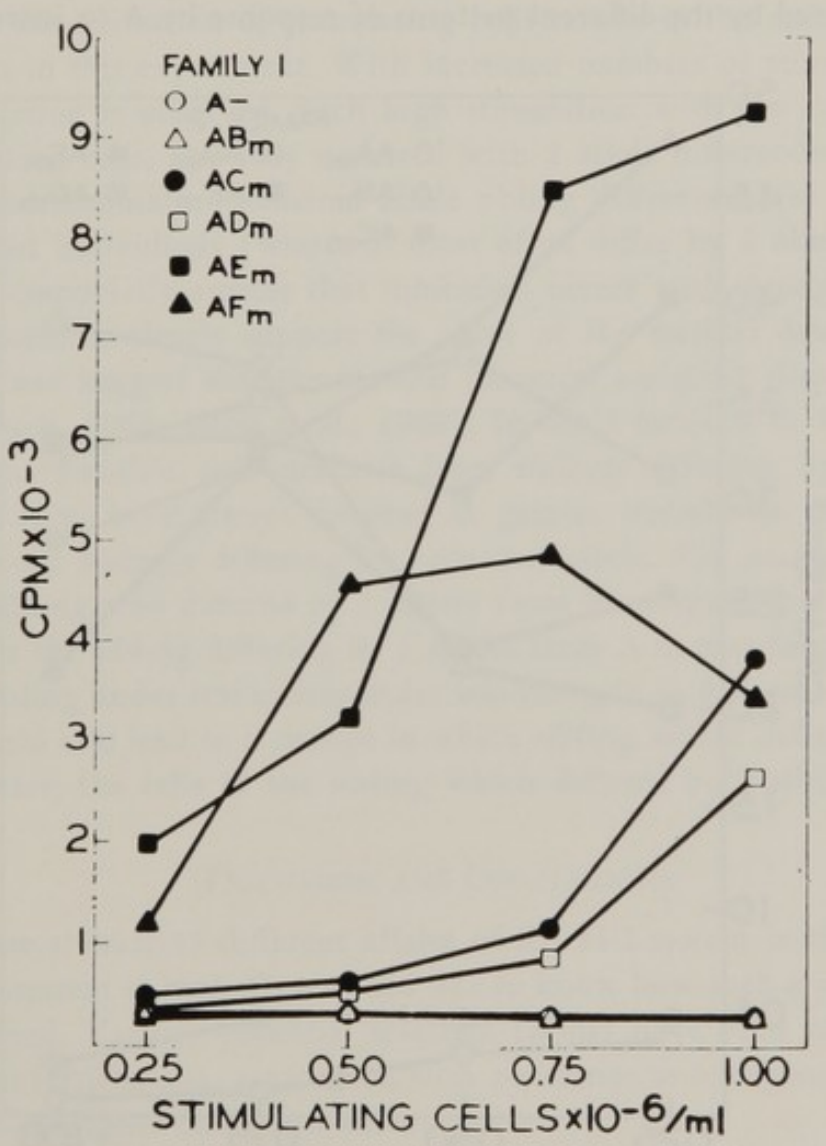



FIG. 9—Protocol as in Figure 5. Cells of the responder are used at a concentration of $3 \times 10^5$ lymphocytes/ml. in all cultures. Stimulating cells are added at the concentrations indicated. A is the responder; B– an MLC identical sibling; C– a parent; D– a sibling differing by "1 allele"; E– a sibling differing by "two alleles"; and F– an unrelated individual.

genotypes is the remaining two families) have been studied (from 2 to 4 times each). In a family, a sibling chosen as responder is tested for his response to cells of all other family members. The previously described technique is amended so that responding cells (A) are tested against several concentrations of stimulating cells ($B_m$).

Results of MLC tests in 2 different families are shown in Figures 9 and 10. In each case the cells of the sibling used as responder (designated A) are used at a constant concentration ($3 \times 10^5$ lymphocytes/ml.) in all cultures. His cells are set up in triplicate with each of 4 different concentrations of stimulating cells from each of the other individuals involved in the experiment. The need to test mixtures at several concentrations of stimulating cells is demonstrated by the different patterns of response by A to increasing con-

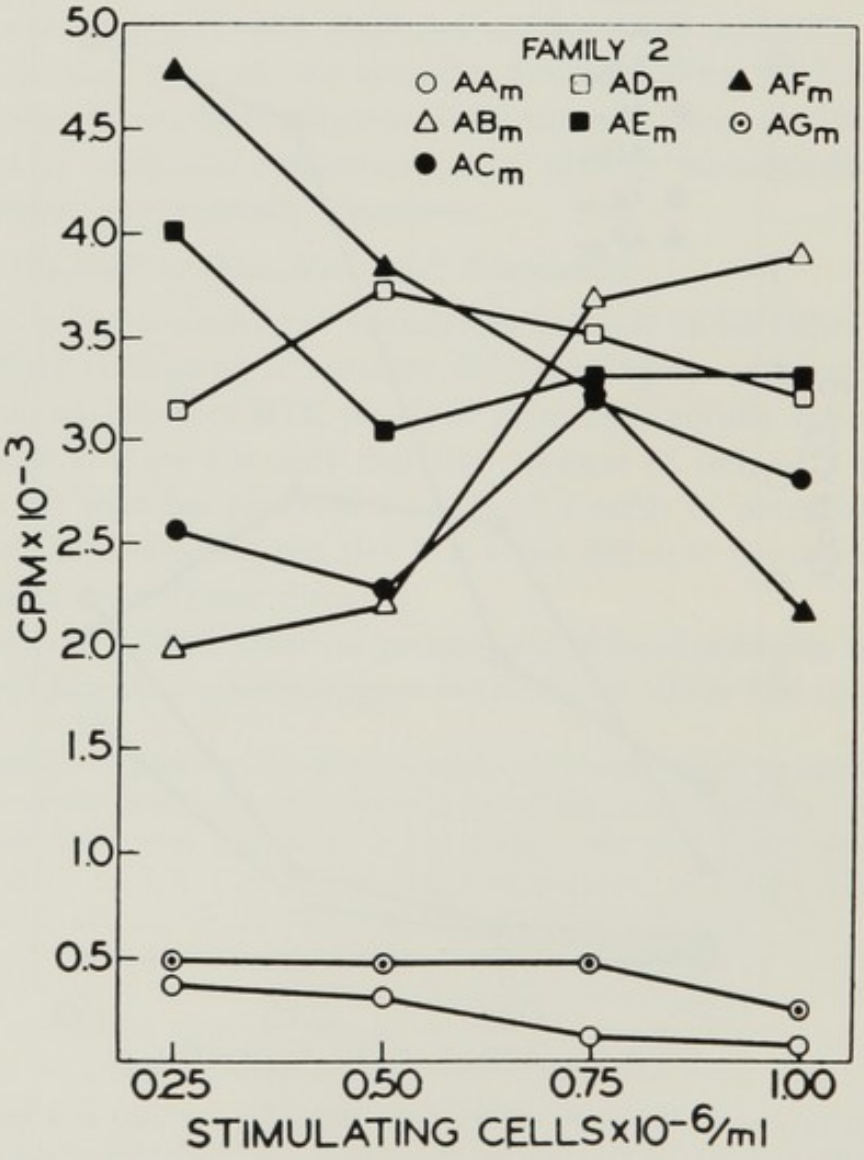



FIG. 10—A different family tested by the protocol given in Figure 9. The responder is again designated A; B, C, D and E each differ by "one allele" from A; F differs by "2 alleles" from A, and G is an MLC identical sibling.

centrations of stimulating cells ($B_m$).

Figure 9 shows that mixtures such as $AC_m$ and $AD_m$, which might be considered non-stimulators with stimulating cell concentrations of 0.25 and 0.5 $\times$ $10^6$ cells/ml., are clearly stimulatory as the stimulating cell concentration is increased. This pattern of response, in which there is little or no response until higher concentrations of stimulating cells are used, has only occurred with cell mixtures from individuals presumed to differ by only one allele, such as $AC_m$ and $AD_m$ in this family. Therefore, before deciding that 2 siblings are non-stimulators, various concentrations of stimulating cells must be tested against the responder in question.

Another response pattern can be seen in Figure 10. In the mixture $AF_m$, the lowest concentration of stimulating cells results in the maximum stimulation seen in this experiment. With increased numbers of stimulating cells, less stimulation is observed. Such high stimulation with low concentrations of stimulating cells, has only occurred with 2 allele differences between responder and stimulator—either 2 allele sibling differences (as in this case) or unrelated individuals (who will most often differ by 2 alleles). On this basis, we temporarily assume that inhibition occurs with strong stimulation.

These patterns clearly support the value of the method described in the appendix, and suggest that the method measures antigenic disparity (Albertini and Bach, 1968; Bach et al., 1968). In the 5 families in which "genotyping" was possible, cell mixtures from siblings differing by 2 alleles at the HL-A locus have always resulted in greater stimulation than cell mixtures between siblings differing by only one allele. For example in Figure 9, E is a sibling who differed by 2 alleles from the responder A, and in Figure 10, F is the sibling differing by 2 alleles from A in that family. In 2 families the sibling under test as responder was changed in different experiments, which would also lead to a change in which sibling would differ by 2 alleles. In every case, the cells of the sibling which differed by 2 alleles stimulated the most.

## DISCUSSION AND CONCLUSIONS

There are at least 15 different alleles of the H-2 system in the mouse and the HL-A system in man. One would like to know how such a polymorphism is maintained. Various potential selective factors have been suggested such as selective fertilization, association with resistance to oncogenic viruses, and control of emergent malignant cells.

Selective fertilization, in which successful fertilization could take place only in combinations differing with respect to at least one allele at the major locus would, of course, provide a very powerful selective force for the maintenance of a very extensive polymorphism, as for example in the case

of self-sterility alleles in various plants.

Recent work supports the concept that H-2 differences may control resistance to viral infection. Nandi (1967) has shown that there is an increased resistance to Bittner virus infection in certain H-2 allelic combinations in the mouse. For instance, he found that virus from cells of the H-$2^a$ donors was incapable of infecting mouse strains with H-$2^d$ or H-$2^k$ genotypes. Previous work had suggested similar possibilities (Lilly, 1966; Tenant and Snell, 1966). One possible explanation for such observations would be that the virus is enveloped in the membrane of its host cell as it emerges from that cell, and that the membrane carries H-2 antigens which would be "incompatible" with the antigens of other cells of different H-2 genotypes.

The Möllers (1966) suggested, on the basis of their own work and that of the Hellstroms (1965), that differences in H-2 phenotype may provide a surveillance mechanism against the proliferation of neoplastic cells. Allogeneic inhibition, as this phenomenon has been termed, is exhibited even when immunological incompatibility is eliminated. Thus, irradiated F-1 leukocytes, or other cells, inhibit the growth of parental strain tumor cells. *In vivo,* the authors hypothesize, if a tumor cell either gains a new antigen (a tumor specific antigen) or loses some antigen, an antigenic incompatibility between the host cells and the tumor cells will exist and this will lead to inhibition of the growth of the tumor cells.

The extent of the H-2 and the HL-A polymorphisms is not in some respects very surprising. As our ability to perform more sensitive analysis of gene products increases (with more refined immunological analysis and better electrophoretic techniques), polymorphisms are detected with increasing frequency (Lewontin and Hubby, 1966). Further, there are loci known with several hundred alleles maintained by selective pressures—such as the self-sterility locus in maize.

At present we can only speculate about the nature of the H-2 or HL-A genes. Shreffler (1967) has reviewed this subject. Some questions which can be asked are: 1) Are the antigens which we detect direct products of the H-2 alleles, or is the situation similar to the ABO system (Watkins, 1967) where the alleles determine enzymes which attach sugars to another molecule, with the antigenic specificities determined by the resulting molecules? 2) Does the entire H-2 system code for a single polypeptide chain or are there "subsystems"—each of which codes for its own polypeptide chain? I would emphasize that there is no way to resolve these questions critically until chemical analysis of the antigen products is available.

At least 2 immunogenetic systems are of great importance for allograft survival in man. Incompatibility for the ABO system poses a stringent barrier across which it is difficult to obtain graft survival, although the amount

of this incompatibility varies with the particular antigens involved (Ceppellini et al., 1966). The HL-A system segregating independently of the ABO system, has been proposed as the major histocompatibility locus in man. Yet incompatibility for antigens at this system are certainly not more problematic than some at the ABO system.

The term "histocompatibility locus," as used above, excludes the ABO system.As such, I think that the concept of a *single* major locus is a valuable one, and of great practical importance. For clarity, it may be better to call it the major human leukocyte antigen system (HL-A). The separation of the major from the multiple other histocompatibility (H) systems, is based on several facts. Skin graft rejection in several species is very prompt (8 to 12 days) in the vast majority of cases when donor and recipient differ at the major locus. However, in the mouse, when a donor-recipient pair is identical at the H-2 locus, but different at another H locus, more prolonged survival of skin grafts results. While differences at multiple minor H loci appear to be additive (Graff, Silvers, Billingham, Hildermann and Snell, 1966), minor loci differences may not represent as strong a barrier to graft survival even when skin is rejected in 8 to 12 days due to only minor loci incompatibilities. Silvers, Wilson and Palm (1967) have shown that immunosuppression is more effective in rat pairs that differ at only minor H loci and reject skin in 8 to 12 days than in rat pairs that differ at the Ag-B locus and reject skin in similar periods of time. This study used animal groups that were comparable with respect to incompatibility since skin graft rejection times were the same in the 2 groups. It elegantly confirmed earlier suggestions that incompatibility at the major histocompatibility locus represented a more severe barrier to both immunosuppression and to the induction of tolerance (Martinez, Smith, Blaese, and Good, 1963) than did differences at the minor locus.

All these experiments suggest that the major locus is quantitatively more important for graft survival than the minor loci. However, these arguments do not set this locus aside in any qualitative way. Recent work with mixed leukocyte culture tests has provided some data which suggest a qualitative difference between the major and the minor H loci.

We have suggested that MLC tests measure incompatibilities at the major histocompatibility locus in man, and in cases where 2 subjects were identical at HL-A, minor loci differences did not cause stimulation. Our reasons for this suggestion were: 1) Skin graft rejection between individuals incompatible at HL-A ranged between 8 and 14 days, while skin between HL-A compatible siblings survived 15 to 42 days (Amos et al., 1966; Bach and Kiskin, 1967). 2) The work of Silvers, Wilson and Palm, mentioned above, demonstrated that in rats, MLC stimulation occurred only between animals differing at the Ag-B locus—not when 2 animals differed at enough minor loci

to effect skin graft rejection in times similar to those of major locus incompatibilities. 3) Survival of kidney homografts in individuals compatible at HL-A seemed better than in HL-A incompatible siblings (Stickel, Amos, Zmyewski, Glenn, and Robinson, 1967). 4) In every other species sufficiently studied, a single major locus had been described.

This lack of stimulation between humans that are identical at HL-A, yet reject skin grafts in only 15 days; and between rats that are identical at Ag-B and reject skin grafts as discussed above, does seem to support the concept that the major histocompatibility locus is, at least in some ways, qualitatively different. It has not, however, been unambiguously established that such a difference does exist, e.g., that the antigens controlled by the major locus are located on the surfaces of cells and those of the minor loci are located elsewhere.

Based on analogy with other animals and from what is known directly in man, pairing for the HL-A system is of the utmost practical importance for graft survival, and I will concern myself in the rest of this discussion only with this system.

Typing procedures can be used to find a donor who has no antigens foreign to the prospective recipient, or less ideally, who has a minimum number of foreign antigens. Two problems arise in this regard. First, 2 individuals who are considered compatible by typing tests may nonetheless be incompatible for donor antigens not detectable by available antisera. It is very probable that not all antigens of HL-A are yet defined. Second, even when the donor is known to carry some antigens foreign to the recipient, there is at present neither a knowledge concerning the "strength" of such antigens, nor the relationship of strength to the histocompatibility genotype of the recipient.

Why can typing procedures predict skin graft survival between siblings but only rarely between unrelated individuals? Given a single locus, 2 parents can have at most 4 different alleles and there is a maximum of 4 genotypes for the siblings. If we designate the father's alleles *"a"* and *"b"* and the mother's alleles *"c"* and *"d,"* then the siblings can be *"ac," "ad," "bc"* and *"bd."* In the mouse each H-2 allele determines several antigens, some of which may be identical with those determined by other alleles. Assuming that this is the case in man, we need not define all the antigens in a sibship to find those siblings who have inherited the same 2 alleles from their parents. This is because we know that each sibling receives one allele from each parent. If we have a single antiserum that differentiates the 2 alleles of a single parent, we will be able to state unambiguously (barring crossing-over within the HL-A system) which allele that parent contributed to a given offspring, regardless of how many antigens are specified by that allele. Hence, with 2 antisera, one

differentiating the 2 alleles of the father and the other those of the mother, we can define completely the genotypes of the offspring with respect to the locus. For instance, if allele *"a"* codes for antigens 1, 2, 3 and 4, allele *"b"* for antigens 1, 2, 5 and 6, allele *"c"* for antigens 1, 3, 7 and 8, and allele *"d"* for antigens 2, 4, 7 and 9, then antisera for antigens 5 and 9 (as one possible pair) will allow us to detect the differences between siblings inheriting the *ac, ad, bc* and *bd* allelic combinations. Thus any 2 siblings not showing a difference with these 2 antisera will be identical at HL-A.

When we deal with parent-child, or unrelated pairs, this is no longer true. While parent and child of necessity share one allele, the second allele is "unrelated." Even if the antigens determined by these unrelated alleles include some that are the same, those which are different will not be detected in the absence of suitable antisera. The same is true, *a fortiori,* for unrelated pairs, since in such cases both alleles are unrelated. Thus a failure to demonstrate antigenic difference between donor and recipient does not guarantee compatibility.

In MLC tests, since stimulation is associated with incompatibility for antigens of the HL-A system, it seems unlikely that there are many alleles in the population which only determine antigens not detectable in MLC tests—otherwise we would find some unrelated matches which do not stimulate. We, therefore, consider reciprocal non-stimulation between 2 individuals as a manifestation of "effective identity" at HL-A with different degrees of stimulation apparently reflecting "degrees of incompatibility" at the HL-A system.

What then are the questions which confront us? First, we must know whether complete compatibility at HL-A is necessary to permit long-term survival of transplanted organs. With the very extensive polymorphism which exists at HL-A, such pairs will be very infrequent. Various estimates have been made to determine the frequency with which a satisfactorily matched unrelated donor could be found for a given recipient ("effective compatibility"). Walford (1967) has based his estimate on the frequency with which skin grafts between unrelated individuals survive 20 days—about 1 in 70. This may well be realistic; however, one might keep in mind that immunosuppression may be more effective when there is no major system incompatibility, and that the time of survival may not be as important as the antigenic system involved. (It is important to realize that "effective compatibility" as discussed here is not necessarily equivalent to "effective genetic identity.") Second, if complete compatibility is not necessary, what degree of incompatibility can be tolerated? To answer these questions we must obtain antisera to measure all the antigens determined by HL-A. Additional antisera are being sought in multiparous individuals as well as by in-

tentional immunizations. Even when this is done there will still be the problem of answering the second question posed above.

I would like to outline the pairing procedure used by the transplantation program at the University of Wisconsin to illustrate one approach to these questions and to emphasize the importance of performing both matching and typing tests at this time.

Potential donors in the family of the recipient are typed and tested by MLC. When an MLC-identical donor is found that individual is used. When no MLC-identical donor exists, the donor that stimulates the cells of the recipient the least is chosen. The amount of incompatibility manifested in that donor-recipient combination tells little, however, about the degree of incompatibility which this represents with respect to some general standard of incompatibility. Therefore, the related donor is chosen, and his cells are tested as stimulating cells against the cells of the recipient along with 15 sets of unrelated stimulating cells.

If all HL-A alleles in the population determine antigens which represent strong incompatibilities for any recipient, most unrelated individuals will differ by 2 alleles; such individuals will be very poor donors and represent close to 100 per cent incompatibility (the worst incompatibility that could be found in the population). A frequency distribution curve of degrees of incompatibility in such a population would be skewed toward great incompatibility (arbitrarily the right). If there are "weak alleles" determining only antigens which are weak incompatibility barriers, then the curve may be symmetrical or even skewed to the left. Whatever the shape of the curve (which can be empirically determined), if 15 sets of unrelated cells are tested with cells of the potential donor, the strongest stimulator of the 15 will give an estimate of the "effective" maximum incompatibility which will be found in the population. The unrelated individual that stimulates the most can be considered as the "standard of incompatibility," and the related donor-recipient match can be expressed as a percentage of the standard.

If complete compatibility at HL-A is not necessary for successful organ transplantation, results using the above approach should tell us the degree of incompatibility which can be tolerated, and how frequently we will find an unrelated individual who falls into the acceptable incompatibility range.

With very rare exceptions, it has so far been impossible to pair unrelated individuals by typing tests, probably for the reasons stated above. It is therefore difficult at the present time to speak of having "good" and "poor" pairing between unrelated individuals, since these words have relatively little meaning. Clearly, progress is being made toward this goal and pairing should be continued using typing and matching tests.

While not all the HL-A antigens are yet defined, antisera measuring some of the remaining ones will become available, perhaps in part by immunization after MLC testing as discussed above. Hopefully, we will obtain the needed information regarding strengths of various antigens by analyzing kidney graft survival, skin graft survival and stimulation in MLC tests (knowing the antigenic constitution of both participants in culture). With the present increase in our knowledge, one might be optimistic that with appropriate pairing, organ transplantation will be widely available as a feasible, therapeutic procedure within the next few years.

## APPENDIX

Following is a description of a matching method previously published (Bach and Voynow, 1966) with details of modifications of that method.

Heparinized blood is allowed to sediment for approximately 2 hours at room temperature. The plasma is removed and centrifuged for 10 minutes at 150 g to obtain leukocytes; the supernatant is recentrifuged at 1000 g to obtain cell-free plasma. Cells to be used as "responding cells" are suspended in Eagle's minimal essential medium modified for suspension culture (MEM-S, Grand Island Biological)and supplemented to contain glutamine, 2 mM; penicillin, 100 units per ml., streptomycin, 100 μg./ml.; and 20 per cent pooled cell free plasma (20 per cent MEM).

Cells to be used as stimulating cells are suspended in 5-10 ml. of 20 per cent MEM, incubated for 20 minutes at 37° C with Mitomycin-C at 25 μg./ml., twice washed in 20 per cent MEM, and resuspended in 20 per cent MEM.

Responding cells are present in all cultures at final concentrations of 0.3-0.5 $\times$ $10^6$ mononuclear cells/ml. For experiments to determine different degrees of stimulation, 4 concentrations of stimulating cells from each potential donor are used—final concentrations are 0.25, 0.50, 0.75 and 1.0 $\times$ $10^6$ cells/ml. In other experiments a single concentration of stimulating cells is used (0.6 to 0.8 $\times$ $10^6$ cells/ml.). Cultures, done in duplicate or triplicate, are distributed in 2.5 ml. volumes in 16 $\times$ 100 mm. glass tubes with metal closures immediately after mixing, and incubated at 37° C in a humidified 4 per cent $CO_2$ atmosphere for 7 days.

After approximately 160 hours of incubation, 2 μc. of tritrated thymidine (specific activity 1.9 c/m mole; Schwart BioResearch) are added to each culture. Replicate cultures are harvested at 5 to 5.5 hours later by addition of 1000-fold excess of non-radioactive thymidine in 0.1 ml. normal saline, emersion of the culture tubes in ice, centrifugation for 10 minutes at 1000 g, and freezing of the cell sediment at –20° C. To determine incorporation of thy-

midine, the cultures are thawed, 5 ml. of cold 5 per cent TCA is added to each, and the tube contents are mixed on a Vortex mixer. Ten minutes later the precipitate is recovered by centrifugation, dissolved in 1 ml. of 0.1 NaOH, and precipitated again with 4.5 ml. of 6.7 per cent TCA. The acid precipitation procedure is repeated twice again. The final precipitate is dissolved in 0.1 ml. of tetraethylammonium hydroxide, mixed with scintillation fluid, and counted. The results are expressed as counts per minute in each sample. The values plotted are averages of the replicate cultures for any given concentration of stimulating cells.

## ACKNOWLEDGMENTS

The author extends his gratitude to his colleagues and students for many stimulating discussions. Special thanks are due Dr. D. B. Amos, who has performed leukocyte typing as mentioned in this paper.

## REFERENCES

Aisenberg, A. C. 1965. Studies of lymphocyte transfer reactions in patients with Hodgkins' disease. J. Clin. Invest. 44: 555.

Albertini, R. J. and F. H. Bach. 1968. Quantitative assay of antigenic disparity at H1-A—the major histocompatibility locus in man. J. Exp. Med. 128: 639-651.

Allen, S. L. 1955. Linkage relations of the genes histocompatibility-2 and fused tail, brachyury and kinky tail in the mouse, as determined by tumor transplantation. Genetics 40: 627-650.

Amos, D. B. 1953. The agglutination of mouse leukocytes by iso-immune sera. Brit. J. Path. 34: 464.

—. 1963. Transplantation antigens in mouse, rat and man. *In* Steinberg, A. G. and Bearn, A. G. (Eds:) Progress in Medical Genetics, Vol. III. p. 106-143.

—, P. A. Gorer and Z. B. Mikulska. 1955. An analysis of an antigenic system in the mouse (the H-2 system). Proc. Royal Soc. [Biol.] 144: 369-380.

—, B. G. Hattler, J. M. MacQueen, I. Cohen and H. F. Seigler. 1967. An interpretation and application of cytotoxicity typing *In* Advance in Transplantations. Munksgaard, Copenhagen, 203-212.

—, P. Hutchin, B. G. Hattler, R. McClowskey and C. M. Zmijewski. 1966. Skin donor selection by leukocyte typing. Lancet 1: 300-302.

—, P. J. Nicks, N. Peacocke and H. O. Sieker. 1965. An evaluation of the normal lymphocyte transfer test in man. J. Clin Invest. 44: 219.

Bach, F. H. 1966a. Histocompatibility testing in man. *In* Metcalf, J. (Ed.): Homotransplantation—Kidney and Other Tissues. National Kidney Foundation Press. 95-105.

—. 1966b. Mixed leukocyte cultures—their assay and genetics. *In* In Vitro. II. Baltimore, Waverly Press, 32-39.

—. 1967. Evaluation of compatibility at the major histocompatibility locus in man. *In* Advance in Transplantation. Munksgaard, Copenhagen, 55-62.

—, R. J. Albertini, T. H. Klostermann and E. Day. 1968. Evaluation of compatibility at the major histocompatibility locus in man. *In* Histocompatibility Testing 1967. Munksgaard, Copenhagen, 55-65.

— and D. B. Amos. 1967. Hu-1: Major histocompatibility locus in man. Science 156: 1506-1508.

—, and H. Hirschhorn. 1964. Lymphocyte interaction: A potential histocompatibility test in vitro. Science 143: 813-814.

— and W. A. Kisken. 1967. Predictive value of results of mixed leukocyte cultures for skin allograft survival in man. Transplantation 5: 1046-1052.

— and T. H. Klostermann. Unpublished observations.

— and N. K. Voynow. 1966. One-way stimulation in mixed leukocyte cultures. Science 153: 545-547.

Bain, B. and L. Lowenstein. 1964. Genetic studies on mixed leukocyte reaction. Science 145: 1315-1316.

—, M. R. Vas and L. Lowenstein. 1964. The development of large immature mononuclear cells in mixed leukocyte cultures. Blood 23: 108-116.

Brent, L. and P. B. Medawar. 1963. Tissue transplantation: A new approach to the "typing" problem. Brit. Med. J. 2: 269.

Bridges, J. M., S. D. Nelson and M. G. McGeown. 1964. Evaluation of lymphocyte transfer test in normal and uremic subjects. Lancet 2: 581.

Ceppellini, R. 1967. A preliminary report on the Third International Workshop on Histocompatibility Testing. *In* Advance in Transplantation. Munksgaard, Copenhagen, 195-202.

—, E. S. Curtoni, P. L. Mattiuz, G. Leigheb, M. Visetti and A. Columbi. 1966. Survival of test skin grafts in man. Effect of genetic relationship and of blood groups incompatibility. Ann. NY. Acad. Sci. 129: 421-445.

—, E. S. Curtoni, G. Leigheb, P. L. Mattiuz, V. C. Miggiano and M. Visetti. 1965. An experimental approach to genetic analysis of histocompatibility in man. *In* Histocompatibility Testing. Munksgaard, Copenhagen, 13-22.

—, P. Franceschini, V. C. Maggiano and G. Tridente. 1965. Directional activation of mixed lymphocyte cultures. A preliminary report in Histocompatibility Testing, 1965. Baltimore, Williams and Wilkins, 1965, 225-228.

Crow, J. F. and M. Kimura. 1964. The number of alleles that can be maintained in a finite population. Genetics 49: 725-738.

Dausset, J. 1954. Leukocyte agglutinins. IV. Leukocyte agglutinins and blood transfusion. Vox. Sang. 4: 190.

—. 1958. Iso-leuko-angicorps. Acta Haemat. (Basel) 20: 156.

—, P. Ivanyi and D. Ivanyi. 1965. Tissue alloantigens in humans. Identification of a complex system (Hu-1). *In* Histocompatibility Testing, 1965. Baltimore, Williams and Wilkins, 1965, 51-62.

—, F. T. Rapaport, P. Ivanyi and J. Colombani. 1965. Tissue alloantigens and transplantation. *In* Histocompaitbility Testing, 1965. Baltimore, Williams and Wilkins, 63-69.

—, F. T. Rapaport and L. Legrand. 1967. Choice of donors by tissue groups of the Hu-1 system. *In* Advance in Transplantation. Munksgaard, Copenhagen, 749-756.

Gleason, R. E. and J. E. Murray. 1967. Report from kidney transplantation registry: Analysis of variables in the function of human kidney transplants. I. Blood group compatibility and splenectomy. Transplantation 5: 343-359.

Gorer, P. A. 1955. The antibody response to skin homografts in mice. Ann. N. Y. Acad. Sci. 59: 365.

— and Z. B. Mikulska. 1959. Some further data on the H-2 system of antigens. Proc. Roy. Soc. [Biol.] 151: 57.

Graff, R. J., W. K. Silvers, R. E. Billingham, W. H. Hildermann and G. D. Snell. 1966. The cumulative effect of histocompatibility antigens. Transplantation 4: 605-617.

Gray, J. G. and P. S. Russell. 1963. Donor selection in human organ transplantation. Lancet 2: 863.

— and P. S. Russell. 1965. The lymphocyte transfer test in man. *In* Histocompatibility Testing. Publication No. 1229: 105-116. National Academy of Sciences—National Research Council, Washington, D. C.

Hattler, B. G. and D. B. Amos. 1965. Reactions obtained with transferred lymphocytes in patients with advanced cancer. J. Nat. Cancer Inst. 35: 927.

Hellstrom, K. E. and I. Hellstrom. 1965. Syngeneic preference and allogeneic inhibition. *In* Palm, J. (Ed.): Isoantigens and Cell Interactions. The Wistar Institute Press 79-.

Hirschfeld, J. 1965. Serological codes: Interpretation of immunogenetic systems. Science 148: 968-971.

Histocompatibility Testing. 1964. Nat. Acad. Sci. Pub. 1329.

— —. 1965. Munksgaard, Copenhagen.

— —. 1967. Munksgaard, Copenhagen.

Ivanyi, D., M. Rychlikova, M. Sasportes, P. Ivanyi and J. Dausset. 1967. Leukocyte antigens and the mixed lymphocyte culture reaction. Vox. Sang. 12: 186.

Johnson, J. G. and P. S. Russell. 1965. The reaction of lymphocytes in culture to components of the medium. Nature 208: 343.

Kasakura, S. and L. Lowenstein. 1965. The effect of irradiation in vitro on mixed leukocyte cultures and leukocyte cultures in phytohemagglutinin. *In* Histocompatibility Testing 1965. Munksgaard, Copenhagen, 211-212.

Lewontin, G. C. and J. L. Hubby. 1966. A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of drosophila pseudoobscura. Genetics 54: 595-609.

Lilly, F. 1966. The histocompatibility—2 locus and susceptibility to tumor induction. Nat. Cancer Inst. Monograph 22: 631-642.

Martinez, C., J. M. Smith, M. Blaese and R. A. Good. 1963. Production of immunological tolerance in mice after repeated injection of disrupted sphere cells. J. Exp. Med. 118: 743.

Mathé, G., A. M. Méry, C. Bréziu, M. Sekiguchi, E. W. Vaubel and J. L. Amiel. 1965. Investigations on a test for histocompatibility for allogeneic grafts. *In* Amos, D. B. and van Rood, J. J. (Eds.): Histocompatibility Testing 1965. Munksgaard, Copenhagen, 79-82.

Medawar, P. B. 1946. Immunity to homologous grafted skin. II. The relationship between the antigens of blood and skin. Brit. J. Exp. Path. 27: 15.

Miescher, P. and M. Fanconnet. 1954. Mise en évidence de différence groupes leukocytaires chez l'homme. Schewiz. Med. Wschr. 84: 597.

Möller, G. and E. Möller. 1966. Interaction between allogeneic cells in tissue transplantation. Ann. NY. Acad. Sci. 129: 735-749.

Nandi, S. 1967. The histocompatibility-2 locus and susceptibility to Bittner virus borne by red blood cells in mice. Proc. Nat. Acad. Sci. USA. 58: 485-492.

Nelson S. D., J. M. Bridges and M. G. McGeown. 1965. Comparison of lymphocyte-transfer test and white-blood-cell grouping for organ donor selections. Lancet I: 1359-1362.

Newth, D. R. 1961. Chance compatibility in homografting. Transplantation Bulletin 27: 452-455.

Palm, J. 1964. Serological evidence of histocompatibility antigens in the rat. Transplantation 2: 603.

Payne, R., H. A. Perkins and J. S. Najarian. 1967. Compatibility for seven leukocyte antigens in renal homografts. *In* Histocompatibility Testing 1967. Munksgaard, Copenhagen, 237-245.

— and M. R. Rolfs. 1958. Fetomaternal leukocyte incompatibility. J. Clin. Invest. 37: 1956.

—, M. Tripp, J. Weigle, W. Bodmer and J. Bodmer. 1964. A new leukocyte isoantigen system in man. Cold Spring Harbor Symposium 19: 285-295.

Rapaport, F. T., J. Dausset, J. Hamburger, D. M. Hume, K. Kano, G. M. Williams and F. Milgrom. 1967. Serological factors in human transplantations. Ann. Surg. 116: 596-608.

—, L. Thomas, I. A. M. Converse and H. S. Lawrence. 1960. The specificity of skin homograft rejection in man. Ann. NY. Acad. Sci. 87: 217.

Russell, P. S., S. D. Nelson and G. J. Johnson. 1966. Matching tests for histocompatibility in man. Ann. NY. Acad. Sci. 129: 368-385.

Shierman, L. W. and A. W. Nordskog. 1964. Immunogenetic studies with fowl: Relationship of blood groups to transplantation immunity and tolerance. Ann. NY. Acad. Sci. 120: 348-355.

Shreffler, D. C. 1965. The Ss system of the mouse—A quantitative serum protein difference genetically controlled by the H-2 region. *In* Palm, J. (Ed.): Isoantigens and Cell Interactions. Philadelphia, Wistar Inst. Press, 11.

—. 1967. Genetic control of cellular antigens. *In* Crow, J. F. and Neel, J. V. (Eds.): Proceedings of the Third International Congress of Human Genetics. Baltimore, Johns Hopkins Press, 217-231.

—, D. B. Amos and R. Mark. 1966. Serological analysis of a recombination of the H-2 region of the mouse. Transplantation 4: 300.

—, and R. D. Owen. 1963. A serologically detected variant in mouse serum: Inheritance and association with the histocompatibility-2 locus. Genetics 48: 9.

Silvers, W. K., D. B. Wilson and J. Palm. 1967. Typing and immunosuppression in rats. Transplantation 5: 1053-1056.

Simonsen, M. 1965. Strong transplantation antigens in man. Lancet I: 415-418.

—. 1966. Immunogenetical speculations on human kidney transplants. Transplantation 4: 354-364.

Snell, G. D. 1958. Histocompatibility genes of the mouse. II. Production and analysis of isogenic resistant lines. J. Nat. Cancer Inst. 21: 843-877.

—, G. Hoeker, D. B. Amos and J. H. Stimpfling. 1964. A revised nomenclature for the histocompatibility-2 locus of the mouse. Transplantation 2: 777-783.

Stickel, D. L., D. M. Amos, C. M. Zmijewski, J. F. Glenn and R. R. Robinson. 1967. Human renal transplantation with donor selection by leukocyte typing. Transplantation 5: 1024-1029.

Stimpfling, J. H. and R. Richardson. 1965. Recombination within the hisocompatibility-2 locus of the mouse. Genetics 51: 831.

Stormont, C. 1955. Linked genes, pseudoalleles and blood groups. The American naturalist 89: 105-116.

Strong, L. C. 1942. The origin of some inbred mice. Cancer Res. 2: 531-539.

Tenant, R. R. and G. D. Snell. 1966. Some experimental evidence for the influence of genetic factors on viral leukemogenesis. Nat. Cancer Inst. Monograph 22: 61-72.

Terasaki, P. I., D. L. Vredevee and M. R. Mickey. 1967. Serotyping for homotransplantation. X. Survival of 196 grafted kidneys subsequent to typing. Transplantation 5: 1057-1070.

Van Rood, J. J. and A. van Leeuwen. 1963. Leukocyte grouping. A method and its application. J. Clin. Invest. 42: 1382-1390.

—, —, J. W. Bruning and K. A. Porter. The importance of leukocyte antigens in renal transplantation. *In* Advance in Transplantation. Munsgaard, Copenhagen, 213-219.

—, — and J. G. Eernisse. 1959. Leukocyte antibodies in sera of pregnant women. Vox Sang. 4: 427.

—, —, A. Schippers, R. Ceppellini, P. L. Mattinz and S. Curtoni. 1966. Leukocyte groups and their relation to homotransplantation. Ann. NY. Acad. Sci. 129: 467-472.

—, —, A. Schippers, W. H. Vooys, E. Fredericks, H. Balner and J. G. Eernisse. 1965. Leukocyte groups. The normal lymphocyte transfer test and homograft sensitivity. *In* Histocompatibility Testing 1965. Munksgaard, Copenhagen, 37-50.

Walford, R. L. 1967. Prospects for donor selection for organ transplantation in man. *In* Advance in Transplantation. Munsgaard, Copenhagen, 573-580.

—, D. C. Martin, G. M. Troup and W. E. Goodwin. 1965. Skin graft survival times in human donor-recipient pairs selected on the basis of lymphocyte typing. *In* Histocompatibility Testing 1965. Munksgaard, Copenhagen, 89-93.

—, E. Shanbrom, G. M. Troup, E. Zeller and B. Ackerman. 1967. Lymphocyte grouping with defined antisera. *In* Histocompatibility Testing 1967. Munsgaard, Copenhagen, 221-230.

Watkins, W. M. 1967. The possible enzymic basis of the biosynthesis of blood group substances. *In* Crow, J. F. and Neel, J. V. (Eds.): Proceedings of the Third International Congress of Human Genetics. Baltimore, Johns Hopkins Press, 171-187.

Wilson, R. E., L. Henry and J. P. Merrill. 1963. A model system for determining histocompatibility in man. J. Clin. Invest. 52: 1497.

CHAPTER 7

# The Genetics of Intestinal Carbohydrate Intolerance


## E. Eggermont

Laboratoire de Chimie Physiologique
Université de Louvain
Louvain, Belgium


A CONGENITAL intolerance to an alimentary sugar may result from an inborn defect at one of the 3 following levels: 1) the breakdown of complex carbohydrates in the digestive tract; 2) the absorption of monosaccharides by the small intestinal mucosa; 3) the intermediary metabolism of galactose and fructose. In this paper, only the first two levels will be considered. Our main interest will be focused on the biochemical nature of the lesion, and on data which give insight on the mode of inheritance of each of these diseases.

## 1. THE DIGESTION OF ALIMENTARY CARBOHYDRATES AND ITS DISORDERS

In adults (Jokl, 1964), as well as in infants and children (Beal, 1953), carbohydrates make up about half of the daily caloric intake. Recent studies show that a minimum of 13 per cent of the total calories must be supplied as carbohydrates to ensure survival (Calloway, 1960). Bloom and Azar fed normal human volunteers a diet that had appropriate amounts of calories derived only from proteins and fat, and observed a series of symptoms, reversible by the addition of a daily supplement of 75 Gm. of carbohydrates: loss of weight, fatigue, postural hypotension, excessive urinary loss of sodium, ketonemia, and an increase of blood nonesterified fatty acids without significant change of blood glucose level (Bloom and Azar, 1963; Azar and Bloom, 1963).

Full comprehension of carbohydrate intolerance makes it necessary to take into account the different types of these compounds in the diet. A tabulation of the content of specific sugars in the individual articles of diet can be found in the study of Hardinge, Swarner and Crooks (1965). Furthermore, the figures available for the U.S.A. (Wohl and Goodhart, 1955), Great Britain (Greaves and Hollingsworth, 1964) and the Netherlands (Mulder, 1964) indicate that lactose supplies 7 to 10 per cent, saccharose 25 to 30 per cent and starch plus dextrins about 50 per cent of the dietary carbohydrates. In the same population the figures may vary widely not only among infants and children (Jeans and Marriott, 1947) but also among adults (Jokl, 1964). The effect of feeding different types of carbohydrates under normal conditions is still an unsettled question. In certain subjects, an increase in serum lipids when sucrose is exchanged for starch has been reported (Kaufmann, Poznanski, Blondheim and Stein, 1966 a, b).

## A. The Normal Breakdown of Complex Carbohydrates

The above data suggest that 80 to 90 per cent of dietary carbohydrates must be hydrolyzed within the gastrointestinal tract before the constituent monosaccharides, glucose, galactose and fructose, can be absorbed. In 1961, Dahlquist and Borgström clearly showed that although starch is hydrolyzed significantly within the intestinal tract, the disaccharides sucrose, maltose and lactose are not. Later, Dahlqvist (1962) demonstrated that the enzymes necessary for the digestion of the oligosaccharides are present in the intestinal mucosa. It seems logical, therefore, to study the intraluminal hydrolysis of starch and the role of the intestinal mucosa in the digestion of oligosaccharides.

### *1. The α-amylolysis of Starch*

Starch consists of 2 glucose polymers, amylose and amylopectin, packed together in plants as well defined structures: starch grains. The grains of different plant species vary in shape and size from 1 to 150 μ in diameter (Bonner, 1950). Badenhuizen and Dutton (1956) have shown that growth of the grains takes place by apposition of shells, and a microgranular structure was depicted by Nikuni (1957) using electron microscopy and x-ray diffraction. These studies suggest the presence of spheric granules of 200-300 Å in diameter and consisting of amylopectin. In contrast, amylose is in an amorphous state and fills the spaces around the microgranules. These naturally occurring structures of starch are considerably destroyed during the processing of food.

Amylose and amylopectin are polymers of glucose; their relative proportions in starch are species-dependent. Amylose, which is practically absent from waxy maize, makes up about 22 to 26 per cent in most starches, and up to 70 per cent in some corn mutants. The molecule is built up of several

hundred glucosyl units linked together by α-1, 4-linkages, with a very small number (perhaps 0.1 per cent) of α-1, 6-linkages (Kjölberg and Manners, 1962). Amylopectin is a branched polymer of several thousand glucosyl units, of which about 4 per cent are attached by an α-1, 6-glucosidic linkage (for a review, see Greenwood, 1956).

Both glucans of starch are fragmented within the gastrointestinal tract by the α-amylase, which acts as an endopolyglucosidase. Walker and Whelan (1960) found that the endproducts of the α-amylolysis of amylose are only maltose and maltotriose in a molar ratio of 2.39 : 1. Maltotetraose is the smallest oligosaccharide on which amylase acts as rapidly as on amylose itself. Maltotriose can also be broken down to maltose and glucose, but this reaction proceeds about 500 times more slowly than the hydrolysis of the higher linear saccharides.

When amylopectin is degraded by α-amylase, a series of branched oligosaccharides originating from the bifurcations present in the parent polysaccharide are liberated, in addition to the oligosaccharides maltose and maltotriose (Roberts and Whelan, 1960). The α-1, 6-glucosidic bonds are not hydrolyzed, and exert a steric hindrance on the cleavage of the adjacent α-1, 4-glucosidic bonds. Due to the random action of α-amylase, either one or 2

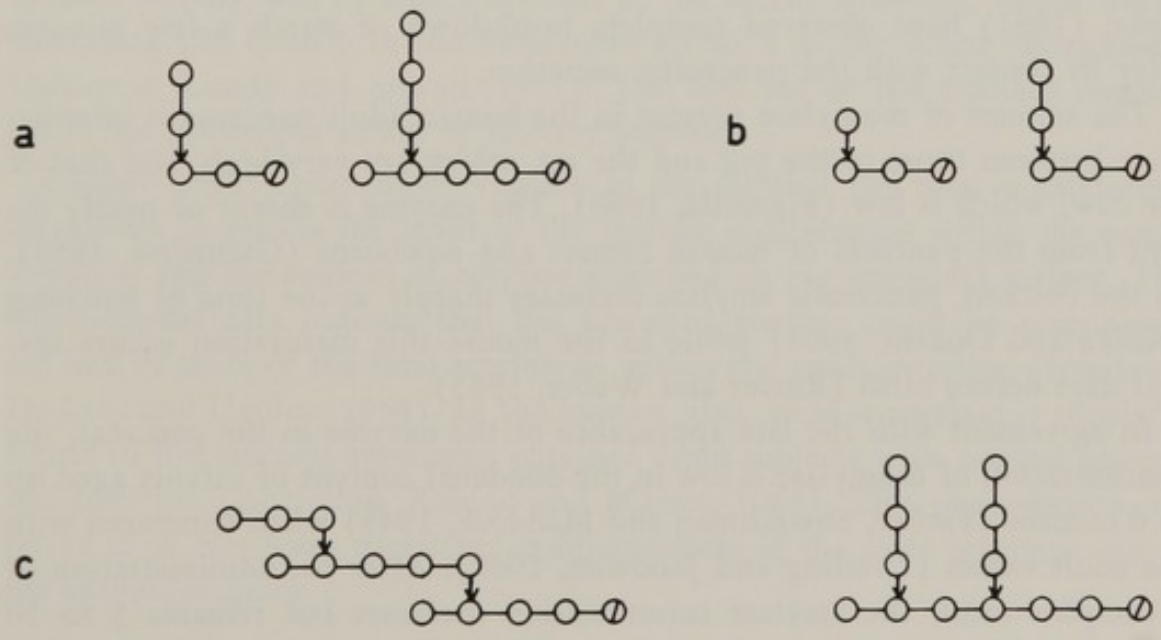



FIG. 1—The branched oligosaccharides formed by α-amylolysis of amylopectin and glycogen. *a*: singly-branched oligosaccharides formed during the first step of α-amylolysis; all intermediary structures between the 2 sugars shown are also produced. *b*: products of the exhaustive degradation by a larger amount of α-amylase. c: doubly-branched oligosaccharides.

Open circle: a glucosyl unit; barred circle: the reducing end; line connecting 2 circles: a 1,4-link; vertical arrow: a 1,6-link. (After Roberts and Whelan, 1960; Walker and Whelan, 1960).

glucosidic units will be undegraded adjacent to the branching points after extensive α-amylolysis (see Fig. 1). When the branches are separated by less than 3 glucosyl units, multiple-branched oligosaccharides arise. For these reasons, neither isomaltose nor panose are normal endproducts.

The starch granules present in rice and maize, but not those of potatoes, are hydrolysed by α-amylase 150 times more slowly than soluble starch. Bile increases the activity on raw starch by 40 per cent (Walker and Hope, 1963). The digestibility of the starch granule is not influenced by its amylose content. However, genetic selection of corn for high yield of amylose has apparently resulted in production of starch that is more resistant to amylolysis (see Nutrition Reviews, 1963).

In man, α-amylase is produced by the salivary glands and by the pancreas; the enzymes from both sources have long been considered identical. Some differences in their electrophoretic behavior have recently been described by Nørby (1965). Both enzymes appear to play a role in the digestion of starch and are responsible for its very complete degradation, at least in the normal human adult. The *in vivo* action of salivary amylase has been studied by Beazell (1941) who has found 40 per cent degradation in the gastric content 30 minutes after the ingestion of a test meal containing 7 per cent starch. In an analogous study of the duodenal content, Dahlqvist and Borgström (1961) have observed complete breakdown of starch a few minutes after its contact with the pancreatic secretion.

The amount of α-amylase present in the human adult pancreas is intermediate between those of the pig and the rat, which are very high, and that of the cow, which is low (Figarella, 1966). The enzyme is absent or nearly absent from the pancreas of human fetuses and newborns (Gschwind, 1950). In the chicken, pancreatic amylase increases sharply at the time of hatching (Kulka and Duksin, 1964) while in the mouse this maturation occurs several days before birth (Rutter and Weber, 1965).

In agreement with the late appearance of the enzyme in the pancreas, the concentration of α-amylase is low in the duodenal content of infants aged up to 6 months (Farber, Shwachman and Maddock, 1943) when compared with the adult values (Dreiling and Janowitz, 1962). After the administration of a complex meal, the amylase concentration increases but remains 5 to 50 times lower in infants than in adults; negligible amounts are found in the premature infant (Borgström, Lindquist and Lundh, 1960 and 1961).

The adequate *in vivo* breakdown of starch necessitates a synthesis of amylase and a synchronization between the gastric emptying and the pancreatic secretion. This regulation is established by neural and hormonal factors originating from the stomach and the upper small intestine (Grossman, 1962). The stimulatory effect of pancreozymin (Harper, Blair and Scratcherd, 1962) and

gastrin (Preshaw, Cooke and Grossman, 1965) on the pancreatic enzyme output is now well documented in the human, while secretin was found to induce the secretion of a bicarbonate-rich fluid from the centroacinar cells (Janowitz and Dreiling, 1962). The natural trigger substances of pancreatic secretion were investigated by Wang and Grossman (1951) in dogs with transplanted pancreas. Hydrochloric acid in contact with the duodenal mucosa greatly stimulates the release of secretin but only weakly that of pancreozymin. The contact of the products of protein digestion with the mucosa of the duodenum markedly increases output of both pancreozymin and secretin. Carbohydrates, either soluble starch, maltose or glucose were inadequate stimuli under the same conditions. Thus far this fact has been neglected in the interpretation of blood glucose level after the oral administration of starch. A diet rich in starch may, however, at long-term selectively increase the output of pancreatic (Reboud, Marchis-Mouren, Paséro, Cozzone and Desnuelle, 1966) but not of salivary (Palla, Ben Abdeljlil and Desnuelle, 1967) α-amylase in the rat. The same effect is not observed in the human (Figarella, 1966).

Other factors governing the degree of α-amylolysis *in vivo* are directly related to the medium conditions. Since α-amylase has its optimal pH at 6.9, its enzymatic activity will be nearly optimal in the succus entericus, which shows increasing pH from 6 in the duodenum up to 8 in the ileum (Borgström, Dahlqvist, Lundh and Sjövall, 1957). The low pH of the stomach content limits the prolonged action of salivary amylase.

Ugolev (1965) presented evidence in the rat that the total α-amylolytic breakdown *in vivo* is the result of the amylase concentration within the succus entericus and the amount of amylase adsorbed on the intestinal surface. The experimental data indicate that the adsorbed enzyme could be responsible for half or more of the total amylolysis within the small intestine (Jesuitova, De Laey and Ugolev, 1964). In the human, also, some α-amylase is regularly found in the mucosal specimens obtained from subjects with normal pancreatic function (Auricchio, Rubino and Mürset, 1965). This phenomenon, referred to as *membrane digestion,* could contribute to the more economic use of the secreted enzymes.

### 2. *The Hydrolysis of Oligosaccharides*

Claude Bernard (1877) was the first to recognize that the small intestine produces an enzyme capable of hydrolyzing saccharose. About 100 years later, Dahlqvist and Borgström (1961) showed that the glycosidase activities of the "succus entericus" of man are too low to account for the physiological digestion of the disaccharides maltose, saccharose and lactose. In the last decade, numerous studies have proven that the hydrolysis occurs at the sur-

face of the intestinal mucosa. The method of the peroral biopsy (Shiner, 1956; Crosby and Kugler, 1957) has made it possible to study the problem accurately in man. In order to reach a proper evaluation of the glycosidase activity of the human intestinal mucosa, a number of factors need to be taken into consideration; they are briefly discussed in this section.

*Technological problems.* The intestinal glycosidases are currently measured by the liberation of glucose from the appropriate substrates with the use of a glucose oxidase reagent. Most workers have used the method of Dahlqvist (1964) with minor modifications. The impurity of the available substrates, the contamination of the commercial preparations of glucose oxidase with glycosidases and the small amounts of tissue obtainable by suction biopsy may be considered as the limiting factors of the technique. The influence of the glycosidases contaminating the glucose oxidase preparations can be successfully eliminated by the use of tris(hydroxymethyl)aminomethane, a known inhibitor of the α-glucosidases (Larner and Gillespie, 1956) or by purification of the glucose oxidase preparations (Messer and Dahlqvist, 1966). Suitable ultramicrotechniques have been developed, which allow the measurement of the glycosidase activities on a few μg of tissue: 120-475μg (Townley, Khaw and Shwachman, 1965), 4-400 μg (Messer and Dahlqvist, 1966), 1-50 μg (Eggermont, 1966 and 1968a). The latter ultramicromethod has been successfully used as a routine procedure for the determination of the glycosidase activities in biopsy specimens.

*The influence of the intestinal surface.* In man (Auricchio, Dahlqvist and Semenza, 1963; Eggermont, 1968a), as well as in other mammals (Borgström and Dahlqvist, 1958; Gitzelmann, Davidson and Osinchak, 1964 and Hübscher, West and Brindley, 1965), the intestinal glycosidases involved in the digestion of carbohydrates are associated with particular structures. Using histochemical (Lojda, 1965; Jos, Frézal, Rey and Lamy, 1967) and immunochemical (Doell, Rosen and Kretchmer, 1965) techniques, they have been localized in the microvillous zone of the enterocytes and therefore probably belong to the brush border or apical membrane of the enterocytes. More direct evidence was obtained by the demonstration of these enzymes in isolated brush border membranes (Miller and Crane, 1961; Hübscher, West and Brindley, 1965) and in the membranous fraction prepared from these structures (Eichholz and Crane, 1965). Recently, Johnson (1967) reported that the invertase and maltase activities are associated within knobs 60 Å in diameter; these knobs are attached to the plasma membrane but are not an integral part of it.

A 20-fold increase in surface area due to the microvillous structure has been estimated by Wilson (1962). Since the intestinal glycosidases belong

to these structures, the morphology of the enterocytes will considerably influence the enzymic activities of individual specimens. Furthermore, the mucosal cells normally cover finger-like processes which project into the lumen and which cause a further increase of the intestinal surface by a factor of 10 (Wilson, 1962). The highest activities of the dissacharidases occur in the apical half of each villus (Dahlqvist and Nordström, 1966; Jos, Frézal, Rey and Lamy, 1967).

These considerations indicate that morphological lesions may considerably influence the enzymic activities. Celiac disease, characterized by the damage of both microvilli and villi, is accompanied therefore by a secondary deficiency of the brush border enzymes (Shmerling, Auricchio, Rubino, Hadorn and Prader, 1964; Plotkin and Isselbacher, 1964; Arthur, 1966).

The release of the maltase, isomaltase and saccharase from the brush border membrane can be obtained by papain digestion in the presence of potassium ions, which protect the enzymes against proteolytic inactivation (Auricchio, Dahlqvist and Semenza, 1963). This treatment solubilizes half of the lactase and only a small amount of trehalase. In this laboratory, the neutral detergent Triton X-100 was found to solubilize the intestinal α-glucosidases and lactase to an extent which always exceeds 84 per cent of the initial activity (Eggermont and Hers, 1968).

*The variation along the intestinal tract.* The distribution of the intestinal disaccharidases along the small bowel of man has been studied on multiple biopsy specimens (Newcomer and McGill, 1966) and segments removed at postmortem examinations (Auricchio, Rubino and Mürset, 1964; Swaminathan and Radhakrishnan, 1965). All glycosidases are maximally active in the jejunum, especially in the adult. The normal variation of the glycosidasic activities along the small intestine makes specification of the site of biopsy sampling essential for the comparison of individual results.

*The variation with age.* The differentiation of enzymes in the human intestinal mucosa starts well before birth, as indicated by the studies of Dahlqvist and Lindberg (1966) on human fetuses aged 11 to 23 weeks, and by those of Auricchio, Rubino and Mürset (1964) and of Eggermont (1966) on fetuses in the latter part of gestation. While all α-glucosidases are well developed months before term, lactase reaches maximum activity only at the very end of normal gestation; and premature infants surviving longer than 24 hours show higher values than those succumbing on the first day (Auricchio, Rubino and Mürset, 1964). Finally, γ-amylase develops gradually during infancy and childhood with wide individual variations (Eggermont, 1968a).

High activities of several of the intestinal glucosidases can be demonstrated in the meconium of premature infants and fetuses (Dahlqvist and

Lindberg, 1966, Eggermont, 1966). This biological material is easily available and could be useful for the early detection of intolerances to carbohydrates. Unfortunately, the glycosidases, in contrast to the alkaline phosphatase, tend to disappear in the full-term infants through the action of the meconium proteases (Eggermont, 1966).

In several species, the disaccharides except lactose are not well hydrolyzed by the newborn animal (Dahlqvist, 1961; Doell and Kretchmer 1964). In the rat, the invertase appears on approximately the 18th day after birth; and its development can be precociously induced by hydrocortisone (Doell and Kretchmer, 1964) or delayed by adrenalectomy (Koldovský, Heringová, Hošková, Jirsová, Noack, Friedrich and Schenck, 1965/66). The induced invertase appears first in the cells lying at the base of the villi, whereas the enzyme content of the differentiated enterocytes is not modified (Doell, Rosen and Kretchmer, 1965). A similar induction has also been observed when hydrocortisone is added to organ cultures of the intestine from neonatal rats (Doell and Kretchmer, 1964) or chick embryos (Hijmans and McCarty, 1966).

In most animals lactase disappears progressively after weaning (Alvarez and Sas, 1961; Doell and Kretchmer, 1962). The probable existence of such a phenomenon in man will be discussed below.

*The intestinal glycosidase activities.* The glycosidase activities, involved in the digestion of the alimentary carbohydrates by the human intestinal mucosa,

TABLE 1. — *The Classification of the α-glucosides and β-galactosides Which Have Been Used to Assess the Glycosidase Activity of the Human Small Intestinal Mucosa*

| | Site of attachment | | | | |
|---|---|---|---|---|---|
| | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $C_6$ |
| α-glucosides of | | | | | |
| Glucose | Trehalose | Kojibiose | | Maltose | Isomaltose |
| Fructose | | Saccharose | Turanose | | Palatinose |
| Oligosaccharides | | | | Malto-triose | Isomalto-triose |
| Glucan | | | | Glycogen | Dextran |
| β-galactosides of | | | | | |
| Glucose | | | | Lactose | |
| Fructose | | | | Lactulose | |
| Aglycone | | | | BNG* | |

* BNG or 6-bromo-2-naphthyl-β-D-galactopyranoside.

can be subdivided in 2 major classes. α-D-glucopyranosidases and β-D-galactopyranosidases. The substrates which have been used to characterize this hydrolytic activity are listed in Table 1; only a few of them are natural substrates.

The human mucosa hydrolyzes the following glucosides: maltose, saccharose, isomaltose, trehalose, turanose and dextran (Dahlqvist, 1962); palatinose and isomaltotriose (Dahlqvist, Auricchio, Semenza and Prader, 1963); maltotriose (Messer and Kerry, 1967); glycogen (Eggermont, 1968a) and kojibiose (Eggermont, unpublished results). It appears that several oligosaccharides which are produced by the α-amylolytic degradation of branched polysaccharides (see Fig. 1) can be converted to glucose by the combined action of an α-1,4-glucosidase (maltase) and of an α-1,6-glucosidase (isomaltase or oligo-1,6-glucosidase).

Lactase shows virtually no activity on lactulose (Dahlqvist and Gryboski, 1965), nor on the heterogalactosides such as 6-bromo-2-naphtyl-β-D-galactopyranoside (Hsia, Makler, Semenza and Prader, 1966), but hydrolyzes the β-glucoside cellobiose, at a speed about 5 times lower than with lactose itself (Semenza and Auricchio, 1962). The mucosa contains another β-galactosidase which preferentially hydrolyzes heterogalactosides (Hsia, Makler, Semenza and Prader, 1966). The latter enzyme has an acid pH optimum and is in the soluble fraction of a homogenate when no precautions are taken to preserve lysosomes (Dahlqvist and Asp, 1967). Furthermore, its postnatal development is different from that of lactase (Koldovský et al. 1965, 1966) and it is not affected in lactase deficiency (Hsia et al., 1966).

The α-galactosides are not hydrolyzed by the mucosa; they are acid-resistant but can be split by the intestinal flora (Gitzelmann and Auricchio, 1965). The potential toxicity of the α-galactosides for children with galactosaemia has been discussed by the same authors.

The β-fructosidic linkage is not attacked by the intestinal mucosa but is cleaved by the intestinal flora. We found that the bound is acid-sensitive even in the normal gastric environment. This would possibly explain the nutritive value of topinambour as recently suggested by Linneweh, Schaumlöffel and Barthelmai (1965) for the treatment of patients with glucose-galactose intolerance.

The glycosidase spectrum of the intestinal mucosa is very much the same in most mammals. Some of the differences observed are the presence of a β-fructosidase in the rat (Carnie and Porteous, 1963) and the absence of lactase and sucrase in the sea lion (Kretchmer and Sunshine, 1964).

Our own data obtained on normal subjects of different age groups are listed in Table 2.

TABLE 2. — *The Enzymic Activities of Several Enzymes, Localized in the Brush Border Membrane, in Relation to Age*

| Subjects | Age | Maltase | Isomaltase | Saccharase | γ-Amylase | Lactase | Trehalase | Alkaline phospha-tase | Proteins |
|---|---|---|---|---|---|---|---|---|---|
| | | Units per gram of mucosa | | | | | | | |
| Saccharose-intolerant Patients*: | | | | | | | | | |
| 1. D.F. | (5½ mo) | 3.5 | 0.9 | 0.2 | 1.8 | 2.2 | 1.0 | 21.1 | 44.4 |
| 2. VB.P. | (1 y 9 mo) | 6.4 | 0.8 | 0.6 | 3.0 | 8.1 | 4.5 | 31.0 | 62.8 |
| 3. S.M. | (1 y 11 mo) | 4.1 | 0.9 | 0.0 | 2.7 | 7.0 | 4.1 | 31.8 | 63.0 |
| 4. St.C | (2 y 3 mo) | 4.8 | 0.8 | 0.2 | 2.7 | 3.5 | 2.8 | 27.6 | 35.4 |
| 5. R.C. | (2 y 5 mo) | 3.6 | 0.5 | 0.0 | 1.7 | 3.5 | 1.1 | 12.4 | 46.5 |
| 6. F.B. | (3 y 5 mo) | 1.8 | 1.2 | 0.0 | 0.7 | 3.4 | 2.7 | 17.8 | 44.7 |
| 7. D.N. | (8 y 5 mo) | 3.2 | 0.7 | 0.0 | 2.3 | 3.9 | 1.8 | 25.9 | 116.0 |
| 8. K.L. | (14 y) | 4.8 | 0.9 | 0.1 | 2.1 | 6.2 | 3.2 | 24.8 | 43.4 |
| Mean | | 4.0 | 0.8 | 0.1 | 2.1 | 4.7 | 2.7 | 24.0 | 57.0 |
| Control subjects | | | | | | | | | |
| Mean | | 32.5 | 7.9 | 10.0 | 5.6 | 5.2 | 2.5 | 28.1 | 61.0 |
| Range | | 15.8-55.6 | 3.8-16.8 | 4.0-17.4 | 0.9-17.2 | 1.3-11.8 | 0.5-5.7 | 8.2-49.0 | 31.6-100.5 |

*The patients are clinically followed by Dr. De Vriendt of Gent, Belgium (cases 1 and 7), by Dr. Loeb of Brussels, Belgium (case 2), by Prof. Fernandes of Utrecht, the Netherlands (cases 3,5 and 8), by Prof. Rey of Paris, France (case 4) and by Dr. Cieters of Gent, Belgium (case 6).

*The identification of the enzymes.* As previously discussed, the intestinal glycosidases are embedded in the brush border membrane of the enterocytes. For these reasons, our definition of these proteins risks being artificial and even incorrect, as the necessary solubilization step may alter the natural configuration of these proteins *in situ.* Experiments using several substrates (Dahlqvist, Auricchio, Semenza and Prader, 1963; Eggermont, 1968; Eggermont and Hers, 1968) and making use of partial heat denaturation (Dahlqvist, 1962), indicate the multiplicity of active centers responsible for the known hydrolytic activities. As Schachman (1963) has shown, an excellent correlation exists between the number of active sites of an enzyme and the number of polypeptide chains involved in its structure.

*The number of active centers.* Experiments of competition between substrates clearly indicate that the ability of the human mucosa to hydrolyze maltose, saccharose, isomaltose and glycogen is due to 3 active centers, each of which acts on maltose and on one of the other 3 substrates. If one assumes that there exist 3 corresponding polypeptide chains, one can call them the maltase-isomaltase, the maltase-saccharase and the γ-amylase. There are also 2 additional active centers for the hydrolysis of trehalose and lactose respectively.

*Heat denaturation.* Dahlqvist (1962) has proposed a classification of the α-glucosidases primarily based on their stepwise heat denaturation in a crude homogenate made up in 0.01 M sodium phosphate buffer, pH 7.0. He postulated that there were 4 maltases: maltase Ia is the most labile and acts on both maltose and isomaltose; maltase Ib is inactivated at 50° and hydrolyzes maltose and saccharose. Maltases II and III are inactivated respectively at 60 and 75 degrees and have apparently no affinity for saccharose or isomaltose.

As will be shown in the next paragraph, even a slight heat denaturation at 45° strongly affects the sedimentation pattern of the subunits, although the activity has not been reduced. It appears difficult, therefore, to draw any conclusion from the treatment at higher temperature. Nevertheless, heat denaturation confirms the existence of a maltase-isomaltase, of a maltase-saccharase and of another maltase fraction which is probably identical with γ-amylase.

Trehalase activity decreases slowly at 52°, following first order kinetics (Dahlqvist, 1962).

*Separation procedures.* The separation of the intestinal glycosidases into subunits has been attempted by three groups of investigators using different techniques of solubilization and isolation.

Chromatography on TEAE-cellulose has been used by Dahlqvist (1965) to separate the α-glucosidases after their solubilization by papain in the presence of $K^+$; 2 peaks of maltase were obtained: the first (fraction A)

contained the whole of isomaltase and saccharase; the second (fraction B) was later analyzed in this laboratory and found to contain the bulk of the γ-amylase. Lactase was also adsorbed and was eluted as a single peak close to fraction A.

Semenza and co-workers (Semenza, Auricchio and Rubino, 1965; Auricchio, Semenza and Rubino, 1965) used filtration on Sephadex to fractionate the mucosal enzymes after their solubilization by papain. Maltase separated in 3 peaks: the first one was devoided of saccharase and practically of isomaltase, and was later found in this laboratory to contain the γ-amylase; on the

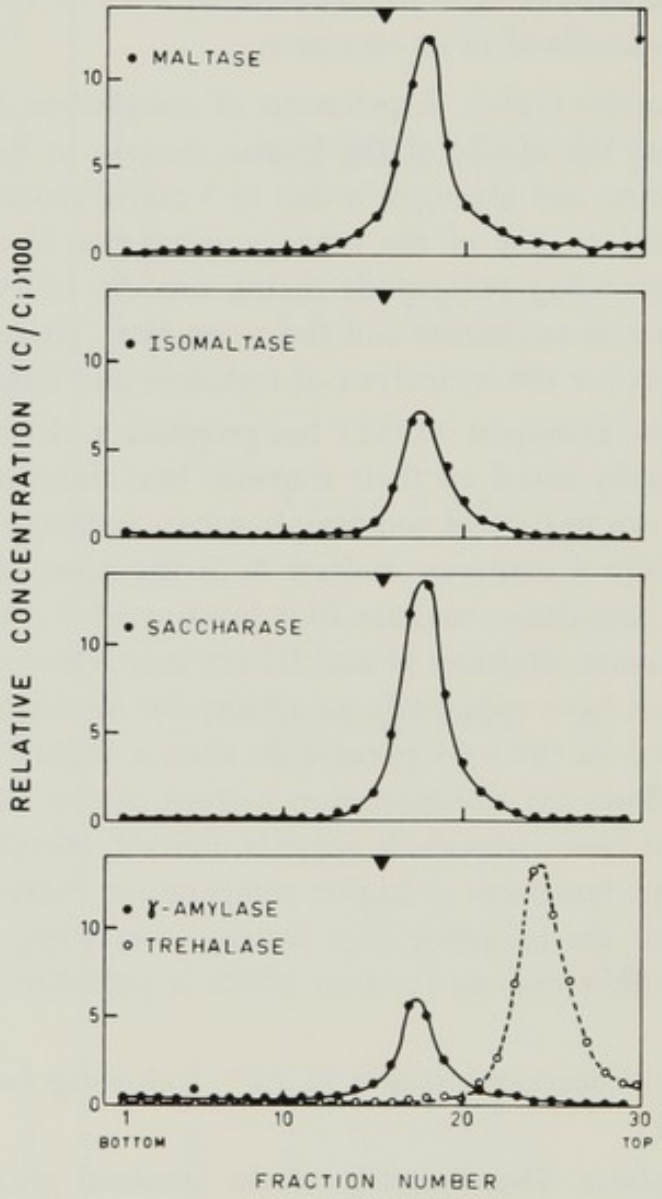



FIG. 2—Sedimentation pattern of the α-glucosidases solubilized with Triton X-100 from the intestinal mucosa of a normal subject. The enzyme concentration of each fraction (C) is expressed in per cent of the initial concentration (Ci) of the preparation layered on the gradient. The vertical arrows in Figs. 2 through 4 indicate the top of the mannitol gradient. The black triangle at the top of each panel indicates the peak of the reference enzyme catalase (mol. wt. 250,000; S20,w 11, 3 s).

basis of heat denaturation, this first fraction was assumed to contain 2 maltases (1 and 2). The second peak (maltase 3) contained also some saccharase activity, while the third one (maltases 4 and 5) contained the isomaltase and the greatest part of the saccharase. Although gel filtration is known to separate molecules on the basis of their size, it was later suggested by Kolínská and Semenza (1967) that, in the present case, isomaltase was presumably retarded by its affinity for dextran and that the saccharase was delayed by its association with the isomaltase.

In this laboratory, the active structures were characterized by their sedimentation behavior in a density gradient (Eggermont and Hers, 1968; Eggermont, 1968b). After their solubilization by Triton X-100, the maltase, isomaltase, saccharase and γ-amylase activities of normal subjects are recovered in a single 10 S peak (Fig. 2) while lactase and trehalase are obtained in the 10.6 S and 3.7 S fractions respectively. In one normal individual, however, among 10 who were analyzed, a second structure of 13.5 S with maltase, isomaltase and γ-amylase activity was repeatedly observed (Pattern B; Eggermont and Hers, 1968).

After treatment at 45° of the mucosal extract containing the α-glucosidases solubilized with Triton X-100, not only is the maltase-isomaltase greatly inactivated (Dahlqvist, 1962) but also is the 10 S structure disintegrated; the γ-amylase now sediments faster (Fig. 3). When the α-glucosidases are separated from the brush border membrane by papain in the presence of potassium ions, which protect the enzymes against proteolytic inactivation, the whole saccharase and most of the maltase, isomaltase and γ-amylase activities are again concentrated in the 10 S fraction. However, some of the maltase, isomaltase and γ-amylase activity is now confined to both faster and slower sedimenting structures. When the solubilization with papain is effected in the absence of $K^+$, all glycosidases are markedly inactivated; the 10 S glucosidase peak is particularly decreased, while the maltase-saccharase pattern becomes complex by the appearance of a novel 7.3 S subunit, and the γ-amylase has a tendency to accumulate in the 13-14 S fraction.

We have also applied our technique to fractions A (maltase-isomaltase + maltase-saccharase) and B (γ-amylase) of Dahlqvist; their sedimentation coefficient was 10 and 13 S respectively. Similar values were obtained for fractions 4 + 5 (maltase-isomaltase + maltase-saccharase) and 1 + 2 (γ-amylase) of Semenza.

An important alteration of the sedimentation pattern of the α-glucosidases has also been observed in saccharose intolerant subjects, and will be described in the next section.

*Conclusion.* The available information indicates that 5 polypeptides, each bearing one active center: a lactase, a threhalase, a maltase-isomaltase, a maltase-

saccharase, and a γ-amylase, are present in the brush border. The last 3 α-glucosidases have been found in various structural associations which may be formed during or after the solubilization step, but which might also pre-exist in the brush border. The existence of these associations is indicated by the change in apparent molecular weight of one chain when another one is specifically inactivated. In the mildest conditions, the 3 enzymes are recovered in the 10 S fraction, in which they might be associated 2 by 2 or constitute a trimer of a higher polymer of relatively smaller units. A dimeric structure is suggested by the existence of a 10 S association of maltase-isomaltase and of maltase-saccharase in the absence of γ-amylase in the meconium (Eggermont, unpublished results) as well as in the fractions which had been partially puri-

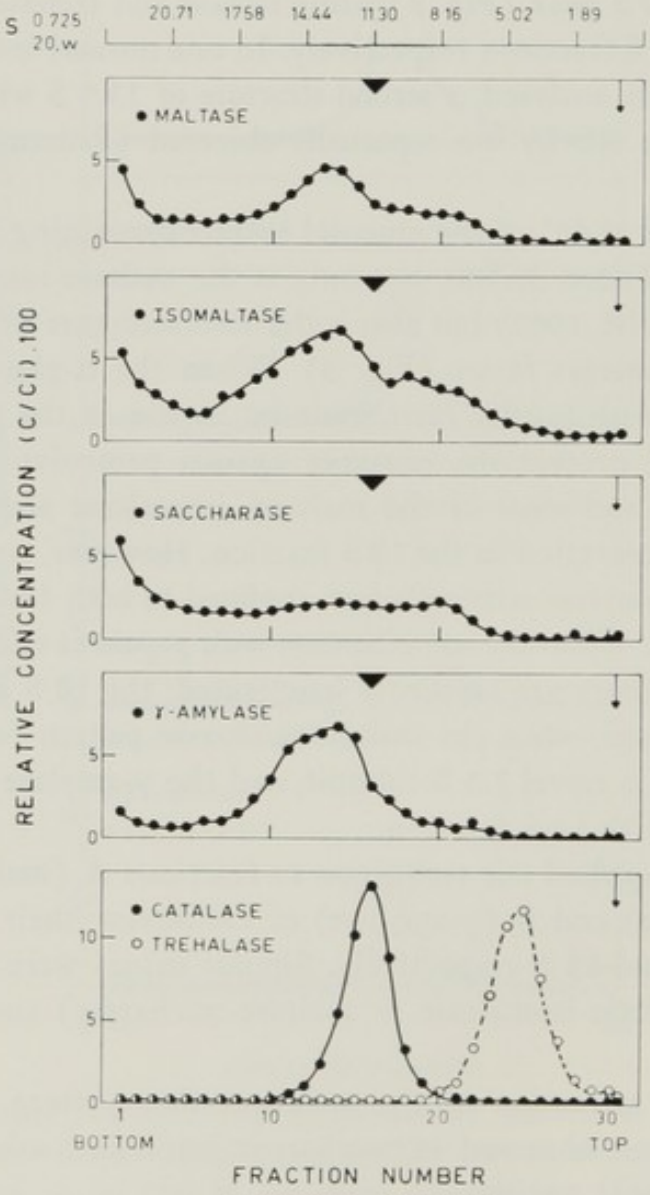



FIG. 3—The effect of heating the mucosal extract at 45° on the sedimentation of the intestinal α-glucosidases including the residual isomaltase. For the significance of the symbols, see Fig. 2 legend.

fied by TEAE-cellulose chromatography or by gel filtration. A 10 S association between the γ-amylase and the maltase-isomaltase is suggested by the fact that, after the thermal inactivation of the latter polypeptide, the former is recovered in an abnormal heavy fraction. It is remarkable that the γ-amylase is recovered as the same heavy structure when it has been previously separated from the 2 other structures by chromatography or by gel filtration. We will see below that, in sucrose intolerance characterized by the complete deficiency of the maltase-saccharase, the γ-amylase and the residual maltase-isomaltase are almost entirely in the 13 S fraction.

Pattern B indicates a qualitative polyenzymic abnormality without quantitative alteration of the catalytic activities. The situation can therefore not be attributed to a mutation of a regulatory gene and is best explained by a single structural alteration of one of the 3 active polypeptides, causing an abnormal association between them.

*Nomenclature.* It now clearly appears that neither heat denaturation nor gel filtration are sufficient to identify the several active structures involved in the hydrolysis of the α-glucosides. Furthermore, the 2 numerical classifications based on these techniques and proposed by Dahlqvist (1962)—"maltases Ia, Ib, II and III"— and by Auricchio, Semenza and Rubino (1965)—"maltases I to 5"—do not correspond. We therefore recommend the adoption of the following terminology:

maltase-isomaltase = maltase Ia = maltase 5
maltase-saccharase = maltase Ib = maltase 4
γ-amylase = maltases II + III = maltases 2 + I.

The independent existence of maltase 3 is doubtful.

## B. The Maldigestion of Carbohydrates

### *1. The Clinical Syndrome*

*History.* In the second half of the 19th Century, breast feeding was still essential; pasteurization of milk was introduced in 1882 and opened the way for the preparation of formulae (Fomon, 1967). Experience in formula feeding led to a greater understanding of carbohydrate tolerance. In 1947 Jeans and Marriott, in their classical work on infant nutrition, summarized the acquired knowledge of that time on the normal tolerance of infants to dietary carbohydrates: "Any sugar, when fed in excessive amounts, may cause gastrointestinal fermentation and diarrhea. Solutions of dextrose stronger than 6 per cent or of milk sugar and cane sugar stronger than 8 per cent are hypertonic and likely to exert some laxative effect, for that reason alone, aside from fermentation. Dextrin and starch, on the other hand, may be given in solutions of a concentration of 20 per cent or more."

Major aspects of infantile dyspepsia, like the existence of pathogenic types of *Escherichia coli* and the disturbances of body fluids and electrolytes, had to be clarified before proper attention could be devoted to other causes of recurrent diarrhea. In 1953, the brilliant work of Dicke, Weijers and van de Kamer elucidated the role of gluten in the intolerance of celiac patients to some starchy foods. In the following years, the clinical syndrome of a specific intolerance to isolated carbohydrates, lactose (Holzel, Schwarz and Sutcliffe, 1959), saccharose (Weijers, van de Kamer, Dicke and Ijsseling, 1961), glucose and galactose (Laplane, Polonovski, Etienne, Debray, Lods and Pissarro, 1962) was recognized.

*Symptoms.* The subjects are normal at birth but, as soon as the non-tolerated sugar is introduced in the diet, they develop clinical symptoms which may vary in severity, even in the same subject.

In the younger age group, diarrhea due to the osmotic action and fermentation of the unabsorbed sugars is the dominant feature; for these reasons, the determination of residual carbohydrates and of organic acids in the stools is a valuable tool for the clinical detection of such patients. Normally, glucose, galactose and lactose are absent from the feces of children and adults but are occasionally found, at a concentration lower than 3 mg. per Gm. in the stools of infants (Gryboski, Zillis and Ma, 1964). The Ames clinitest tablets may be used to demonstrate the excess carbohydrate (Kerry and Anderson, 1964) whereas paper chromatography will allow the identification of the unabsorbed sugars (Durand, Martino and Lamedica, 1961). These chromatographic studies have revealed the presence of glucuronic acid in the meconium and transitional stools of the first 3 days of life as well as of fucose in the feces of breast-fed infants (Baar and Bickel, 1964). Lactulose or 4-o-β-D-galactopyranosyl-D-fructose can be demonstrated in the stools of 65 per cent of artificially fed infants (Gryboski, Zillis and Ma, 1964). Lactulose is not present in fresh cow milk but is formed during its processing. Furthermore, some pentoses and hexoses derived from fruits and vegetables may appear in the stools of older children and adults.

Part of the unabsorbed sugar is metabolized by the intestinal flora into a number of organic acids, of which lactic acid is the most characteristic. However, during the onset of a fermentative diarrhea, acetic acid occurs before lactic acid in the stools. One assumes therefore that the lactic acid-forming flora increases with the load of unabsorbed sugar (Weijers and van de Kamer, 1964) or with the fall of pH (Tabaqchali and Booth, 1967). In the feces of children with fermentative diarrhea but also of normal breast-fed children the mean lactic acid concentration is as high as 350 mg. per cent, whereas the value observed in artificially fed children is well below 50 mg.

per cent. When the daily production of feces is taken into account (20-30 gm. for breast fed babies, 40-50 gm. for artificially fed babies, 70-90 gm. for toddlers but 100-300 gm. for children with fermentative dyspepsia), the daily fecal loss of lactic acid of children with carbohydrate intolerance is well above that of breast-fed infants (Weijers and van de Kamer; 1963 and 1964).

In the older child and adult, the actual clinical experience is almost limited to the intolerance to lactose. In most subjects, the symptoms of diarrhea are less predominant. The lactase deficient people are sometimes free of symptoms or may have only minor complaints such as flatulence, meteorism, abdominal cramps and loose stools. Some of them recognize the deleterious effect of milk and deliberately omit it from the diet. (Haemmerli et al., 1965; Dunphy et al., 1965; Klotz and Lubos, 1967). An excessive lactic acid excretion was not found in the adult (McMichael, Webb and Dawson, 1965). Nevertheless, special investigations can show the osmotic action of the unabsorbed sugar (Kern and Struthers, 1966).

When an individual is suspected of intolerance to a specific carbohydrate, additional information can be obtained by loading the subject with the suspected sugar and by measuring the subsequent changes in the level of blood glucose and of carbohydrates and lactic acid in the feces (Weijers and van de Kamer, 1963; Auricchio, Prader, Mürset and Witt, 1961; Girardet, 1965). If osmotic and or fermentative diarrhea develops, or if the blood glucose level does not increase more than 20 mg. per cent, it is still important to show that the same symptoms do not appear after the administration of the corresponding monosaccharides, or of other sugars, and that proteins and fat are well tolerated. The situation is further complicated by the fact that isolated deficiencies of saccharase (Rey, Frézal, Jos, Bauche and Lamy, 1963) and lactase (Lifshitz, 1966) may induce secondary lesions of the mucosa, causing a complex picture in which the isolated defect may be obscured.

It is our opinion that, in spite of the correlation found between intestinal enzyme activities and increase of blood glucose (Haemmerli et al., 1965; McMichael, Webb and Dawson, 1966), the result of a sugar load will not give a definitive diagnosis concerning the cause of the maldigestion syndrome. In most cases, the determination of enzymes in an intestinal biopsy is required. For these reasons, the discussions on the genetics of the different forms of glycosidase deficiencies will be limited to those cases which have been analyzed at the enzyme level.

*2. The Intolerance to Starch*

As discussed by Davidson (1967), the concept of starch intolerance can be traced to the misinterpretation of the correct observation of Herter (1908) that patients with celiac disease are made worse by ingestion of some starchy foods. The report of Andersen (1947) on the low level of α-amylase in the

duodenal content of some children with celiac disease further increased the confusion, and seemed to indicate that starch intolerance is a mild form of celiac disease. The confusion ended when Dicke, Weijers and Van de Kamer (1953) demonstrated that the precipitating factor of the enteropathy of subjects with celiac disease is not the starch but the gluten of some starchy foods. For this reason, the earlier reports on starch intolerance have to be interpreted cautiously.

The clinical diagnosis of starch intolerance due to α-amylase deficiency is suspected from dietary history but hard to demonstrate by starch loads since starch itself is a poor stimulus for pancreatic secretion and is difficult to measure in stools (Davidson and Bauer, 1958; Neven and Fouassin, 1962). Lowe and May (1951) appear to be the only authors who have reported a selective pancreatic deficiency of amylase. The patient, a boy of 13 years, was underdeveloped (31 Kg.) and had up to 6 foul-smelling, light-colored bowel movements a day. The abdomen was slightly distended. Amylase was present in the saliva, but not in the material aspirated from the duodenum, which contained decreased trypsin and normal lipase activity. Since the family history was non-contributory, a genetic origin of this isolated defect of α-amylase is not demonstrated.

Although the theory that α-amylase deficiency is responsible for celiac disease is now discarded, it should not be overlooked that Andersen (1947) has demonstrated a transitory deficiency of pancreatic α-amylase in some of the children with starch intolerance. Recently, Martin du Pan and Infante (1963) have reported similar observations and we were also able to study an analogous patient, of which several sibs had presented the same clinical symptoms during infancy. In view of the present data on the maturation of the pancreas (see p. 244) and on the possible dissociation of the amount of enzymes elaborated by the gland (Sarles, Figarella, Prezelin and Souville, 1966), this clinical entity might be partially ascribed to a delay in the postnatal synthesis of pancreatic α-amylase.

*3. The Intolerance to Saccharose*

Weijers, van de Kamer, Dicke and Ijsseling (1961) have published the first clinical report on intestinal intolerance to saccharose. Shortly thereafter, the Zürich group (Prader, Auricchio and Mürset, 1961; Auricchio, Prader, Mürset and Witt, 1961) made the observation that patients affected by this disease are also intolerant to starch despite a normal α-amylase activity and a normal tolerance to maltose. They postulated that the intolerance to starch could be due to a simultaneous defect of isomaltase (Auricchio, Dahlqvist, Mürset and Prader, 1963). In 1963, Anderson, Messer, Townley and Freeman obtained proof of the double lesion, by the demonstration that both

saccharase and isomaltase are deficient in such patients, and that the mucosa is normal histologically. Later, the integrity of the microvilli of the enterocytes was verified with the electron microscope by Sonntag, Brill, Troyer, Welsh, Semenza and Prader (1964) and by Neale, Clark and Levin (1965).

The quantitative determination of intestinal glucosidases in sucrose intolerance (Burgess, Levin, Mahalanabis and Tonge, 1964; Auricchio, Rubino, Prader, Rey, Jos, Frézal and Davidson, 1965; Kerry and Townley, 1965; Rey and Frézal, 1967) has clearly shown that the saccharase is virtually absent. Furthermore, several other α-glucosidases are severely affected: the activity of isomaltase, maltase and γ-amylase (Eggermont and Hers, 1968) are reduced to approximately 10, 20 and 35 per cent of the normal, respectively. The residual maltase activity is mostly due to the γ-amylase and appears to be usually sufficient for a normal tolerance to maltose. The residual isomaltase activity has maintained its typical heat sensitivity (Eggermont and Hers, 1968). Our personal data on 8 cases of saccharose intolerance are shown in Table 3.

The reconciliation of the complex enzymic lesion of hereditary saccharose intolerance with the "one gene, one protein" hypothesis has puzzled many investigators. Various explanations of the multiple defects have been advanced: the existence of several enzymes with a common polypeptide or a mutation affecting the regulatory gene (Rey, Frézal, Jos, Bauche and Lamy, 1963); a single enzyme with multiple glucosidase activities (Launiala, Perheentupa, Visakorpi and Hallman, 1964); a common precursor (Semenza, Auricchio, Rubino, Prader and Welsh, 1965); and finally the presence of a common inhibitor (Prader and Auricchio, 1965).

Recently we have obtained information which indicates that the polyenzymic lesion, characteristic of saccharose intolerance, is qualitative as well as quantitative and can therefore not be explained by a mutation of a regulatory gene. As shown in Figure 4, the sedimentation pattern of the residual maltase-isomaltase and γ-amylase after their solubilization by Triton X-100 is abnormal, in that the 10 S fraction has disappeared and has been replaced by two new peaks of 12.6 and 6.2 S respectively. (Eggermont and Hers, 1968). This situation is reminiscent of that induced by the treatment of the extract at 45°, which causes the disintegration of the 10 S peak, with a simultaneous inactivation of the maltase-isomaltase. We have concluded that the data are best explained on the basis of a simple mutation which would inactivate the structural gene for maltase-saccharase, with the assumption that in the absence of the latter enzyme, the 2 other active polypeptides form an abnormal association in which they are less active than normal. If this is correct, it is not necessary to postulate a more elaborate theory!

At the present time, there exists also some evidence that all patients with

TABLE 3. — *Enzymic Activities and Protein Content Measured in the Intestinal Mucosa of 8 Saccharose Intolerant Subjects and of a Control Group of 35 Children*

| | Fetuses (9)+ | Pre-term infants (5) | Full-term infants (5) | Infants & children (35) | Adults (9) |
|---|---|---|---|---|---|
| Enzymes: μmoles/min. and Gm. mucosa | | | | | |
| Maltase | 16.7±2.9 | 16.6±4.0 | 23.4±6.4 | 32.5±1.9 | 43.6±5.8 |
| Isomaltase | 3.5±0.6 | 4.1±1.0 | 4.6±1.1 | 7.9±0.6 | 13.4±1.9 |
| Saccharase | 6.0±1.0 | 5.9±1.4 | 7.9±2.0 | 10.0±0.7 | 13.5±1.8 |
| γ-Amylase | 1.4±0.4 | 0.6±0.2 | 0.7±0.1 | 5.6±0.7 | 12.2±2.1 |
| Lactase | 0.6±0.2 | 3.8±1.1 | 5.1±1.5 | 5.2±0.5 | 6.8±1.3 |
| Trehalase | 2.7±0.6 | 1.5±0.4 | 3.2±1.1 | 2.5±0.2 | 4.6±1.0 |
| Alkaline phosphatase | 9.1±1.7 | 36.0±11.4 | 38.8±7.2 | 28.1±1.9 | 32.8±4.8 |
| Proteins: mg./Gm. mucosa | 50.2±6.9 | 45.8±11.3 | 52.3±8.0 | 61.0±3.5 | 63.0±7.3 |

sucrase-isomaltase deficiency may not be affected by the same mutation. In some cases, for instance, the residual saccharase activity is not negligible and may reach 10-20 per cent of the mean normal value (Launiala, Perheentupa, Visakorpi and Hallman, 1964; Welsh and Brown, 1966). Francois, Frédérich, Vicens-Calvet, Bertrand and Ruitton-Ugliengo (1963) reported also, but only on the evidence of sugar loads, that the isomaltase was not affected in a patient with saccharose intolerance.

The hereditary nature of saccharose intolerance is now well established. The clinical observations on the condition of the parents and sibs of the affected children strongly suggest that saccharose intolerance is inherited as a simple recessive autosomal trait. In a recent review on the subject, Rey and

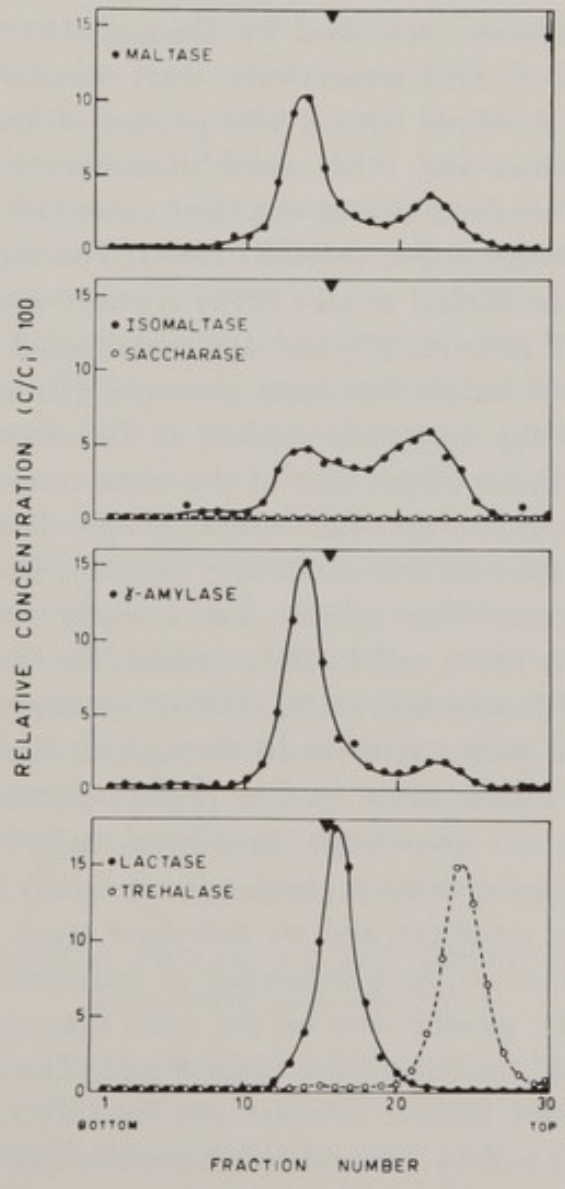



FIG. 4—The sedimentation pattern of the α-glucosidases and lactase solubilized with Triton X-100 from the intestinal mucosa of a saccharose intolerant child. For the significance of symbols, see Fig. 2 legend.

Frézal (1967) discussed this problem from the documented cases published in the literature: the parents appear normal, the sex ratio of boys to girls in the group of affected children is 1.5. Among the siblings 23 per cent are affected. The direct determination of enzymic activities in the small-intestinal mucosa obtained from the parents of such patients substantiates this conclusion, since the saccharase and isomaltase activities of the parents are found to be intermediate between those of normal individuals and of the homozygotes (Kerry and Townley, 1965) and since qualitatively, the sedimentation pattern of the glucosidases of the mother of one patient was the superposition of the normal and of the abnormal profile (Eggermont and Hers, 1968).

*4. The Intolerance to Lactose*

Two clinical syndromes, described by Durand (1959) and by Holzel, Schwarz and Sutcliffe (1959) respectively, were tentatively attributed to a congenital defect of intestinal lactase. The patient of Durand suffered diarrhea, transient lactosuria and other renal disturbances, when lactose was present in the diet. Intestinal lactase was later measured in a similar patient and found to be normally active (Holzel, 1967). The symptoms of the clinical entity described by Holzel et al. (1959) are very similar to those of sucrose intolerance. In 2 patients, $2\frac{1}{2}$ and $4\frac{1}{2}$ months- old respectively, a nearly complete absence of lactase has been observed (Launiala, Kuitunen and Visakorpi, 1966); in the 5-year-old patient of Davidson et al. (1964) the residual activity was about 18 per cent of the mean control value (Auricchio et al. 1965). In our opinion, the mild clinical symptoms and the residual lactase activity of the latter patient could also be explained by the precocious disappearance of lactase. The subject was recently reviewed by Durand (1964), Holzel (1965, 1967) and Lifshitz (1966). For a certain diagnosis of hereditary congenital lactase deficiency, it seems necessary that the enzymatic defect be isolated and persist from birth throughout life with, preferably, a familial incidence of similar cases. For the present discussion, we retain only the 3 cases which, to our knowledge, have been studied at the direct enzymatic level. In these cases, there is absolutely no family history of a similar disorder among other members, and we therefore reach the conclusion that a genetically determined primary deficiency of intestinal lactase has not yet been documented. The genetic data on the cases diagnosed on the basis of clinical symptoms and sugar loads are equally rare. The disorder was recognized among siblings by Holzel, Schwarz and Sutcliffe (1959); Weijers and van de Kamer (1962) and by Durand and Lamedica (1962). The occurrence of acquired selective deficiency of intestinal lactase in the adult complicates the interpretation of the observations made in the parents (Weijers and van de Kamer, 1962). Consanguinity of the parents has so far not been reported.

The selective deficiency of intestinal lactase in the adult is a frequent condition, first recognized in 1963 by Auricchio, Rubino, Semenza, Landolt and Prader and by Dahlqvist, Hammond, Crane, Dunphy and Littman. Its frequency reaches about 10 per cent in the white population (Sheehy and Anderson, 1965); Bayless and Rosensweig, 1966) but may be as high as 50 per cent among Negroes living in Africa (Cook and Kajubi, 1966) as well as in the United States (Bayless and Rosensweig, 1966). This enzyme deficiency is sometimes complicated by an intolerance to milk, which was not manifested during childhood and which is therefore acquired. The decrease of lactose tolerance occurs during the first 4 years of life in the Baganda children (Cook, 1967) and probably after the age of 6 in the American Negro population (Huang and Bayless, 1967). This situation resembles the fall of lactase activity after weaning observed in many mammals (see page 247). Lactase deficiency may also represent a "functional scar" in connection with a number of gastrointestinal disorders such as celiac disease (Shmerling, Auricchio, Rubino, Hadorn and Prader, 1964; Arthur, 1966) and gastrectomy (Gryboski, Thayer, Gryboski, Gabrielson and Spiro, 1963; Hooft, Van Hauwaert, De Laey and Adriaenssens, 1963). In all conditions, the acquired nature of the defect precludes a lesion of the structural gene(s) coding the polypeptide of the lactase enzyme. Nevertheless, the large racial variations indicate that the loss of lactase may be genetically controlled. In this regard, Bayless and Rosensweig (1967) postulated that the precocious disappearance of activity in the Negro may be another example of genetic polymorphism. Ferguson and Maxwell (1967) suggested the existence of 3 alleles: L, $l_1$ and $l_2$ which could possibly occupy the locus controlling the synthesis of lactase: L, the gene active throughout life; $l_1$, the gene coding the enzyme only during the period of physiological requirements, and $l_2$, an abnormal gene. With the latter assumption, normal lactose tolerance would be possible with the genotype LL, $Ll_1$ and $Ll_2$; isolated deficiency after infancy would appear in the subjects with genotype $l_1$ $l_1$ or $l_1$ $l_2$ while congenital hereditary lactase deficiency would correspond to the genotype $l_2$ $l_2$.

## II. ABSORPTION AND MALABSORPTION OF CARBOHYDRATES

### A. The Physiological Process of the Absorption of Glucose, Galactose and Fructose

The gastrointestinal lumen is lined with a lipoprotein membrane permeable through pores with a radius of about 4 Å, as measured in experiments in which lipid-insoluble non-electrolytes were used (Lindemann and Solomon, 1962). Since the hexoses are insoluble in lipids and have an effective hydrodynamic radius of 4.2 Å. (Schultz and Solomon, 1961), their passage through the pores is negligible. In accordance, the gastric absorption of glu-

cose occurs only when its concentration exceeds 11 per cent (Warren, Karr, Hoffman and Abbott, 1940) and the absorption of glucose across the colon is also insignificant (Long, Geiger and Kinney, 1967). However, when glucose, galactose and fructose enter the small intestine, they are absorbed very rapidly (Groen, 1937) due to the presence of mediators built into the membrane (for a review, see Mitchell, 1967). The difference in concentration on both sides of the membrane seems the only driving force for the translocation of fructose. Since glucose and galactose not only pass the membrane but accumulate in the mucosal cells against a concentration gradient, an adequate energy supply is necessary for their transport.

*1. The Absorption of Fructose*

Experiments performed *in vitro* on isolated small-intestinal segments have shown that the absorption of fructose proceeds proportionately to its concentration on the mucosal site (Riklis and Quastel, 1958) and that the ketosugar is not concentrated in the serosal compartment (Wilson and Vincent, 1955). During this process, a significant amount of fructose is converted into lactate (Kiyasu and Chaikoff, 1957) or glucose by the mucosa, at least in the species with an intestinal glucose-6-phosphatase (Ginsburg and Hers, 1960).

In man, the kinetics of fructose uptake has been studied *in vivo* by Holdsworth and Dawson (1964) who found a linear relationship with the intraluminal concentration; at a concentration of 0.25 M (5 per cent), fructose is resorbed at a rate of 0.35 Gm. per minute, and 30 cm. of small intestine. Since sorbose, a structurally related ketosugar, is not absorbed (Holdsworth and Dawson, 1965), the absorption of fructose is presumably mediated by a specific carrier. A negligible conversion of fructose into glucose by the human intestine was found by Holdsworth and Dawson (1965); Öckerman and Lundborg (1965) reported that up to 70 per cent of the sugar absorbed and recovered as hexose in the mesenteric veins consisted of glucose. In agreement with this finding, the presence of glucose 6-phosphatase was demonstrated in the human intestinal mucosa (Öckerman, 1965) in which fructose appears to be metabolized as in the guinea pig small intestine (White and Landau, 1965). During the absorption of fructose in man, some lactate is also produced (Holdsworth and Dawson, 1965).

Up to the present time, no specific defect of the absorption of fructose has been recognized.

*2. The Absorption of Glucose and Galactose*

Glucose and galactose not only pass through the intestinal mucosa but accumulate within the mucosal cells; the process displays Michaelis-Menten kinetics. The apparent *Km* for the accumulation of glucose and galactose in intestinal strips of the golden hamster is 1.5 mM and 2.2 mM respectively (Crane,

1960). With the use of the intestinal perfusion technique, the kinetics of glucose and galactose absorption could also be measured in man: in the jejunum, the apparent maximum velocity (*V max*) and affinity constant (*Km*) for glucose are 0.73 Gm./min./30 cm. small-intestine and 220 mM respectively; the corresponding values for galactose are 1 Gm./min./30 cm. and 330 mM (Holdsworth and Dawson, 1964).

The active transport of both monosaccharides occurs at the apical or brush-border membrane of the enterocytes (McDougal, Little and Crane, 1960; Crane, 1962). Their translocation is mediated by a common carrier bearing a carrier-center, a term used in analogy with the active center of an enzyme (Mitchell, 1967). This center accepts other sugars which fulfill such minimal requirements as the pyranose ring structure, the equatorial hydroxyl group in the $C_2$ position and the variably substituted methyl group on $C_5$ (for a review see Wilson, 1962). In recent years, the two latter conditions were found not to be strictly necessary since an active transport of mannose (Csáky and Ho, 1966) and xylose (Salomon, Allums and Smith, 1961; Csáky and Ho, 1965; Alvarado, 1966) by the sugar carrier could be demonstrated in certain conditions.

In 1958, Riklis and Quastel made the observation that the movement of glucose was dependent on the presence of $Na^+$ in the medium. In 1962, Bihler, Hawkins and Crane could demonstrate that the reduction of the tissue energy supplies eliminates the accumulation against a concentration difference but not the $Na^+$-dependent equilibration of glucose. They postulated that the sugar-carrier possesses a binding site for cations, and later demonstrated that in the presence of $Na^+$ the carrier manifests about 100 times more affinity for its substrate than in the presence of $K^+$ (Crane, Forstner and Eichholz, 1965). Further evidence of the interaction of $Na^+$ on the sugar-carrier was obtained by the phenomenon of counter-transport (Crane 1964); when isolated villi with blocked energy-dependent transport of $Na^+$ are first loaded with $Na^+$ and sugar and subsequently transferred in a medium without $Na^+$, the sugar leaves the villi in spite of the concentration difference. In the rabbit, however, the $Na^+$-activation of sugar transport is characterized by a large increase in maximal velocity with little change in the apparent *Km*. (Schultz and Zalusky, 1964).

Since the carrier is assumed to migrate freely within the brush border membrane and to be exposed alternatively to the extracellular medium, rich in $Na^+$, and to the cytoplasma, rich in $K^+$, the sugar can be transported against its concentration gradient as a result of the ionic asymmetry.

The maintenance of the active transport of glucose will therefore depend on the continuous extrusion of $Na^+$. Since the membranous enzyme, the ouabain-sensitive ($Na^+ + K^+$) activated ATP-ase, shares many properties

with the system involved in the active transport of $Na^+$ and $K^+$ across the cellular membranes, it has been tentatively identified with the sodium pump (Skou, 1965; Opit and Charnock, 1965). The hydrolysis of ATP by this system is considered as the energy-producing step. Since ouabain, a potent inhibitor of the sodium pump, does not penetrate the mucosal cells and inhibits the sugar transport only when present in the serosal compartment, Csáky and Hara (1965) tentatively localized the ($Na^+ + K^+$) activated ATP-ase in the serosal facing membranes.

The active transport of amino acids (Robinson, 1966) appears also to be $Na^+$-dependent. In recent years, it was observed that the actively transported sugars and amino acids interfere with each other in a manner which was not explained by competition for the available energy. (Bingham, Newey and Smyth, 1966; Alvarado, 1966). A number of observations also suggests that the sugar transport system may be closely related to the sucrase (Semenza, Tosi, Valotton, Delachaux and Mülhaupt, 1964).

In man, the capacity of the jejunum to concentrate glucose appears as early as 10 to 16 weeks of intrauterine development (Jirsová, Koldovský, Heringová, Hošková, Jirásek and Uher, 1965/1966).

Detailed reviews on the active transport of sugars and other compounds were recently published by Crane (1960, 1962, 1965), Wilson (1962), Wiseman (1964), Csáky (1965), Newey (1967) and by Mitchell (1967).

### B. The Malabsorption of Glucose and Galactose

Glucose and galactose malabsorption causing a life-threatening diarrhea from birth was described in 1962 almost simultaneously by Laplane, Polonovski, Etienne, Debray, Lods and Pissarro and by Lindquist and Meeuwisse. Up to now, about 14 cases have been published (Anderson, Kerry and Townley, 1965; Linneweh, Schaumlöffel and Barthelmai, 1965; Lindquist, 1965; Schneider, Kinter and Stirling, 1966; Marks, Norton and Fordtran, 1966; Dubois, Loeb, Eggermont and Mainguet, 1966; Abraham, Levin, Oberholzer and Russell, 1967. The clinical condition improves abruptly when oral feeding is stopped or when fructose is the only sugar present in the diet. Although this is a rare disorder, the information is now available on the etiology and the physiopathological mechanism as well as on the mode of inheritance of the disease.

The reduced uptake of glucose together with the normal absorption of fructose was directly demonstrated by measuring the disappearance of the monosaccharides from the luminal content (Lindquist and Meeuwisse, 1963; Dubois, Loeb, Eggermont and Mainguet, 1966). From balance studies, Anderson et al. (1965) could calculate that after a single dose of either glucose, galactose or fructose 59 per cent, 37 per cent and 0.4 per cent respectively of

the administered monosaccharide could be recovered in the feces; later, Linneweh et al. (1965) and Schneider et al. (1966) reported somewhat lower values for both glucose and galactose, varying between 14 and 21 per cent. With the use of radioactive sugars, Linneweh, Schaumlöffel and Barthelmai (1965) could demonstrate that the uptake of glucose or galactose does not exceed 10 per cent of the administered dose, whereas the normal child resorbs over 90 per cent of it. Since diarrhea appears even in the absence of fermentation, the osmotic effect of the unabsorbed sugar seems the principal elicitor of the excessive stools.

The intestinal mucosa of these patients is normal both by histological (Anderson et al., 1965; Schneider et al., 1966) and electron microscopic (Schneider et al., 1966) criteria and manifests no deficiency of the disaccharidases (Anderson et al., 1965; Dubois et al., 1966; Abraham et al., 1967). Schneider et al. (1966) using autoradiographic techniques have shown that *in vitro,* contrary to the normal, the mucosal cells of a glucose-galactose-intolerant patient do not accumulate $^{14}C$-label from $^{14}C$-galactose.

A more direct investigation of the transport mechanism in a mucosal fragment of a patient was undertaken by Eggermont and Loeb (1966) and their conclusions have been confirmed by Meeuwisse and Dahlqvist (1966). The data of this are presented in Fig. 5; it appears that intestinal biopsy specimens from normal subjects accumulate glucose and L-leucine when incubated with the respective substrates in the presence of $Na^+$ but not in the presence of $K^+$. Under the same conditions, the glucose-galactose-intolerant infant accumulates L-leucine but not glucose. The energy supply seems there-

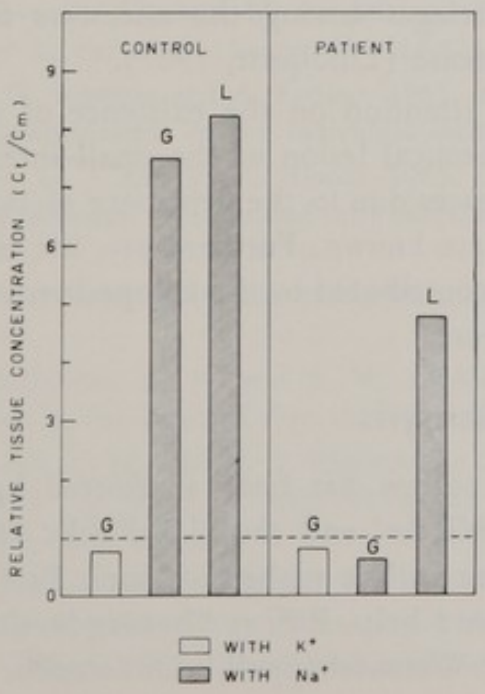



FIG. 5—An *in vitro* study of intestinal transport in glucose-galactose intolerance. Mucosal fragments weighing a few mg were incubated for 15 min at 37° in a medium containing either $Na^+$ or $K^+$ and [$C^{14}$] labelled glucose (G) or leucine (L). At the end of incubation, the concentration of radioactive material in the tissue (Ct) was measured and compared with that in the medium (Cm). Paper chromatography of the radioactive material accumulation within the tissue showed that 36% and 65% of it were made of glucose or leucine respectively (After Eggermont and Loeb, 1966).

fore normal, and one reaches the conclusion that the disease is probably due to the defect of the specific carrier for sugars.

Intravenous glucose tolerance tests were performed in some patients in good nutritional condition; the glucose level dropped normally (Lindquist, 1965; Dubois et al., 1966; Abraham et al., 1967). At routine examinations all except 2 (Lindquist, 1967, Dubois et al., 1966) the 14 described patients showed slight glucosuria; and careful studies of the maximal rate of tubular transport indicated that the renal threshold for glucose is lowered. Patients with renal glucosuria have, however, a normal intestinal transport of glucose (Thier 1967); and the independent handling of glucose and galactose by the kidney indicate that the renal and the intestinal sugar-carrier are different. In patients with glucose-galactose intolerance, the complex relationship between urinary glucose excretion and blood glucose concentration suggests the existence of two renal sugar-carriers, as already demonstrated for lysine; only one of them should then be related to the intestinal sugar-carrier Scriver, 1967).

The available data concerning the patients' relatives permit the conclusion that congenital glucose-galactose malabsorption is inherited by an autosomal recessive mode (Lindquist, Meeuwisse and Melin, 1963). The parents are healthy and do not suffer from the same disorder. The limited information on the siblings of the reported patients indicate that 2 out of 8 are also affected (Laplane et al., 1962; Anderson et al., 1965; Linneweh et al., 1965; Abraham et al., 1967). The disorder occurs in both sexes, although 75 per cent of the affected subjects are females. The recessive mode of inheritance is further documented by the detailed pedigrees of 6 Swedish patients; the high frequency of consanguineous marriages among the ancestors firmly establish the hereditary origin of the disease (Lindquist, 1967).

Only 10 years ago, Durand oriented attention on the existence of sugar intolerance secondary to a specific biochemical lesion of the small intestine. Today, a number of well-recognized diseases due to the deficiency of lactase, saccharase and the mobile sugar-carrier are known. Furthermore, the enthusiastic workers in the field have greatly contributed to the comprehension of normal intestinal digestion and absorption.

## ACKNOWLEDGMENTS

The original work included in this review has been supported by the "Fonds de la Recherche Scientifique Médicale" and the U.S. Public Health Service (Research Grant AM 9235). The author wishes to thank Professor H. G. Hers for his continuous interest and help. E.E. is "Bevoegdverklaard Navorser" of the "National Fonds voor Wetenschappelijk Onderzoeb."

## REFERENCES

Abraham, J. M., B. Levin, V. G. Oberholzer and A. Russell. 1967. Glucose-galactose malabsorption. Arch. Dis. Child. 42: 592.597.

Alvarado, F. 1966. Transport of sugars and amino acids in the intestine: evidence for a common carrier. Science 151: 1010-1013.

—. 1966. D-xylose active transport in the hamster small intestine. Biochim. Biophys. Acta. 112: 292-306.

Alvarez, A., and J. Sas. 1961. β-galactosidase changes in the developing intestinal tract of the rat. Nature 190: 826-827.

Andersen, D. H. 1947. Celiac syndrome. VI. The relationship of celiac disease, starch intolerance, and steatorrhea. J. Pediat. 30: 564-582.

Anderson, C. M., K. R. Kerry and R. R. W. Townley. 1965. An inborn defect of intestinal absorption of certain monosaccharides. Arch. Dis. Child. 40: 1-6.

—, M. Messer, R. R. W. Townley and M. Freeman. 1963. Intestinal sucrase and isomaltase deficiency in two siblings. Pediatrics 31: 1003-1010.

Arthur, A. B. 1966. Intestinal disaccharidase deficiency in children with coeliac disease. Arch. Dis. Child. 41: 519-524.

Auricchio, S., A. Dahlqvist, G. Mürset and A. Prader. 1963. Isomaltose intolerance causing decreased ability to utilize dietary starch. J. Pediat. 62: 165-176.

—, — and G. Semenza. 1963. Solubilization of the human intestinal disaccharidases. Biochim. Biophys. Acta 73: 582-587.

—, A. Prader, G. Mürset and G. Witt. 1961. Saccharose-intoleranz. Durchfall in folge hereditären Mangels an intestinaler Saccharaseaktivität. Helv. paediat. Acta 16: 483-505.

—, A. Rubino, and G. Mürset. 1965. Intestinal glycosidase activities in the human embryo, fetus, and newborn. Pediatrics 35: 944-954.

—, —, A. Prader, J. Rey, J. Jos, J. Frézal and M. Davidson. 1965. Intestinal glycosidase activities in congenital malabsorption of disaccharides. J. Pediat. 66: 555-564.

—, —, G. Semenza, M. Landolt and A. Prader. 1963. Isolated intestinal lactase deficiency in the adult. Lancet II: 324-326.

—, G. Semenza and A. Rubino. 1965. Multiplicity of human intestinal disaccharidases. II. Characterization of the individual maltases. Biochim. Biophys. Acta 96: 498-507.

Azar, G. J. and W. L. Bloom. 1963. Similarities of carbohydrate deficiency and fasting. II. Ketones, nonesterified fatty acids, and nitrogen excretion. Arch. Intern. Med. 112: 338-343.

Baar, S., and H. Bickel. 1964. Oligosaccharides in infants' faeces. J. Clin. Path. 17: 273-274.

Badenhuizen, N. P., and R. W. Dutton. 1956. The mechanism of growth of starch granules in the Potato Tuber, studied with carbon-14. Biochem. J. 62: 13P-14P.

Bayless, T. M., and N. S. Rosensweig. 1966. A racial difference in incidence of lactase deficiency. A survey of milk intolerance and lactase deficiency in healthy adult males. J.A.M.A. 197: 968-972.

— and —. 1967. Incidence and implications of lactase deficiency and milk intolerance in white and negro populations. Johns Hopkins Med. J. 121: 54-64.

Beal, V. A. 1953. Nutritional intake of children. J. Nutr. 50: 223-234.

Beazell, J. M. 1941. The reexamination of the role of the stomach in the digestion of carbohydrate and protein. Amer. J. Physiol. 132: 42-50.

Bernard, C. 1877. Leçons sur le diabète et la glycogenèse animale. Paris: Cercle du Livre Précieux, pp. 163-178.

Bihler, I., K. A. Hawkins and R. K. Crane. 1962. Studies on the mechanism of intestinal absorption of sugars. VI. The specificity and other properties of $Na^+$-dependent entrance of sugars into intestinal tissue under anaerobic conditions, in vitro. Biochim. Biophys. Acta 59: 94-102.

Bloom, W. L., and G. J. Azar. 1963. Similarities of carbohydrate deficiency and fasting. I. Weight loss, electrolyte excretion, and fatigue. Arch. Intern. Med. 112: 333-337.

Bonner, J. 1950. Plant Biochemistry. New York, Academic Press Inc., pp. 48-65.

Borgström, B., and A. Dahlqvist. 1958. Cellular localization, solubilization and separation of intestinal glycosidases. Acta Chem. Scand. 12: 1997-2006.

—, —, G. Lundh and J. Sjövall. 1957. Studies of intestinal digestion and absorption in the human. J. Clin. Invest. 36: 1521-1536.

—, B. Lindquist and G. Lundh. 1960. Enzyme concentration and absorption of protein and glucose in duodenum of premature infants. Amer. J. Dis. Child. 99: 338-343.

—, — and —. 1961. Digestive studies in children. Studies under normal and pathological conditions. Amer. J. Dis. Child. 101: 454-466.

Burgess, E. A., B. Levin, D. Mahalanabis and R. E. Tonge. 1964. Hereditary sucrose intolerance: levels of sucrase activity in jejunal mucosa. Arch. Dis. Child. 39: 431-443.

Calloway, D. H. 1960. Nutritional aspects of the all-purpose survival ration. A critical appraisal. U. S. Armed Forces Med. J. 11: 403-417.

Carnie, J. A., and J. W. Porteous. 1963 Rat intestinal invertase. Biochem. J. 89: 100 P.

Cook, G. C. 1967. Lactase activity in newborn and infant Baganda. Brit. Med. J. 1: 527-530.

—and S K. Kajubi. 1966. Tribal incidence of lactase deficiency in Uganda. Lancet I: 725-729.

Crane, R. K. 1960. Studies on the mechanism of the intestinal absorption of sugars. III. Mutual inhibition, in vitro, between some actively transported sugars. Biochim. Biophys. Acta 45: 477-482.

—. 1960a. Intestinal absorption of sugars. Physiol. Rev. 40: 789-825.

—. 1962. Hypothesis for mechanism of intestinal active transport of sugars. Fed. Proc. 21: 891-895.

—. 1964. Uphill outflow of sugar from intestinal epithelial cells induced by reversal of the $Na^+$ gradient: its significance for the mechanism of $Na^+$-dependent active transport. Biochem. Biophys. Res. Commun. 17: 481-485.

—. 1965. $Na^+$ dependent transport in the intestine and other animal tissues. Fed. Proc. 24: 1000-1006.

—, G. Forstner and A. Eichholz. 1965. Studies on the mechanism of the intestinal absorption of sugars. X. An effect of $Na^+$ concentration on the apparent Michaelis constants for intestinal sugar transport, in vitro. Biochim. Biophys. Acta 109: 467-477.

Crosby, W. H., and H. W. Kugler. 1957. Intraluminal biopsy of the small intestine. Amer. J. Dig. Dis. 2: 236-241.

Csáky, T. Z. 1965. Transport through biological membranes. Ann. Rev. Physiol. 27: 415-450.

— and Y. Hara. 1965. Inhibition of active intestinal sugar transport by digitalis. Amer. J. Physiol. 209: 467-472.

— and P. M. Ho. 1965. Intestinal transport of D-xylose. Proc. Soc. Exp. Biol. Med. 120: 403-408.

— and —. 1966. Active transport of D-mannose in the small intestine. Life Sci. 5: 1025-1030.

Cuatrecasas, P., D. H. Lockwood and J. R. Caldwell. 1965. Lactase deficiency in the adult. A common occurrence. Lancet I: 14-18.

Dahlqvist, A. 1961. Intestinal carbohydrases of a new-born pig. Nature 190: 31-32.

—. 1962. Specificitity of the human intestinal disaccharidases and implications for hereditary disaccharide intolerance. J. Clin. Invest. 41: 463-470.

—. 1964. Method for assay of intestinal disaccharidases. Anal. Biochem. 7: 18-25.

—. 1965. Assay of intestinal disaccharidases in normal humans and patients with disaccharidase deficiency. *In* Ruyssen, R. and Vandendriessche L. (Eds.): Enzymes in Clinical Chemistry. Amsterdam; Elsevier Publishing Company, pp. 136-145.

— and N. G. Asp. 1967. Rat small-intestinal β-galactosidases. Influence of pH on the hydrolysis of different substrates. Biochem. J. 103: 86-89.

—, S. Auricchio, G. Semenza and A. Prader. 1963. Human intestinal disaccharidases and hereditary disaccharide intolerance. The hydrolysis of sucrose, isomaltose, palatinose (isomaltulose), and a 1,6-α-oligosaccharide (isomaltooligosaccharide) preparation. J. Clin. Invest. 42: 556-562.

— and B. Borgström. 1961. Digestion and absorption of disaccharides in man. Biochem. J. 81: 411-418.

— and J. D. Gryboski. 1965. Inability of the human small-intestinal lactase to hydrolyze lactulose. Biochim. Biophys. Acta 110: 635-636.

—, J. B. Hammond, R. K. Crane, J. V. Dunphy and A. Littman. 1963. Intestinal lactase deficiency and lactose intolerance in adults. Gastroenterology 45: 488-491.

— and T. Lindberg. 1966. Development of the intestinal disaccharidase and alkaline phosphatase activities in the human foetus. Clin. Sci. 30: 517-528.

— and C. Nordström. 1966. The distribution of disaccharidase activities in the villi and crypts of the small-intestinal mucosa. Biochim. Biophys. Acta 113: 624-626.

Davidson, M. 1967. Disaccharide intolerance. Pediat. Clin. N. Amer. 14: 93-107.

— and C. H. Bauer. 1958. The value of microscopic examination of the stool for extracellular starch in the diagnosis of starch intolerance. Pediatrics 21: 565-572.

Dicke, W. K., H. A. Weijers and J. H. van de Kamer. 1953. Coeliac disease: II. The presence in wheat of a factor having a deleterious effect in cases of coeliac disease. Acta Paediat. Scand. 42: 34-42.

Doell, R. G., and N. Kretchmer. 1962. Studies of small intestine during development. I. Distribution and activity of β-galactosidase. Biochim. Biophys. Acta 62: 353-362.

— and —. 1964. Intestinal invertase: precocious development of activity after injection of hydrocortisone. Science 143: 42-44.

—, G. Rosen, and N. Kretchmer. 1965. Immunochemical studies of intestinal disaccharidases during normal and precocious development. Proc. Nat. Acad. Sci. USA. 54: 1268-1273.

Dreiling, D. A., and H. D. Janowitz. 1962. The measurement of pancreatic secretory function. *In* A. V. S. De Reuck, and M. P. Cameron, (Eds.): Ciba Foundation Symposium on the Exocrine Pancreas. 1961. London. London, J. and A. Churchill Ltd., pp. 225-258.

Dubois, R., H. Loeb, E. Eggermont and Mainguet. 1966. Etude clinique et biochimique d'un cas de malabsorption congénitale du glucose et du galactose. Helv. Paediat. Acta 21: 577-587.

Dunphy, J. V., A. Littman, J. B. Hammond, G. Forstner, A. Dahlqvist and R. K. Crane. 1965. Intestinal lactase deficit in adults. Gastroenterology 49: 12-21

Durand, P. 1959. Lactosurie et saccharosurie. Moderne Probleme der Pädiatre 4: 496-502.

—. 1964. Lactose intolerance. *In* Durand, P. (Ed): Disorders due to intestinal defective carbohydrate digestion and absorption. Roma, Il Pensiero Scientifico, pp. 105-140.

— and G. M. Lamedica. 1962. Disaccharide intolerance. Helv. Paediat. Acta 17: 395.

—, A. M. Martino and G. M. Lamedica. 1961. Diagnosis of carbohydrate-intolerance diarrhoeas by stool chromatography. Lancet II: 374.

Eggermont, E. 1966. Enzymic activities in meconium from human foetuses and newborns. Biol. Neonat. 10: 266-280.

—. 1968a. The glucoamylase of the small intestinal mucosa in man. In preparation.

—. 1968b. The intestinal α-glucosidases of normal human subjects and of patients with saccharose intolerance. II. Sedimentation properties of the enzymes solubilised with papain. In preparation.

— and H. G. Hers. 1968. The intestinal α-glucosidases of normal human subjects and of patients with saccharose intolerance. I. Sedimentation properties of the enzymes solubilised with Triton X-100. In preparation.

— and H. Loeb. 1966. Glucose-galactose intolerance. Lancet II: 343-344.

Eichholz, A., and R. K. Crane. 1965. Studies on the organization of the brush border in intestinal epithelial cells. I. Tris disruption of isolated hamster brush borders and density gradient separation of fractions. J. Cell Biol. 26: 687-691.

Farber, S., H. Shwachman and C. L. Maddock. 1943. Pancreatic function and disease in early life. I. Pancreatic enzyme activity and the celiac syndrome. J. Clin. Invest. 22: 827-838.

Ferguson, A., and J. D. Maxwell. 1967. Genetic aetiology of lactose intolerance. Lancet II: 188-190.

Figarella, C. 1966. Composition et stabilité de l'équipement enzymatique du pancreas de l'homme et de divers animaux. Bull. Soc. Chim. Biol. (Paris) 48: 97-115.

Fomon, S. J. 1967. Infant Nutrition. Philadelphia and London, W. B. Saunders Company, 299 p.

Ford, J. D., and J. C. Haworth. 1963 The fecal excretion of sugars in children. J. Pediat. 63: 988-990.

François, R., Frédérich, E. Vincens-Calvet, M. Bertrand and Ruitton-Ugliengo. 1963. Intolérance isolée au saccharose. Pédiatrie 18: 563-579.

Ginsburg, V., and H. G. Hers. 1960. On the conversion of fructose to glucose by guinea pig intestine. Biochim. Biophys. Acta 38: 427-434.

Girardet, P. 1965. Absorption et Malabsorption du Lactose. Bern and Stuttgart, H. Huber, 98 p.

Gitzelmann, R., and S. Auricchio. 1965. The handling of soya alpha-galactosides by a normal and galactosemic child. Pediatrics 36: 231-235.

—, E. A. Davidson and J. Osinchak. 1964. Disaccharidase of rabbit small intestine: intracellular distribution, solubilization, purification and specificity. Biochim. Biophys. Acta 85: 69-81.

Greaves, J. P., and D. F. Hollingsworth. 1964. Changes in the pattern of carbohydrate consumption in Britain. Proc. Nutr. Soc. 23: 136-143.

Greenwood, C. T. 1956. Aspects of the physical chemistry of starch. Advances Carbohyd. Chem. 11: 335-393.

Groen, J. 1937. The absorption of hexoses from the upper part of the small intestine in man. J. Clin. Invest. 16: 245-255.

Grossman, M. I. 1962. Nervous and hormonal regulation of pancreatic secretion. *In* A. V. S. De Reuck, and M. P. Cameron (Eds.): Ciba Foundation Symposium on the Exocrine Pancreas. 1961. London. London: J. and A. Churchill Ltd., pp. 208-224.

Gryboski, J. D., W. R. Thayer, W. A. Gryboski, I. W. Gabrielson and H. M. Spiro. 1963. A defect in disaccharide metabolism after gastrojejunostomy. New Eng. J. Med. 268: 78-80.

—, J. Zillis and O. H. Ma. 1964. A study of fecal sugars by high voltage electrophoresis. Gastroenterology 47: 26-31.

Gschwind, R. 1950. Das Verhalten der Pankreasenzyme bei Frühgeburten. Ann. Paediat. 175: 169-184.

Haemmerli, U. P., H. Kistler, R. Ammann, T. Marthaler, G. Semenza, S. Auricchio and A. Prader. 1965. Acquired milk intolerance in the adult caused by lactose malabsorption due to a selective deficiency of intestinal lactase activity. Amer. J. Med. 38: 7-30.

Hardinge, M. G., J. B. Swarner and H. Crooks. 1965. Carbohydrates in Foods. J. Amer. Diet Ass. 46: 197-204.

Harper, A. A., E. L. Blair, and T. Scratcherd. 1962. The distribution and physiological properties of pancreozymin. *In* A. V. S. De Reuck, and M. M. Cameron (Eds.): Ciba Foundation Symposium on the Exocrine Pancreas. 1961. London. London: J. and A. Churchill Ltd., pp. 168-185.

Hijmans, J. C. and K. S. McCarty. 1966. Induction of invertase activity by hydrocortisone in chick embryo duodenum cultures. Proc. Soc. Exp. Biol. Med. 123: 633-637.

Holdsworth, C. D., and A. M. Dawson. 1964. The absorption of monosaccharides in man. Clin. Sci. 27: 371-379.

— and —. 1965. Absorption of fructose in man. Proc. Soc. Exp. Biol. Med. 118: 142-145.

Holzel, A. 1965. Development of intestinal enzyme systems, and its relation to diarrhoea. Pediat. Clin. N. Amer. 12: 635-654.

—. 1967. Sugar malabsorption due to deficiencies of disaccharidase activities and of monosaccharide transport. Arch. Dis. Child. 42: 341-352.

—, V. Schwarz and K. W. Sutcliffe. 1959. Defective lactose absorption causing malnutrition in infancy. Lancet I: 1126-1128.

Hooft, C., J. Van Hauwaert, P. De Laey and K. Adriaenssens. 1963. Malabsorption after total gastrectomy in childhood. Helv. Paediat. Acta 18: 502-517.

Huang, S. S., and T. M. Bayless. 1967. Lactose intolerance in healthy children. New Eng. J. Med. 276: 1283-1287.

Hübscher, G., G. R. West and D. N. Brindley. 1965. Studies on the fractionation of mucosal homogenates from the small intestine. Biochem. J. 97: 629-642.

Huggett, A. St. G., and D. A. Nixon. 1957. Enzymic determination of blood glucose. Biochem. J. 66: 12 P.

Hsia D. Y. Y. 1966. The development of intestinal β-galactosidase and β-glucuronidase in the newborn. Biochim. Biophys. Acta 122: 550-553.

—, H. B. Hadorn, M. Makler, A. Prader, G. Semenza and G. Zoppi. 1966. β-galactosidase activity in human intestinal mucosa. J. Pediat. 68: 853-854.

—, M. Makler, G. Semenza and A. Prader. 1966. β-galactosidase activity in human intestinal lactases. Biochim. Biophys. Acta /113: 390-393.

Janowitz, H. D., and D. A. Dreiling. 1962. The pancreatic secretion of fluid and electrolytes. *In* A. V. S. De Reuck, and M. P. Cameron (Eds.): Ciba Foundations Symposium on the Exocrine Pancreas. 1961. London. London, J. and A. Churchill Ltd., pp. 115-137.

Jeans, P. C., and Mck. W. Marriott. 1947. Infant Nutrition (ed. 4). St. Louis, The C. V. Mosby Company, 516 p.

Jesuitova, N. N., P. E. De Laey and A. M. Ugolev. 1964. Digestion of starch in vivo and in vitro in a rat intestine. Biochim. Biophys. Acta 86: 205-210.

Johnson, C. F. 1967. Disaccharidase: localization in hamster intestine brush borders. Science 155: 1670-1672.

Jokl, E. 1964. Nutrition, Exercise and Body Composition. Springfield, Ill, Charles C Thomas.

Jos, J., J. Frézal, J. Rey and M. Lamy. 1967. Histochemical localization of intestinal disaccharidases: application to peroral biopsy specimens. Nature 213: 516-518.

Kaufmann, N. A., R. Poznanski, S. H. Blondheim and Y. Stein. 1966a. Effect of fructose, glucose, sucrose and starch on serum lipids in carbohydrate induced hypertriglyceridemia and in normal subjects. Israel J. Med. Sci. 2: 715-726.

—, —, — and —. 1966b. Changes in serum lipid levels of hyperlipemic patients following the feeding of starch, sucrose and glucose. Amer. J. Clin. Nutr. 18: 261-269.

Kern, F., and J. E. Struthers. 1966. Intestinal lactase deficiency and lactose intolerance in adults. J.A.M.A 195: 927-930

Kerry, K R., and C. M. Anderson. 1964. A ward test for sugar in faeces. Lancet I: 981-982.

— and R. R. W. Townley. 1965. Genetic aspects of intestinal sucrase-isomaltase deficiency. Aust. Paediat. J. 1: 223-235.

Kiyasu, J. Y., and I. L. Chaikoff. 1957. On the manner of transport of absorbed fructose. J. Biol. Chem. 224: 935-939.

Kjölberg, O., and D. J. Manners. 1962. The fine-structure of amylose. Biochem. J. 84: 50P.

Klotz, A. P., and M. C. Lubos. 1967. Gastrointestinal symptoms and intestinal lactase deficiencies. A word of caution. Amer. J. Dig. Dis. 12: 421-423.

Koldovský, O., A. Heringová, J. Hošková, V. Jirsová, R. Noack, M. Friedrich and G. Schenck. 1965/66. The postnatal development of enzyme activities of the small intestine. Biol. Neonat. 9: 33-43.

Kolínská, J. and G. Semenza. 1967. Studies on intestinal sucrase and on intestinal

sugar transport. V. Isolation and properties of sucrase-isomaltase from rabbit small intestine. Biochim. Biophys. Acta 146: 181-195.

Kretchmer, N., and P. Sunshine. 1967. Intestinal disaccharidase deficiency in the sea lion. Gastroenterology 53: 123-129.

Kulka, R. G., and D. Duksin. 1964. Patterns of growth and α-amylase activity in the developing chick pancreas. Biochim. Biophys. Acta 91: 506-514.

Laplane, R., C. Polonovski, M. Etienne, P. Debray, J. C. Lods and B. Pissarro. 1962. L' intolérance aux sucres à transfert intestinal actif. Ses rapports avec l'intolérance au lactose et le syndrome coeliaque. Arch. Franc. Pédiat 19: 895-944.

Larner, J., and R. E. Gillespie. 1956. Gastrointestinal digestion of starch. II. Properties of the intestinal carbohydrases. J. Biol. Chem. 223: 709-726.

Launiala, K., P. Kuitunen and J. K. Visakorpi. 1966. Disaccharidases and histology of duodenal mucosa in congenital lactose malabsorption. Acta Paediat. Scand. 55: 257-263.

—, J. Perheentupa, J. Visakorpi and N. Hallman. 1964. Disaccharidases of intestinal mucosa in a patient with sucrose intolerance. Pediatrics 34: 615-620.

Lifshitz, F. (1966). Congenital lactase deficiency. J. Pediat. 69: 229-237.

Lindemann, B., and A. K. Solomon. 1962. Permeability of luminal surface of intestinal mucosal cells. J. Gen. Physiol. 45: 801-810.

Lindquist, B. 1965. Blockage of Active Transport of glucose. XI. International Congress of Pediatrics. Group Panel Discussions. 1965. Tokyo. Tokyo: University of Tokyo Press, pp. 85-93.

—. 1967. Symposium on intestinal absorption and malabsorption. 1967. Zürich.

— and G. W. Meeuwisse. 1962. Chronic diarrhoea caused by monosaccharide malabsorption. Acta Paediat. Scand. 51: 674-685.

—, —, and K. Melin. 1963. Osmotic diarrhoea in genetically transmitted glucose-galactose malabsorption. Acta Paediat. Scand. 52: 217-219.

— and —. 1963. Intestinal transport of monosaccharides in generalized and selective malabsorption. Acta Paediat. Scand. suppl. 146: 110-115.

Linneweh, F., E. Schaumlöffel and W. Barthelmai. 1965. Angeborene Glucose– und Galaktose– Malabsorption. Klin. Wschr. 43: 405-409.

Lojda, Z. 1965. Some remarks concerning the histochemical detection of disaccharidases and glucosidases. Histochemie 5: 339-360.

Long, C. L., J. W. Geiger and J. M. Kinney. 1967. Absorption of glucose from the colon and rectum. Metabolism 16: 413-418.

Lowe, C. U. and C. D. May 1951. Selective pancreatic deficiency. Absent amylase, diminished trypsin, and normal lipase. Amer. J. Dis. Child. 82: 459-464.

Marks, J. F., J. B. Norton and J. S. Fordtran. 1966. Glucose-galactose malabsorption. J. Pediat. 69: 225-228.

Martin du Pan, R. et F. Infante. 1963. Diarrhée chronique par insuffisance d'amylase pancréatique. Rapport du XIXe congrès de l'association des pédiatres de langue française, 1963. Paris. Paris: Expansion Scientifique Française, 4, pp. 130-133.

McDougal, Jr. D. B., K. D. Little and R. K. Crane. 1960. Studies on the mechanism of intestinal absorption of sugars. IV. Localization of galactose concentrations within the intestinal wall during active transport, in vitro. Biochim. Biophys. Acta 45: 483-489.

McMichael, H. B., J. Webb and A. M. Dawson. 1965. Lactase deficiency in adults. A cause of "functional" diarrhoea. Lancet I: 717-720.

—, — and —. 1966. Jejunal disaccharidases and some observations on the cause of lactase deficiency. Brit. Med. J. 2: 1037-1041.

Meeuwisse, G., and A. Dahlqvist. 1966. Glucose-galactose malabsorption. Lancet II: 858.

Messer, M., and A. Dahlqvist. 1966. A one-step ultramicro method for the assay of intestinal disaccharidases. Anal. Biochem. 14: 376-392.

— and K. R. Kerry. 1967. Intestinal digestion of maltotriose in man. Biochim. Biophys. Acta 132: 432-443.

Miller, D., and R. K. Crane. 1961. The digestive function of the epithelium of the small intestine. II. Localization of disaccharide hydrolysis in the isolated brush border portion of intestinal epithelial cells. Biochim. Biophys. Acta 52: 293-298.

Mitchell, P. 1967. Translocations through natural membranes. Advances Enzym. 29: 33-87.

Mulder, T. 1964. De voeding in Nederland. Voeding 25: 604-608.

Neale, G., M. Clark and B. Levin. 1965. Intestinal sucrase deficiency presenting as sucrose intolerance in adult life. Brit. Med. J. 2: 1223-1225.

Neven, M., and A. Fouassin. 1962. Méthode de dosage de l'amidon dans les matières fécales. Nutr. Dieta (Basel) 4: 241-250.

Newcomer, A. D., and D. B. McGill. 1966. Distribution of disaccharidase activity in the small bowel of normal and lactase-deficient subjects. Gastroenterology 51: 481-488.

Newey, H. 1967. Absorption of carbohydrates. Brit. Med. Bull. 23: 236-240.

Nikuni, Z. 1957. Fine structure of starch granules. Studies with electron microscope and X-ray diffractometer. Chem. Absts. 51: 17210 i.

Nørby, S. 1964. Electrophoretic non-identity of human salivary and pancreatic amylases. Exp. Cell Res. 36: 663-702.

Nutr. Rev. 1963. Digestibility of high-amylose corn starches. 21: 27-28.

Öckerman, P. A. 1965. Glucose-6-phosphatase in human jejunal mucosa. Properties demonstrating the specific character of the enzyme activity. Biochim. Biophys. Acta 105: 22-33.

— and H. Lundborg. 1965. Conversion of fructose to glucose by human jejunum. Absence of galactose-to-glucose conversion. Biochim. Biophys. Acta 105: 34-42

Opit, L. J., and J. S. Charnock. 1965. A molecular model for a sodium pump. Nature 208: 471-474.

Palla, J. C., A. Ben Abdeljlil and P. Desnuelle. 1967. Comparative study of the control of amylase biosynthesis in rat pancreas and parotid glands. Biochim. Biophys. Acta 136: 563-565.

Plotkin, G. R., and K. J. Isselbacher. 1964. Secondary disaccharidase deficiency in adult celiac disease (nontropical sprue) and other malabsorption states. New Eng. J. Med. 271: 1033-1037.

Prader A., and S. Auricchio. 1965. Defects of intestinal disaccharide absorption. Ann. Rev. Med. 16: 345-358.

Preshaw, R. M., A. R. Cooke and M. I. Grossman. 1965. Pancreatic secretion induced by stimulation of the pyloric gland area of the stomach. Science 148: 1347-1348.

Reboud, J. P., G. Marchis-Mouren, L. Paséro, A. Cozzone and P. Desnuelle. 1966. Adaptation de la vitesse de biosynthèse de l'amylase pancréatique et du chymotrypsinogène à des régimes riches en amidon ou en protéines. Biochim. Biophys. Acta 117: 351-367.

Rey, J., and J. Frézal. 1967. Les anomalies des dissacharidases. Arch. Franc. Pédiat 24: 65-101.

—, —, J. Jos, P. Bauche and M. Lamy. 1963. Diarrhée par trouble de l'hydrolyse intestinale du saccharose, du maltose et de l'isomaltose. Arch. Franc. Pédiat. 20: 381-400.

Riklis, E., and J. H. Quastel. 1958. Effects of cations on sugar absorption by isolated surviving guinea pig intestine. Canad. J. Biochem. Physiol. 36: 347-362.

Roberts, P. J. P., and W. J. Whelan. 1960. The mechanism of carbohydrase action, V. Action of human salivary α-amylase on amylopectin and glycogen. Biochem. J. 76: 246-253.

Robinson, J. W. L. 1966. Les relations entre les ions de sodium et l'absorption intestinale d'acides aminés. Biochim. Biophys. Acta 126: 61-72.

Rutter, W. J., and C. S. Weber. 1965. Specific proteins in cytodifferentiation. *In* Developmental and Metabolic Control Mechanisms and Neoplasia. Baltimore, Williams and Wilkins Co., pp. 195-218.

Salomon, L. L., J. A. Allums, and D. E. Smith. 1961. Possible carrier mechanism for the intestinal transport of D-xylose. Biochem. Biophys. Res. Com. 4: 123-126.

Sarles, H., C. Figarella, G. Prezelin and C. Souville. 1966. Comportement différent de la lipase, de l'amylase et des enzymes protéolytiques pancréatiques après différents modes d'excitation du pancréas humain. Bull. Soc. Chim. Biol. (Paris) 48: 951-957.

Schachman, H. K. 1963. Considerations on the tertiary structure of proteins. Cold Spring Harbor Symp. Quant. Biol. 28: 409-430.

Schneider, A. J., W. B. Kinter and C. E. Stirling. 1966. Glucose-galactose malabsorption. Report of a case with autoradiographic studies of a mucosal biopsy. New Eng. J. Med. 274, 6: 305-312.

Schultz, S. G., and A. K. Solomon. 1961. Determination of the effective hydrodynamic radii of small molecules by viscometry. J. Gen. Physiol. 44: 1189-1199.

— and R. Zalusky. 1964. Ion transport in isolated rabbit ileum. II. The interaction between active sodium and active sugar transport. J. Gen. Physiol. 47: 1043-1059.

Scriver, C. R. 1967. Symposium on intestinal absorption and malabsorption. 1967. Zürich.

Semenza, G., and S. Auricchio. 1962. Chromatographic separation of human intestinal disaccharidases. Biochim. Biophys. Acta 65: 172-175.

—, — and A. Rubino. 1965. Multiplicity of human intestinal disaccharidases. I. Chromatographic separation of maltases and two lactases. Biochim. Biophys. Acta 96: 487-497.

—, —, —, A. Prader and J. D. Welsh. 1965. Lack of some intestinal maltases in a human disease transmitted by a single genetic factor. Biochim. Biophys. Acta 105: 386-389.

—, R. Tosi, M. C. Vallotton-Delachaux and E. Mülhaupt. 1964. Sodium activation of human intestinal sucrase and its possible significance in the enzymic organization of brush borders. Biochim. Biophys. Acta 89: 109-116.

Sheehy, T. W., and P. R. Anderson. 1965. Disaccharidase activity in normal and diseased small bowel. Lancet II: 1-5.

Shiner, M. 1956. Duodenal biopsy. Lancet I: 17-19.

Shmerling, D. H., S. Auricchio, A. Rubino, B. Hadorn and A. Prader. 1964. Der sekundäre Mangel an intestinaler Disaccharidase-aktivität bei der Cöliakie. Helv. Paediat. Acta 19: 507-527.

Skou, J. C. 1965. Enzymatic basis for active transport of $Na^+$ and $K^+$ across cell membrane. Physiol. Rev. 45: 596-617.

Sonntag, W. M., M. L. Brill, W. G. Troyer, J. D. Welsh, G. Semenza and A. Prader. 1964. Sucrose-isomaltose malabsorption in an adult woman. Gastroenterology 47: 18-25.

Sunshine, P., and N. Kretchmer. 1964. Intestinal disaccharidases: absence in two species of sea lions. Science 144: 850-851.

Swaminathan, N., and A. N. Radhakrishnan. 1965. Distribution of α-glucosidase activities in human and monkey small intestine. Indian J. Biochem. 2: 159-163.

Tabaqchali, S., and C. C. Booth. 1967. Relationship of the intestinal bacterial flora to absorption. Brit. Med. Bull. 23: 285-290.

Thier, S. 1967. Unpublished results.

Thomas, J. E. 1964. Mechanisms of action of pancreatic stimuli studied by means of atropinelike drugs. Amer. J. Physiol. 206: 124-128.

Townley, R. R. W., K. T. Khaw and H. Shwachman. 1965. Quantitative assay of disaccharidase activities of small intestinal mucosal biopsy specimens in infancy and childhood. Pediatrics 36: 911-921.

Ugolev, A. M. 1965. Membrane (Contact) Digestion. Physiol. Rev. 45: 555-595.

Walker, G. J., and P. M. Hope. 1963. The action of some α-amylases on starch granules. Biochem. J. 86: 452-462.

— and W. J. Whelan. 1960. The mechanism of carbohydrase action. 7. Stages in the salivary α-amylolysis of amylose, amylopectin and glycogen. Biochem. J. 76: 257-263.

Wang, C. C., and M. I. Grossman. 1951. Physiological determination of release of secretin and pancreozymin from intestine of dogs with transplanted pancreas. Amer. J. Physiol. 164: 527-545.

Warren, R., W. G. Karr, O. D. Hoffman and W. O. Abbott. 1940. Incubation studies of the human small intestine. 15. The absorption and expulsion of glucose from the stomach. Amer. J. Med. Sci. 200: 639-648.

Weijers, H. A. and J. H. van de Kamer. 1962. Diarrhoea caused by deficiency of sugar-splitting enzymes. II. Acta Paediat. Scand. 51: 371-374.

— and —. 1963. Aetiology and diagnosis of fermentative diarrhoeas. Acta Paediat. Scand. 52: 329-337.

— and —. 1964. Fermentative diarrhoeas. *In* Durand, P. (Ed.): Disorders due to intestinal defective carbohydrate digestion and absorption. Roma: Il Pensiero Scientifico, pp. 57-104.

—, —, W. K. Dicke and J. Ijsseling. 1961. Diarrhoea caused by deficiency of sugar splitting enzymes. Acta Paediat. Scand. 50: 55-71.

Welsh, J. D., and R. C. Brown. 1966. Sucrase-Palatinase deficiency. Lancet II: 342-343.

White, L. W., and B. R. Landau. 1965. Sugar transport and fructose metabolism in human intestine in vitro. J. Clin. Invest. 44: 1200-1213.

Wilson, T. H. 1962. Intestinal Absorption. Philadelphia and London, W. P. Saunders Company. 263 p.

— and T. N. Vincent. 1955. Absorption of sugars in vitro by the intestine of the golden hamster. J. Biol. Chem. 216: 851-866.

Wiseman, G. 1964. Absorption from the Intestine. London and New York, Academic Press, 564 p.

Wohl, M. G., and R. S. Goodhart. 1955. Modern Nutrition in Health and Disease. London, Henry Kimpton, p. 144.

Zieve, L., S. E. Silvis, B. Mulford and W. D. Blackwood. 1966. Secretion of pancreatic enzymes. I. Response to secretin and pancreozymin. Amer. J. Dig. Dis. 11: 671-684.

# Author Index


Abbott, W. O., 264
Abraham, J. M., 266, 268
Abrami, P., 145
Acheson, E. D., 74
Achord, J. C., 79
Ackerman, B., 215
Adams, M. S., 31, 109, 135
Adriaenssens, K., 263
Allums, J. A., 265
Alvarado, F., 265 266
Aggeler, P. M., 179
Aisenberg, A. G., 208
Al-Aish, M. S., 28
Albertini, R. J., 213, 229
Albright, F., 2
Alepa, F. P., 188
Alexander, B., 189
Alexander, D. S., 4, 11, 15, 16
Alfieri, A., 23
Allen, S. L., 205
Almy, T. P., 74, 75
Althoff, W., 72
Alvarez, A., 248
Ames, B. N., 170
Amos, D. B., 203, 205, 208, 213, 215, 217, 223, 231, 232
Andersen, D. H., 257, 258, 266
Andersen, H. J., 102, 104
Anderson, C. M., 256, 258, 266, 268
Anderson, H., 8, 10
Anderson, J. R., 133, 140
Andrade, D., 28
Andreani, D., 98
Angle, C. R., 35
Antonius, I. I., 5
Arakaki, D. T., 38
Arapakis, G., 143
Ardeman, S., 134, 137
Arfors, K. E., 69
Armendares, S. L., 15
Armstrong, M. D., 191
Arneil, G. C., 108
Arthur, A. B., 247
Ascher, K. W., 97
Ashford, P. W., 21
Asp, N. G., 249
Aster, R. A., 101
Atkin, N. B., 39
Atkins, L. M., 21
Auerbach, V. H., 187, 191
Aula, P., 21, 22
Aurichio, S. A., 176, 245, 246, 247, 249, 251, 253, 257, 258, 259, 262, 263
Ausannaire, 36
Austen, K. F., 142
Azar, G. I., 241
Azevedo, E., 102

Baar, S., 256
Bach, F. H., 201, 208, 209, 210, 213, 219, 223, 229, 231, 235
Bachmann, K. D., 35
Back, F. P., 24, 43
Bahner, F. G., 11, 12
Badenhuizen, N. P., 242
Baglioni, C., 182, 183
Baguley, B. C., 170, 172, 192
Baikie, A. G., 6, 7, 8, 16, 41, 43, 44
Bailey, D., 79
Bain, A. D., 30
Ball, P. A. J., 67, 68
Ballon, C., 39
Balner, H., 217
Bamater, F., 141
Bamford, K. F., 69
Barclay, D. L., 17
Barker, W. F., 74
Barr, M. L., 1, 2, 3, 4, 5, 8, 9, 17
Bartel, K., 35
Bartels, E. D., 105, 106, 128, 129, 131
Barter, R. H., 37, 38
Barthelmai, W., 249, 266, 267
Bartholomew, L. G., 80
Baschieri, L., 98
Bashore, R. A., 17
Bateman, A. J., 33
Battin, J. J., 10
Bauche, P., 257
Bauer, C. H., 257
Bauman, P., 24
Bayless, T. M., 263
Baylet, R., 39
Beal, V. A., 241
Beall, G. N., 105
Bearn, A. G., 21, 44, 172, 173, 174
Beazell, J. M., 244
Becak, W. M., 21, 28
Beckers, C., 98
Beckman, L., 69
Begrand, L., 217
Beierwalter, W. H., 101, 108
Beischer, N. A., 39
Ben Abdelilil, A., 245
Benedict, J. D., 179
Benirschke, K., 38
Benson, J. T., 102
Benson, P. F., 10
Bent-Hansen, K., 145
Berg, J. M., 18
Bergada, C., 16
Bergemann, E., 2, 9
Berger, R., 28, 29, 30, 36
Bernard, C., 245
Bertrand, J., 10
Bertrand, M., 261
Bessis, M., 174
Bessman, S. P., 191
Betke, K., 182
Bianco, I., 181
Bias, W. B., 31
Bickel, H., 191, 256
Bielschowsky, M., 147
Biesele, J. J., 21
Biggs, J. G. S., 15
Bihler, I., 265
Billingham, R. E., 23
Binder, V. E., 74
Binopoulos, D., 98
Biro, C. E., 44
Bishop, P. M. F., 9
Bitan, A., 10, 11
Blaese, M., 231
Blair, E. L., 244
Blake, M. N., 44
Blank, C. E., 4, 5, 6, 7
Blau, J. N., 28
Blizzard, R. M., 102, 140, 141, 142, 143
Blömback, B., 188
Blondheim, S. H., 242
Bloom, G. E., 31, 43
Bloom, W. L., 241
Bock, A. M., 170, 172, 192
Bochkov, N. P., 2, 4
Boczkowski, K., 16, 17
Bodfish, R. E., 108
Bodmer, W., 216
Boeswillwald, M., 28
Bogaert, L. van, 192
Boley, S. J., 82
Bond, J., 2, 4, 7, 10
Bonnette, J., 29
Bonnier, G., 16
Bongiovanni, A. M., 132
Booth, C. C., 256
Borgfors, N., 107
Börgstrum, B., 242, 244, 245, 246
Botella, Llusia J., 16
Bottura, C., 44
Boué, J. G., 38
Bourgoin, P., 39
Bowen, P., 34, 38, 43
Boyd, E., 8, 13
Bover, S. H., 182
Boyle, J. A., 101, 139
Boylen, J. B., 191
Braend, M., 109
Brancati, C., 182
Brandt, N. J., 185
Brandt, N. V., 21
Breg, W. R., 21, 22, 29, 30, 37
Brent, L., 208
Bréziu, C., 208
Bridges, J. M., 208
Brigas, J. H., 9, 18
Brill, M. L., 259
Brindley, D. N., 246
Brissaud, 36
Brittain, R. P., 6
Brodian, M., 71, 73
Brodie, H. R., 26
Brody, J., 9
Brown, I. H., 71
Brown, R. C., 261
Brown, C. D., 21
Brogger, A., 17, 35
Bruhl, H., 4
Brulé, M., 145
Brunning, J. W., 217
Brunton, M., 6
Bryans, A. M., 4
Buchanan, W. W., 124
Büchner, Th., 35
Bühler, E., 34, 35
Buckton, K. E., 6, 21, 37, 41, 43, 44
Buentello, L., 15
Bunin, I., 144
Burch, P. R. J., 103, 104, 105, 106, 107, 133, 148
Burgess, E. A., 259
Burke, G., 128
Burket, 102
Burnet, F. M., 117, 121, 133, 147, 148, 149
Burwell, R. G., 104, 105, 107
Byrd, J. R., 16, 17

Cahill, G. F., 187
Calabresi, F., 22
Calkins, E., 107
Callender, J. T., 135
Calloway, D. H., 241
Calvo, R., 145
Cameron, A. H., 25, 26
Campbell, J., 8
Canlorbe, P., 30
Cant, J. S., 29
Carballo, J. D., 78
Carbone, P. P., 43
Carmel, R., 134
Carnevalle-Lopez, A., 2, 13
Carnie, J. A., 249
Carpenter, C. C. J., 141, 143
Carr, E. A., 101, 108
Carr, D. H., 1, 3, 4, 5, 8, 10, 12, 13, 17, 21, 23, 24, 26, 27, 31, 37, 38, 39, 40, 153
Carrell, R. E., 31
Caskey, C. T., 180
Carter, C. O., 21, 63, 71, 73
Case, M. P., 43
Casey, M. D., 4, 5, 6, 7, 34
Cassano, C., 98
Castleman, B., 143
Celano, M. J., 145
Cepellini, R., 207, 208, 210, 216, 217, 218, 222, 223
Chaikoff, I. L., 264
Chamarin, I., 134
Chandler, R. W., 102
Charles, D., 37
Charnock, J. S., 266
Chauffard, A., 145
Chaundhry, A. P., 78
Chee, D., 140, 141, 142, 143, 144
Chew, A. R., 104, 105
Chiat, H., 82
Childs, B., 102
Chiorboli, E., 9
Chirigos, M. A., 192
Christian, C. D., 80
Clark, M., 259
Clarke, C. A., 64, 67, 97
Clarke, C. M., 18, 23
Clavero-Nunez, J. A., 16
Clement, W. E., 95
Clendenin, J. M., 38
Cleveland, W. W., 16
Climenko, H., 126
Cline, A. L., 170, 172, 192
Close, H. G., 6, 7, 8

Cockburh, K. G., 15
Coghill, N. F., 139
Cohen, M. M., 15, 17 30, 34, 43, 44
Cohen, R. J., 145
Cole, R. K., 126, 127
Coleman, D. L. 192
Colover, J., 140
Colombani, J., 217
Columbi, A., 207
Comar, D., 94
Conley, C. L., 182
Conney, A. M., 187
Cunnings, D. E., 182
Conen, P. E., 4, 11, 15, 25, 27, 43
Cook, G. C., 263
Cooke, A. A., 245
Cooper, M. D., 118
Corcoran, P. A., 31
Cordova, M. S., 145
Cori, G. T., 187
Corney, G., 11
Cornu, P., 189
Costra, A., 98
Cotter, L. H., 4
Cottino, F., 98
Cotton, D. W. K., 176
Cotton, J. B., 31
Court Brown, W. M., 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 16, 31, 37, 41, 43, 44
Cozzone, A., 245
Craig, P. E., 123
Crane, R. K., 246
Crawford, J. D., 126
Creasman, W. T., 37
Crohn, B. B., 75
Crombrughe, B. de, 98
Crome, L., 192
Crooks, H., 242
Crosby, W. A., 246
Cross, H. E., 102
Cross, W. M., 25, 26
Crow, J. F., 220
Csáky, T. L., 265, 266
Curling, J. B., 99
Currie, A. R., 95
Curtoni, E. S., 207, 208, 217

Dacie, J. V., 145
Dagg, J. H., 139
Dahlqvist, A., 176, 242, 244, 245, 246, 247, 248, 249, 251, 267
Dahnke, G. S., 43
Daikos, D. K., 99
Dallaire, L., 22. 26, 151, 152, 153
Damashek, W., 145
Darnell, A., 31
Dauset, J., 210. 215, 216, 217, 221, 223
Davidson, M., 246, 257
Davidson, R., 101
Davidson, R. G., 191
Davies, P., 33
Davis, W., 140, 141, 142, 143
Dawson, A. M., 257, 264
Day, E., 213
Day, R. W., 8, 9, 18, 21, 24, 104, 152
de Almeida J. C., 9
Debray, P., 256, 266
Decourt, L., 9
Defrenne, R., 10
DeGroot, L. J., 96, 123
de la Chapelle, A., 5, 6, 13, 14
de la Cruz, F., 28
Delachaux, 266
De Laey, P., 245, 263
Delhanty, J. D. A., 18, 21
Del Porto, F., 22
Del Porto Mercuri, A., 22, 23
de Mayo, A. P., 21
Denborough, M. A., 135
Desnuelle, P., 245
Després, J. P., 81
Destine, M. L., 30
Dewhurst, J., 16, 17
De Yissher, M., 98
Di George, A. M., 30, 34, 187
Dutrillaux, B., 30
Diamond, L. K., 183
Diamond, M. T., 122
Dicke, W. K., 256, 257
Dingle, P. R., 151
Djanian, A. Y., 144
Dobbs, C. E., 145
Dodson, V. N., 101
Doebler, T. K., 121
Doell, R. G., 64
Domiach, D., 103, 105, 106, 119, 120, 123, 124, 126, 127, 130, 131, 132, 133, 134, 137, 138, 139, 144, 147, 151, 152, 155
Dorfman, I. A., 106
Dormer, P., 24
Dowling, J. T., 108
Dreiling, D. A., 244, 245
Dreyfus, J. C., 169, 174
Dubois, R. H., 267, 268
Dukes, C. E., 78
Duksin, D., 244
Duncan, B. P., 34
Dunning, E. J., 123
Dunphy, J. V., 257, 263
Dupont, A., 35
Durand, P., 256, 262, 268
Dutton, R. W., 242

Eagle, H., 192
East, J., 149
Eberlein, W. R., 132
Ecozcue, J., 43
Edington, C. W., 13
Edington, G. M., 182
Edwards, F. C., 75
Edwards, J. H., 13, 18, 23, 25, 26, 33,
Enisse, J. G., 215, 217
Eggermont, E., 241, 246, 247, 248, 249, 251, 253, 254, 259, 262, 266, 267
Ehrlich, M., 192
Ehrlich, P., 117
Eichholz, A., 265
Eidelman, A. I., 30
Eisen, J. D., 22, 35
Ellebjerg, J., 15
Ellis, J. R., 18, 27, 29
Elman, D. S., 107
Elmore, S. M., 30
Emanuel, B., 72
Emanuel I., 18
Engel, E., 14, 30, 104, 151, 152
Engelberth, O., 151, 152
Epstein, W. V., 127, 142, 143, 145, 155
Erkman, B., 4, 25, 27, 43
Esselborn, V. M., 102
Etienne, M., 256, 266
Evans, D. A. P., 65, 67, 68, 69, 70, 104, 105
Eugster, J., 100
Eylan, E., 123
Eylar, E. H., 188
Ezrin, C., 103

Fagerhol, M. K., 109
Faiman, C., 30
Faint, S., 10
Fanconnet, M., 215
Farber, S., 244
Fareed, J., 30
Farids, E., 39
Farmer, R. G., 80
Fathalla, M. F., 17
Faucher, J. P., 8
Favara, B., 34
Felix-Davies, D., 123
Feltkamp, J. E. W., 144
Fenner, F., 117
Ferguson, A., 263
Ferguson, J., 7, 8, 35
Ferguson-Smith, M. A., 4, 5, 6, 13, 14, 15, 16, 29, 109, 152
Fernandes, J. M., 9
Ferragu, O., 34
Ferrari, I., 44
Ferrari, V., 191
Fessas, P., 183
Fialkow, P. J., 104, 117, 124, 127, 145, 149, 150, 152, 153, 154, 155
Figarella, C., 244, 245, 258
Finley, W. H., 23, 34
Firsheim, I. L., 29
Fisher, R. A., 106, 107, 128, 131, 134, 135
Fischgrund, A., 34
Fitzgerald, M. G., 9, 39
Flora, R. M., 191
Florsheim, W. H., 108
Flynn, D., 151
Fodor, O., 67
Fogelson, L. I., 106
Fölling, A., 189
Fomon, S. J., 255
Forbes, A. P., 14, 104, 149, 151, 152
Ford, C. E., 9, 18
Fordstram, J. S., 266
Forget, M., 10
Forrest, F., 16
Forssman, H., 21, 23
Forstner, G., 265
Fortner, J. G., 107
Fortune, D. W., 39
Fouassin, A., 257
Fraccaro, M., 5, 18, 24, 33, 34
Franceschini, P., 210
Fraser, G. R., 89, 90, 91, 93, 94, 95, 97, 101, 102, 106, 107
Fraser, J. H., 8, 22
Frasier, S. D., 17
Fredericks, E., 217
Freeman, M., 258
Fredrickson, D. S., 173
Frei, E., 43
Freund, J., 122
Freycon, M. Th., 31
Frézal, J., 176, 246, 247, 257, 262
Friedman, A. I., 106
Friedrich, M., 248
Froland, A., 3, 12, 13, 15, 35, 104
Frosch, W. A., 44
Fudenberg, H., 118, 121, 127, 145, 147, 155
Fukuschima, T., 39
Furuyama, J-i, 16
Futterweit, W., 4

Gabrielson, 263
Gallop, C., 145
Galloway, W. H., 106
Gamstorp, I., 107
Gannon, P. G., 77, 79
Gardner, E. J., 78, 80, 81
Gardner, L. I., 30, 39, 102
Garson, O. M., 7, 8
Gartler, S. M., 102, 154
Gauld, I. K., 31
Gautier, M., 4, 8, 17
Gedda, L., 22
Geiger, J. W., 264
George, P. A., 107
Gerald, P. S., 30, 31, 34, 183
German, J. L., 16, 21, 29, 43, 44
Gerstl, B., 192
Giannelli, F., 21, 27
Gilgenkrantz, S., 8, 26
Gillespie, R. E., 246
Ginsburg, V., 264
Girardet, P., 257
Gitlin, D., 189
Gitzelman, R., 246, 249
Glass, J. H., 11
Glassman, E., 193
Glenn, J. F., 232
Glynn, L. E., 140
Goldberg, M. N., 17, 31, 127
Goldsmith, L. A., 28, 69
Goldstein, G., 144
Goldstein, M. N., 6, 189
Good, R. A., 118, 175, 231
Goodman, R. M., 15, 28
Goodwin, W. E., 217
Gordon, E. B., 145
Gorlin, R. J., 4, 34,

78
Gorer, P. A., 205, 215
Goudie, R. B., 133, 140
Gough, M. H., 10
Graff, R. I., 231
Graham, J. B., 109
Graiver, L., 69
Granick, S., 179
Grattenpanche, H., 39
Gray, J. G., 208
Gray, K. G., 104, 133, 145
Grayzel, A. I., 192
Greenblatt, R. B., 14, 16, 17
Greene, D. M., 150
Greenwood, C. T., 243
Griesbach, W. E., 107
Grossman, M. I., 244, 245
Grouchy, J. de, 10, 11, 21, 26, 29, 30, 31, 36
Grumbach, M. M., 4, 9, 34, 151
Gryboski, J. D., 249, 256, 263
Gschwind, R., 244
Guerineau, P., 8
Guerst, G. M., 106
Gumpel, R. C., 78
Gustavson, K. H., 24, 34

Haddad, H. M., 11
Hadjidakis, S. G., 99
Hadorn, H. B., 247
Haemmerli, V. P., 257
Haggar, R. A., 12
Hahn, H. B., Jr., 132
Hale, C. G., 5
Hall, P. F., 124, 127, 131, 133
Hall, R., 24, 38
Hallman, N., 261
Halsted, J. A., 82
Hambert, G., 2, 3, 8
Hamerton, J. L., 21, 22, 28
Hamburger, J., 217
Hamlin, E., 126
Hammond, J. B., 263
Hara, Y., 266
Hard, W. L., 22
Hardinge, M. G., 242
Harris, H., 175
Hasson, K. M., 31
Harden, R. Mc. G., 139
Harnden, D. G., 1, 2, 3, 5, 6, 7, 8, 10, 11, 12, 13, 25, 26, 41, 43
Harper, A. A., 244
Harris, H., 97, 130
Hart, A. G., 12
Hartman, W. H., 107, 124, 132
Harwald, B., 128, 130
Hashimoto, H., 119
Hassan, T. H. A., 138
Hasson, J. E., 6
Hattler, B. G., 208, 217
Hauge, M., 128, 130
Hauschka, T. S., 6
Hauschteck, E., 35
Haust, M. D., 4
Hawk, W. A., 80
Hawkins, K. A., 265
Hayashi, T., 21
Hayles, A. B., 132
Hecht, F., 5, 24, 27, 43
Heilmeyer, L., 175
Heinz, H. A., 11, 12, 21
Heller, R. H., 13, 15
Hellstrom, K. E., 230
Helver, B. J., 147, 148
Hempel, J. M., 22, 31, 35, 38
Henry, L., 207
Henstell, H. H., 96
Heptinstall, R. H., 96
Herbert, V., 134
Heringova, A., 248, 266
Herman, E. C., 182
Herr, H. M., 7
Hers, H. G., 187, 247, 251, 262, 264
Hersh, A. H., 74
Herter, 257
Herveg, J. P., 98
Hicks, J. D., 148
Hijmans, J. C., 248
Hildebrandt, H. M., 34
Hilderman, W. H., 231
Hill, O. W., 103, 151
Hirschfeld, J., 215
Hirschhorn, K., 29, 44, 209, 210
Hjelt, L., 21, 22
Hjort, T., 103
Ho, P. M., 265
Hodgkin, W. E., 37, 175
Hodson, A. W., 69
Hoffenberg, R., 9
Hoffman, E., 123
Hoffman, M., 16
Hoffman, O. D., 264
Holdsworth, C. D., 264, 265
Hollander, C. S., 102
Hollingsworth, D. F., 242
Holkes, G., 205
Holman, H. R., 118, 155
Holmes, M. C., 147, 148
Holst, H. E., 108
Holzel, A., 262
Holzel, D. Y. Y., 256
Hooft, C., 263
Hook, L. B., 21, 22, 24, 26, 27
Hope, P. M., 244
Hope, R. M., 244
Hortling, H., 5, 6
Horton, B. F., 182
Horwich, L., 65, 66, 67, 68
Hoskova, J., 248, 266
Hotchin, J., 155
Houston, E. W., 5, 44
Howell, Evans, A. W., 97, 105, 128, 129
Howie, J. B., 147, 148
Hsia, D. Y. Y., 249
Hsu, C. C., 19
Huang, S. S., 263
Huang, S. W., 19, 21
Hubby, J. L., 230
Hubscher, G., 246
Huguley, C. M., 176
Hume, D. M., 217
Huisman, T. H. J., 182
Hung, W., 123, 149, 155
Hungerford, D. A., 41, 43
Hunter, R. L., 108
Hunter, W. E., 9
Hussey, C. V., 189
Hustinx, W. J., 21, 22, 28
Hutchin, P., 217
Hutchinson, D. L., 37
Hutchison, J. H., 108

Iber, F. L., 189
Ijsseling, J., 256
Infante, F., 258
Ingbar, S. H., 128
Ingram, V. M., 173, 181
Inhorn, S. L., 26, 38
Innella, F., 145
Insley, J., 30
Irwin, S., 43
Irvine, W. J., 121, 122, 134, 137, 140, 141, 142, 143, 155
Isselbacher, K. J., 247
Israelstrom, D. M., 44
Ivanyi, D. M., 210, 217, 221, 223

Jackson, A. D. M., 94
Jackson, I. M. D., 139
Jackson, J. F., 9, 16, 21
Jacob, F., 169, 173, 186, 192
Jacobs, P. A., 2, 3, 5, 6, 7, 8, 10, 11, 12, 14, 16, 37, 41, 43, 44, 185
Jacobsen, P., 31, 35, 37
Jacobson, C. B., 38
Jacoby, G. A., 191
Jagiello, G., 14, 30
Jalling, B., 34
Janowitz, H. D., 244, 245
Jayson, M. I. V., 128, 129, 130, 138
Jeandet, J., 188
Jeans, P. C., 242
Jeffs, R. D., 4
Jenkins, W. J., 145
Jensen, M. K., 34
Jervis, G. A., 189
Jett, M., 143
Jeune, M. J., 19, 188
Jirasek, 266
Jirsova, V., 248, 266
Joffe, B., 122
Johansson, C. J., 6
Johnson, C. F., 246
Johnson, J. G., 207, 210
Johnston, M. W., 103
Jokl, E., 241, 242
Jones, H. W., 6, 9, 13, 15, 16, 105, 107
Jones, J. H., 66
Jos, J., 246, 247, 257
Josso, N., 10, 11
Juif, J. G., 94

Kadetani, T., 38
Kaijser, K., 5, 18
Kajubi, S. K., 263
Kallen, B., 38
Kalmus, H., 97, 130
Kalow, W., 133
Kano, K., 217
Kaplan, J. C., 174
Kaplan, M., 34
Karr, W. G., 264
Kasakura, S., 210
Kaufman, B. N., 15, 28
Kaufman, N. A., 242
Kellershohn, C., 94
Kelley, W. N., 180
Kennedy, J. S., 95, 107
Kenney, F. F., 16
Kern, F., 257
Kerr, J. H., 144
Kerr, M., 17, 38
Kerry, K. R., 249, 256, 262, 266
Kesaree, N., 9
Kessel, A. M. v., 39
Keuth, U., 35
Keynes, G., 144
Khaw, K. I., 246
Khorsandi, M., 4
Kieler, J., 145
Kimura, M., 220
Kingsmith-Flynn, D., 152, 153
Kinney, J. M., 264
Kinter, W. B., 266
Kirch, R. A., 13
Kisken, W. A., 213, 231
Kissmeyer-Nielsen, F., 145
Kite, J. H., 121
Kitchin, F. D., 97, 107
Kjolberg, O., 243
Kleihauer, E., 182
Klinefelter, H. F., 2 213
Klinger, H. P., 34, 39
Klosterman, T. H., 213
Klotz, A. P., 257
Knowles, H., 106, 143
Knox, J. D. E., 37, 44
Knox, W. E., 192
Knox, W. G., 77
Knudsen, A., 79
Koepf, G. F., 6
Kohner, E. M., 106
Koldovsky, O., 248, 266
Koler, R. D., 43
Kolinska, J., 253
Konigsberg, U. R., 29
Kopper, P. H., 188
Kosenow, W., 6
Kosowicz, J., 10
Kostamis, P., 98, 99
Koutras, D. A., 98, 99
Kralios, A. C., 99
Krauss, S., 143
Kreines, K., 143
Kretchmer, N., 246, 248, 249
Krevans, J. R., 182
Krieger, H., 102
Krooth, R. S., 177
Kuensbergs, E. V., 37, 44

Kugler, H. W., 240
Kuitumen, P., 262
Kulka, R. G., 244
Kunkel, H. G., 118
Kusakabo, T., 90, 95
Kutscher, A. H., 79
Kyiasi, J. Y., 264
Kyle, M., 140

Labie, D., 183
Labrune, B., 30
La Du, B. N., 191
Lafourcade, J., 28, 29, 30, 31
Lamber, R., 76
Lamedica, G. M., 256, 262
Lamy, M., 10, 11, 14, 30, 36
Landau, B. R., 264
Landing, B. H., 102, 106, 143
Landott, M., 263
Lang, T., 100
Langdon, R. G., 187
Lange-Nielsen, G. E., 95
Langman, M. J. S., 64, 65, 66, 68, 69
Lannard-Jones, J. E., 74
Laplane, R., 256, 266, 268
Lapolla, J. J., 35
Larner, J., 246
Larrieu, M. J., 189
Larson, W., 8, 9
Laumala, K., 261, 262
Laurance, B. M., 34
Laurent, C., 31
Lawrence, R., 4
Laxdal, O. E., 34
Leao, J. C., 30, 39
Lee, C. S. N., 34, 43
Lehman, O., 21, 23
Leigheb, G., 207, 208
Lejeune, J., 17, 28, 29, 30, 34, 36, 184
Lemli, L., 10
Lenhard, L. W., 7
Lennox, B., 8, 9, 16
Lerman, J., 107
Lerner, I. M., 102, 122
Lessof, M. H., 9
Lestradet, H., 188
Leveque, B., 8
Levin, B., 159, 266
Levin, W. C., 44
Levine, P., 145
Levis, 98
Levitt, T., 105
Levrat, M., 76
Levy, R. P., 109
Lewczak, P., 39
Lewis, F. J., 10
Lewis, G., 105
Lewit, S. G., 106
Lewontin, 230
Liao, S. K., 19
Lichzieher, I. B., 106
Liden, G., 108
Lifschitz, F., 257, 262
Lilly, F., 230
Lindberg, T., 248
Lindquist, B., 244, 266, 268
Lindsley, D. L., 13
Lindsten, J., 5, 10, 13, 14, 18, 24, 34
Linderbloom, G. E., 91
Linneweh, F., 192, 249, 266, 267, 268
Lion, D. T., 30
Linsao, L., 34
Liptn, M. M., 122
Little, K. D., 265
Littman, A., 263
Lods, J. C., 256, 266
Loeb, H., 266, 267
Lojda, Z., 246
Long, C. L., 264
Long, L. D., 96
Lord, P. M., 34
Loughman, W. D., 44
Loulit, R. T., 191
Lowe, C. U., 257
Lowbeer, L., 123
Lowenstein, L., 209
Lowrey, G. H., 101
Lubos, M. C., 257
Luce, A., 23
Lundborg, H., 264
Lundth, G., 244, 245
Lykke, A., 12
Lyon, M. F., 174

Ma, O. H., 256
Maddock, C. L., 244
Magasamik, B., 180
Magenis, R. E., 27
Maggiano, V. C., 208, 210
Mahalanabis, D., 259
Mahesh, V. B., 16, 17
Mahler, R., 187, 188
Mainguet, 266
Makda, H., 16
Makino, S., 3, 38, 39
Makler, M., 249
Malamos, B., 98, 99
Malvaux, P., 7
Mandry, J., 94
Manners, D. J., 242
Mantle, I. I., 2, 3, 4, 5, 7, 8, 10, 11, 12, 13
Mantzos I., 98
Marchis-Mouren, G., 245
Marinello, M. J., 43
Marketos, S., 98, 99
Mark, R., 205
Marks, I. F., 266
Marks, P. A., 181
Markson, J. L., 134, 139
Marquez-Monter, H., 2, 13
Marriot, Mck. W., 242
Marsh, W. L., 145
Marshall, J. S., 109, 144
Marshall, R., 27
Martin du Pan, R., 258
Martin, D. C., 217
Martin, L., 106, 107, 128, 131
Martin, R. G., 170
Martinez, C. J. M., 231
Martino, A. M., 256
Masi, A. T., 124, 132
Mathe, G., 208
Matioli, G. T., 188
Matsumoto, K., 16
Mattiuz, P. L., 207, 217
Maxwell, J. D., 263
Macy, C. D., 258
Mayer, M., 21, 22, 24, 26, 27
Maynard Smith, J., 133
McBride, J. A., 6, 74, 76
McCarty, K. S., 248
McClemont, W. F., 6
McClowskey, R., 217
McConahey, W. M., 123
McCornel, R. B., 63, 67, 68, 71, 97, 139
McCurdy, P. R., 173
McDonald, L. T., 31
McDonough, P. G., 16, 17
McDougal, C. O. M., 105
McDougal Jr., D. B., 265
McDougal, J. H., 5
McFayden, I. J., 139
McGavin, D. D. M., 29
McGee, B. J., 30
McGeown, M. G., 208
McGill, D. B., 247
McGillivray, R. C., 8, 15
McGirr, E. M., 95, 101, 107, 108
McGrath, P. J., 7
McGregor, A. R., 126
McGregor, T. N., 7
McGuire, P., 5
McIlree, M. E., 33
McIndoe, P. H., 96
McIntyre, P. A., 135
McKay, E., 106, 134
McKay, R. J., 37
McKusick, V. A., 79, 81, 102
McMaster, P. R. B., 122
McMichael, H. B., 257
Meadow, S. R., 24
Means, H., 96, 106
Medawar, P. B., 208, 215
Meek, J. C., 105
Meeuwisse, G., 266, 267
Meister, L., 108
Melchior, J. Chr., 34
Mele, D., 21
Melin, K., 268
Mella, G., 28
Melmann, W. J., 185
Mellon, J. P., 150
Mellors, R. C., 147, 149
Melnyk, J., 31
Melville, M. M., 6
Merikas, G., 65
Merrill, J. P., 207
Merritt, A. D., 31
Mery, A. M., 208
Messer, M., 246, 249, 258
Metcalfe, S., 37
Methlin, G., 94
Meyer, W. J., 16
Michel, R., 91
Mickey, M. R., 217
Mier, P. D., 176
Miesher, P., 215
Migeon, B. R., 16, 29, 31, 34
Mikkelson, M., 15, 21, 35
Mikulska, Z. B., 205
Miles, C. P., 24
Milgrom, F., 140, 217
Milholland, R. J., 191
Miller, D., 296
Miller, O. J., 21, 22, 29, 31, 35, 38
Ming, P. L., 34
Miras, K., 98
Mitchel, J. M., 6
Mitchel, P., 264, 266
Miyake, T., 90, 95
Mohamed, S. D., 105, 106
Mohr, J., 35
Moller, G., 230
Monardo, A., 11
Money, J., 11
Monod, J., 169, 173, 186, 192
Montmollin, M. E. de, 30
Moore, J. M., 134, 139, 143
Moore, K. L., 2
Morgans, M. E., 94
Morgenroth, J., 117
Morishima, A., 4, 151
Morris, I. M., 16
Morris, W. R., 107
Morson, B. C., 77
Morson, F. G. W., 79
Mosbach, J., 135
Moses, S. W., 187
Mosier, H. D., 4, 17
Motulsky, A. G., 142, 143, 151, 180, 182
Mueller, P. S., 122
Mukherjee, B. B., 22
Muldal, S., 4
Mulder, T., 242
Mulhaupt, E., 266
Muller, H., 18
Munro, T. A., 81
Murphy, E. S., 82, 139
Murphy, J. W., 31
Murset, G., 35, 245, 247, 258
Mutton, D. E., 28

Nadler, H. L., 37
Najarian, J. S., 217
Nance, W. E., 30
Nandi, S., 230
Nastuk, W. L., 143
Neale, G., 259
Neel, J. V., 77, 78, 101, 102, 180, 182
Neilson, I. M., 134, 143, 155
Neiman, N., 176
Nelson, S. D., 207, 208, 223
Nerup, J., 140
Neu, R. L., 30, 39
Neven, M., 257
Newcomer, A. D., 247
Newey, H., 266
Newman, E., 67, 68
Newnham, R. E., 27
Newsam, J. E., 33
Newth, D. R., 217
Nicks, P. J., 208
Nicoloff, J. T., 108
Nicuni, Z., 242
Nielsen, J., 104
Niemi, M., 5, 6
Nierlich, D. P., 180
Nikolai, T. F., 109

Nillson, I. M., 189
Nilsson, L. R., 105, 107, 124, 126, 131, 132, 144
Nitowsky, H. M., 29
Nivelon, A., 31
Nack, R., 248
Nogales, F., 16
Norby, S., 244
Nordskog, A. W., 205
Nordstrom, C., 247
Norins, L. C., 147
Norton, J. B., 266
Nowell, P. C., 41, 43
Nozaki, R., 16

Oberholzer, V. G., 266
Ockerman, P. A., 264
Ockey, C. R., 4
Olanders, S., 8
Olbrich, E., 24
Oldfield, M. C., 81
Oosterhuis, H. J. G. H., 144
Opit, L. J., 265
O'Reilly, R. A., 179
Ortiz, M., 15
Osserman, K. E., 143, 144
Osinchak, J., 246
Oski, F. A., 188
Osler, W., 100
Oster, J., 23
O'Sullivan, M. A., 21
Owen, R. D., 206
Owen, S. G., 103, 105, 123, 124, 126, 131

Page, E. W., 40
Palla, J. C., 245
Palm, J., 205, 231
Palmer, C. G., 30
Pardee, A. B., 179
Pare, C. M. B., 191
Parker, W. C., 172, 173, 174
Pasero, L., 245
Passarge, E., 71, 72, 73
Patau, K., 26, 38
Patton, D. D., 108
Pawlowitzki, I. H., 16
Pay, B. Y., 150
Payne, R., 215, 216, 217
Peacocke, N., 208
Peagler, F., 4
Pearson, C. M., 187
Pedersen, G. T., 103
Penrose, L. S., 18, 21, 23, 152, 153
Pergament, E., 38
Perheentupa, J., 261
Perkins, H. A., 217
Peterson, R. D. A., 118
Peterson, W. C., 4
Petterson, G., 16
Pettit, M. D., 102, 106
Pevzelon, R., 76
Pfeifer, R. A., 6, 16, 21, 29, 35
Pfitzer, P., 30
Philip, J., 14, 16, 17
Pierson, M., 8, 26
Pinsky, L., 34, 177
Pissarro, C., 256, 266
Pitt, D. B., 35
Platter, F., 99
Plescia, O. J., 143
Plotkin, G. R., 247
Plummer, H. S., 106
Plunkett, E. R., 1, 4, 5, 9, 13, 17
Po, B. T., 79
Poate, H. R. G., 96
Polani, P. E., 9, 13, 18, 26
Polonovski, C., 256, 266
Porritt, A., 96
Porteus, J. W., 249
Porter, F. S., 177
Porter, I. H., 21
Porter, K. A., 217
Poser, C. M., 192
Potter, E. B., 107
Poznanski, R., 242
Pozsonyi, J., 5
Prabhaker, S., 2
Prader, A., 35, 247, 249, 251
Pratt, R. T. C., 144
Preshaw, R. M., 245
Prezelin, G., 258
Price, W. H., 7, 33
Pringle, R. A., 67
Probst, J. H., 10
Proctor, H. D., 79
Pryles, C. V., 21
Punnett, H. H., 34
Purves, H. D., 107

Quasel, J. H., 191, 264
Quick, A. J., 189

Reboud, J. P., 245
Reed, T. E., 77, 78
Rees, C., 134
Reifenstein, E. C., 2
Reisman, L. E., 24, 31
Rendel, J., 69
Renold, A. E., 187
Rethore, M. O., 29, 30
Richards, R. C., 80
Richardson, R., 205
Richardson, W. R., 71
Rigas, D. A., 43
Rigopoulos, G. A., 98, 99
Riklis, E., 264, 265
Rimoin, D. L., 102
Ritzman, S. E., 44
Rivarola, M. A., 16
Riviera, R., 94
Ro, J., 82
Roberts, K. D., 107, 155, 243
Robson, M. K., 35
Robinson, J. C., 28, 69
Robinson, J. W. L., 266
Robinson, K. S., 191
Robinson, R. R., 232
Roche, J., 91
Rohde, R. A., 30, 34
Roit, I. M., 105, 106, 119, 120, 121, 123, 124, 126, 127, 130, 131, 133, 134, 137, 138, 139, 144, 147, 151, 155
Romei, L., 23
Rose, F. C., 106
Rose, N. R., 119, 120
Rosen, F., 191
Rosen, G., 246, 248
Rosenblum, A., 34
Rosenkrans, C. J., 23
Rosenkrantz, W., 8, 23
Rosenzweig, N. S., 263
Roubin, M., 21
Rowell, R. N., 103, 104, 106
Roxburgh, R. A., 82
Roy, J. R., 16
Royer, P., 30
Rubin, A., 245, 247, 252, 263
Rubinoff, A., 37
Rucknagel, D. L., 184
Ruitton-Ugliengo, 261
Rundal, T. S., 31
Russel, P. S., 207, 208, 210
Rutter, W. J., 244
Rychlikova, M., 223
Rywkin, L. A., 106

Sacrez, R., 94
Saez, J. M., 16
St. Pierre, F., 181
Salm, S. B., 38
Salmon, Ch., 29, 30, 34
Salomon, L. L., 265
Sanchez, J., 15
Sandberg, A. A., 15, 43
Sandler, M., 191
Sanger, R., 13
Sapelier, J., 26
Sargent, T. W., 44
Sarles, H., 258
Sas, 248
Sasaki, M., 39
Sasportes, M., 223
Sasso, W., 9
Savage, P. T., 79
Savitsky, A. D., 43
Saxena, K. M., 105, 124, 126, 128, 131
Scarth, L., 134, 140, 141, 142, 143
Schwachman, H., 244
Scott, J. S., 4, 17
Scratcherd, T., 244
Scully, A. L., 17
Schachman, H. K., 251
Schade, S. G., 134
Schaumloffel, E., 249, 266, 267
Scheinberg, I. H., 189
Schenk, G., 248
Schimke, R. N., 107
Schippers, A., 217
Schlegel, R. J., 38, 39
Schmickel, R. D., 21
Schmid, R., 187
Schneider, A. J., 266, 267
Schulz, S. G., 263, 265
Schwartz, M., 134, 145
Schwartz, V., 256, 262
Schwartz, G., 112
Scriver, C. R., 268
Seal, U. S., 109
Seelen, J. C., 39
Segal, L. J., 4, 5, 6, 7
Seki, T., 16
Sekiguchi, M., 208
Semenza, G., 246, 247, 249, 251, 263, 266
Serejsky, M. J., 106
Sergovich, F. R., 3, 21, 23, 44
Seringe, P., 28
Shanbrom, E., 215
Shannon, D. C., 142
Shapiro, M., 145
Shaver, E. L., 4
Shaw, M. W., 17, 21, 22, 30, 34, 37
Sheba, Ch. 123
Sheehy, T. W., 263
Shepard, T. H., 97, 102
Shepperd, J. A., 79
Sherlock, P. B. M., 74, 75
Shich, L. Y., 185
Shierman, L. W., 205
Shindler, A. M., 30
Shine, I. B., 11
Shinton, N. K., 145
Siowall, J., 245
Shmerling, D. H., 247, 263
Shreffler, D. C., 69, 205, 206, 230
Shulman, L. E., 124, 132
Sidbury, J. B., 63
Sieber, W. K., 72
Sieker, H. O., 208
Silvers, W. K., 231
Silverstrom, E., 181
Simonsen, M., 218
Simpson, J. A., 143, 144
Simpson, R. D., 79
Sindhvananda, N., 29
Singer, I. D., 31
Singer, K., 181
Singh, R. P., 10, 12, 37
Sipova, I., 3
Skillman, T. B., 102
Skinner, J. L., 7
Skou, J. C., 265
Smallpiece, V., 18, 23
Smart, G. A., 103, 124, 126
Smith, D. W., 10, 26, 38
Smith, J. F., 108, 153
Smith, J. M., 231
Smith, L. H., Jr., 76
Smout, M. S., 13
Smyth, C. J., 180, 266
Snell, G. D., 203, 205, 231
Snyder, L. H., 102
Sohval, A. R., 16
Sokal, J. E., 43
Solomon, A. K., 263
Solomon, N., 105
Soltan, H. G., 4, 5, 17, 21, 26, 27
Sonntag, W. M., 259
Soulier, J. P., 189
Souville, C., 258
Spann, J. L., 123
Sparkes, R. S., 31, 151
Spiro, H. M., 263
Stafford, N. R., 101
Stalder, G. R., 34

Stamatoygannopoulos, G., 183
Stamos, H. F., 135
Stanage, W. F., 22
Stanbury, J. B., 90, 91, 92, 96, 123, 173
Staples, W. I., 22, 31, 34
Starkman, M. N., 30
Steele, M. W., 29, 30, 37
Stein, Y., 242
Steinberg, A. G., 22, 109
Steinberg, V. L., 76
Steiner, J. W., 103, 140
Stenchever, M. A., 31, 38
Stent, G. S., 170
Sternberg, W. H., 17
Stevens, L. J., 27
Stewart, A. G., 134, 140, 141, 142, 143
Stewart, J. S. S., 16
Stickel, D. L., 232
Stimpfling, J. H., 205
Stirling, C. E., 266
Storey, G., 76
Stotte, L. A. M., 39
Stoufflet, E. J., 44
Strand, A., 17
Strauss, A. J. L., 143
Strong, J. A., 2, 6, 185
Strong, L. C., 203
Struthers, J. E., 257
Stupperich, E., 35
Subray, N., 2
Sukliffo, K. W., 256, 262
Sunshine, P., 249
Sutherland, J. M., 102
Sutton, H. E., 174
Swaminathan, N., 247
Swarner, 242
Swift, M. R., 43
Swyer, G. I. M., 9
Szulman, A. E., 38, 39

Tabaqchali, S., 256
Tada, K., 191
Tagaki, N., 15
Tagher, P., 24
Tanton, S. H., 101
Tassopoulos, C. N., 98
Taylor, A. I., 10, 25, 26, 27, 28
Taylor, K. B., 119, 120, 130, 134, 137, 138, 139
Teplitz, R. L., 31
Terasaki, P. I., 213, 217
Terzakis, T. A., 30
Teter, J., 16, 17
te Velde, K., 136, 137
Thayer, W. R., 263
Therkelsen, A. J., 5
Therman, E., 26, 38
Thiede, H. A., 37, 38
Thieme, E. T., 107
Thier, S., 268
Thier, S. O., 123
Thiriet, M., 26
Thomas, L., 207
Thovicht, S., 30
Tiepolo, L., 24
Tijdink, G. A. J., 39
Tisdale, V., 43
Tjio, J. H., 43
Tominaga, T., 40
Tompkins, R., 30
Tonge, R. E., 259
Torrigiani, G., 121
Torrioli-Riggio, G., 121
Tosi, R., 266
Tough, I. M., 37, 41, 43, 44
Townes, P. L., 7
Townley, R. R. W., 246, 258, 259, 262, 266
Tridente, G., 210
Tripp, 216
Troisier, J., 145
Trolle, D., 16
Trotter, W. R., 94, 130
Troup, G. M., 215, 217
Troxel, D. B., 145
Troyer, W. G., 259
Trubowitz, S., 185
Truelove, S. C., 75
Tschudy, D. P., 179
Tubiana, M., 91
Tucker, C. C., 23
Tudhope, G. R., 134
Tulloch, W. Selby, 33
Tunnett, 30
Turcot, J., 81
Turnbull, R. B., 80
Turner, B., 31, 37
Turpin, R., 17, 28, 34
Tyler, F. H., 191

Uchida, J. A., 23, 24, 26, 27, 31, 34, 149, 150, 152, 153, 154
Ugolev, A. M., 245
Uher, 266
Umansky, I., 31
Ungar, B., 143
Uozumi, T. R., 16
Urcan, S., 67
Ursel, M., 23

Vajda, I., 145
Valentine, G. H., 344
Vallotton, M. B., 104, 149, 152, 266
Van de Kamer, J. H., 256, 257, 262
van den Berghe, H., 7
van der Geld, H., 144
van der Hagen, C. B., 21
Vander Latan, W. P., 105
van der Verff ten Bosh, J. J., 10
Van Hauwaert, J., 263
van Leuwen, J., 215, 217
Van Middlesworth, L., 98
Van Rood, J. J., 213, 215, 216, 217, 221, 223
van Wyk, J. J., 9
Vas, M. R., 209
Vaubel, E. W., 208
Veale, A. M. O., 77, 78
Veiga, A. A., 44
Verger, P., 10
Vesell, M., 16
Vetters, J. M., 104
Vialette, J., 28
Vickery, A. L., 126
Vincens-Calvet, E., 261
Vincent, T. N., 264
Visakorpi, J. K., 261, 262
Visetti, M., 207, 208
Vislie, H., 35
Visscher, M. de, 7, 98
Vital, C., 10
Volpe, J., 28
Volpe, R., 103, 124
Von Halle, E. S., 13
Vooys, W. H., 217
Vos, G. H., 145
Voynow, N. K., 210, 235
Vredevee, D. L., 217

Walter, K., 11, 12
Wagner, F., 188
Wagner, H. P., 26
Walford, R. L., 215, 217, 233
Walker, G. J., 243, 244
Walzer, S. B., 34
Wang, C. C., 245
Wang, H. C., 31, 34, 43
Warburg, E., 4, 12
Warner, S., 31
Warburton, D., 29
Warkany, J., 21
Warren, R., 264
Waters, A. H., 139
Watkins, W. M., 230
Watson, C. J., 177, 178
Waxman, S. H., 38
Weakley, D. R., 187
Webb, G. C., 35
Webb, J., 257
Weber, G., 192, 294
Weber, W. W., 25
Wehn, A. M., 35
Weigle, J., 216
Weigle, W. O., 121
Weiiers, H. A., 256, 257, 262
Weinberg, T., 29
Weiner, L. B., 143, 145
Weinhouse, R., 18
Weiss, L., 30
Welch, J. P., 7
Weleber, R. G., 7
Welsh, J. D., 259, 261
Wennstrom, J., 5, 6
Wertelecki, W., 30
West, C. D., 102
Weste, S. M., 7, 8
Whatmore, 7
Wheelan, W. J., 243
Wheeler, J. T., 182
Whitaker, J., 142
White, L. W., 264
Whittingham, S., 144
Widal, F., 145
Wiener, J. D., 91, 145
Wiens, R. G., 4, 5, 17
Wiens, R. L., 106
Wiesli, B., 2
Wilkins, L., 9, 11, 16
Wilkinson, J. F., 135
Williams, D. L., 151, 152
Williams, J. P., 79
Williams, G. M., 217
Williams, J., 6
Wilson, A. C., 179
Wilson, G. M., 134
Wilson, R. E., 207, 231, 247
Wilson, T. H., 246, 264, 266
Wingate, L., 37, 38
Winship, T., 123
Winter, J., 132
Wiseman, G., 266
Witebsky, E., 119, 140, 144
Witt, G., 257
Witte, E. H., 37
Wolff, O. H., 25, 26
Wong, J., 35
Woodrow, J. C., 105
Wooley, P. V., 9
Woolf, C. M., 80
Woolf, L. I., 189
Woolmer, L. B., 132
Wren, P. J., 104, 151
Wright, S. W., 8, 9, 18, 21, 31, 104, 134, 135, 144, 152
Wuepper, K. D., 141, 142
Wyngaarden, J. B., 173, 179

Yarnis, H., 75
Yataganas, X. A., 99
Ying, K. L., 5
Yuceoglu, A. M., 4
Yunis, J. J., 21, 22, 24, 26
Yuwiler, A., 191

Zachu-Christiansen, B., 12
Zaleski, W. A., 5, 34
Zaluski, R., 265
Zeigler, N. A., 7
Zeller E., 215
Zerefos, N., 98, 99
Zetterquist, P., 34
Zillis, J., 256
Zmijewski, C. M., 217, 232
Zmucky, R., 123
Zourlas, P. A., 16
Zuckerkandl, E., 180
Zuppinger, K., 3

# Subject Index


Addison's disease
  idiopathic, 134, 139-140
  with idiopathic hypoparathyroidism, 141-142
Adenomatosis
  multiple endocrine, 107
Agammaglobulinemia, 121
Amenorrhea, 7, 13, 14, 15, 16
Anemia
  autoimmune hemolytic, 144-145, 147, 155
  and idiopathic chronic gastritis, 133-134
  pernicious, 64, 133-134
  relationship between blood group A and, 64
Anomaly
  "aneusomy of recombination," 36
  cardiovascular, 10
  chromosome, 10, 12
  of lymphatic system, 10
  mosaic, 10, 13
    *See also* Mosaicism
  skeletal, 5
  types of, 43
  XO, 11, 12, 13
  XXX, 8
  XXXXX, 9, 11
  XXY, 3, 7, 12
  XXYY, 4
  XXXXY, 5, 9
  XYY sex chromosome, 6-7
  *See also* Sex
Anophthalmia, 26
Antigens
  leukocyte, 214-216
Atrophy
  juvenile diabetes with optic, 105
  testicular, 2
Autoimmunity
  and chromosomal aberrations, 149-150
  gastric, 137-138, 139
  genetic aspects of, 117-156
  thyroid, 119, 122, 123, 130, 137-138
Azoospermia, 2

Blood group(s)
  A, 64
  AB, 65
  ABO, 63, 64, 69
  B, 65, 69
  duodenal ulcer in association with, 70
  and gastroenterology, 63-71
  Lewis, 68
  O, 64, 65
  pleiotropic effect of ABO, 71
  relationship between alimentary disease and ABO, 70
  secretor status and ABO, 70

Carcinoma
  gastric, 67
  of stomach, 64
  thyroid, 107
Carditis rheumatic, 67
Cell(s)
  chromosome of fetus by culturing, 37
  control cultures containing, 210
  differentiation and genetic diseases, 185-188
  45, X, 13
  germ, 12, 27
  ii and iii chromosomes in, 33
  Leydig, 5
  line XO, 9
  line XY, 4
  triple-X, 15
  XO, 11, 15
  XXY, 5
  XY, 17
Chromatic
  – negative girls, 9
  – negative males, 7
  – positive buccal smears, 4
  – positive Klinefelter's syndrome, 2
  – positive males, 3, 5
  – sex, 7
Chromosomal abnormalities
  in phenotypic males, 1-6
Chromosomal disorders, 151-152
Chromosomal variants
  in gonadal dysgenesis, 9-24
Chromosome
  abnormalities, 184-185
  acrocentric, 17, 19, 35
  anomalies and ovarian function, 12
  anomalies and spontaneous abortions, 37-40
  anomalies in abortuses, 42
  behavior at meiosis, 31
  breaks among users of LSD, 43-44
  complexes of triploidy, XXY, XXX and XYY, 39
  18, 29-30
  4, 29
  G – group, 28, 41
  in mongolism, 17
  morphology of, 36
  Philadelphia, 41, 42, 44
  ring, 30-31
  sex, 1-6, 13
  studies in populations of normal subjects, 44
  translocation, 19, 22, 23, 24
  translocation between the X and Y, 6
  X in female, 7, 13
  X, 2
  Y, 2, 13, 17
Cirrhosis
  of liver, 64
Colitis, 73, 76
  *See also* Gastrointestinal tract
Contraceptives
  oral, 44
Cretinism
  athyreotic, 101
  endemic, 99-100
  sporadic goitrous, 89-96, 105
  sporadic non-goitrous, 100-103
    *See also* Hypothyroidism
Crohn's disease, 73-76
  *See also* Gastrointestinal tract

Deafness
  with retinitis pigmentosa, 95
Diabetes
  mellitus, 64, 67, 142-143
  with optic atrophy, 105
Down's syndrome, 150, 153
Drosophila
  Y chromosome in, 13
Dysgenesis
  gonadal, 9-24
  mixed and pure gonadal, 16
  in phenotypic females, 9
  and primary amenorrhea, 14
  XO gonadal, 9-13, 16, 17
  XY gonadal, 17
    *See also* Pseudohermaphroditism

Encephalomyelitis, 122
Enzymes
  enzymic activities of, 250
Escherichia coli, 256
Etiology
  autoimmunoc, 106
  and pathogenesis, 120-122
  role of genetic factors in, 89

Fibrosis
  peritibular, 4

Gastritis
atrophic, 137
idiopathic chronic, 133-134
Gastroenterology
and blood groups, 63-71
Gastrointestinal tract
Hirschprung's disease of, 71-73
inheritance and diseases of, 72-73
jejunal mucosa in, 69
phosphatases in, 69
polyposis in, 76-78
relationship of Lewis blood type to disease of, 68
secretor character in disease of, 67
ulcerative colitis and Crohn's disease of, 73-76
Gene(s)
controller, 171
intervention of regulator, 177-179
structural, 172-173
Genetics
and gastrointestinal system, 63-83
bacterial, 169-193
of glycogen storage diseases, 63
of intestinal carbohydrate tolerance, 241-268
medical, 63
of thyroid disease, 89-110
Gonosome
XY, 17
Grave's disease, 127, 128
*See also* Thyrotoxicosis
Gynecomastia, 2

Hashimoto
disease, 100, 105
disease in monovular twins, 122
Hematemesis
and melena, 64-65
high O blood group in, 64-65
Hemizygotes, 109
Heterozygosis
in man, 34, 96
translocation, 37
Heterozygotes, 91, 97, 109, 174
Hirschsprung's disease, 71-73
*See also* Gastrointestinal tract
Histocompatibility
in man, 201-236
studies in man, 206-216
studies in mouse, 203-206
testing, 216-217
Hemoglobin disease, 180
Homozygosis, 96
Homozygotes, 95, 174
Hormone(s)
abnormal thyrotrophic, 105
production, 91
thyroid, 89, 108
urinary follicle-stimulating, 2
Hyperplasia
cause of thyroid, 107
Hypoparathyroidism
Addison's disease with idiopathic, 141-142
Hypothyroidism
adult, 103-104
deiodination in, 90
diagnosis of, 109
endemic goiter and, 98-99
infantile with goiter, 89-96
partial, 127
polygenic inheritance of, 102
primary, 125-126
sporadic non-toxic goiter and, 96-98

Intestinal mucosa, 260
Intolerance
to lactose, 262-263
to saccharose, 258-259
to starch, 257-258
Isotopes
radioactive, of iodine, 89

Klinefelter's syndrome, 2-6

Leukemia
myeloid, 41, 43
Lewis blood type, 68
Lymphedema, 10

Melena, 64-65
*See also* Hematemesis
Micrognathia, 29
Mongolism
chromosome in, 17
in women, 8
Monosony, 28
Mosaicism, 3, 11, 12
effect on phenotype, 4
45, X, 15
ovarian failure and chromosome, 13-16
in woman, 8
XO, 10
in XO cell line, 9
XO/XX, 13, 15
XO/XY type of, 15, 16
Myasthenia gravis, 143-144, 155
Myxedema
adult, 103
comparison of juvenile CLT and juvenile, 132
hereditary factors in, 127
juvenile, 101
primary, 125-126

Neoplasia
genetic factors determining, 107
malignant, 107-108

Oligosaccharides
hydrolysis of, 245-246
Oligospermia, 24
Operons
in higher animals and man, 176-177

Pathogenesis
and etiology, 120-122
Pentasomy X
in females, 9
Phenylketonuria, 190-192
Physiology
alimentary, 64
gastrointestinal, 68-69
Polyposis, 76-78
colonic and tumors of nervous system, 81
colonic with development of duodenal polyp, 82-83
gastric, 82
multiple of colon and stomach, 81-82
*See also* Gastrointestinal tract
Polyps fi
colonic with sebaceous cysts, 81
of colon and rectum, 80-81
Polysomy-X
females with, 7-9, 10
Porphyria
acute, 178
erythropoietic, 178
hepatic, 178
Proteins
inherited variation in thyroxine-binding, 108-109
Pseudohermaphroditism, 9, 16
and mixed gonadal dysgenesis, 17

Sex
chromatic, 1, 2, 7
chromosome abnormalities in phenotypic males, 1-6
chromosome anomalies, 3
chromosome anomaly in female, 7
chromosome complement in males, 4
chromosome XO complement, 9, 13
chromosome XO complex, 15
chromosome XXX complex, 7
chromosome XXY complement, 4, 7
chromosome XXY complex, 5
chromosome XXXXY constitution, 5
chromosome XXYY complement, 5

chromosome variants, 1, 9
Spermatogenesis, 4
Spondylitis
ankylosing, 76
Stasis dermatitis, 4
Stenosis
hypertrophic pyloric, 63, 72, 73
Strabismus, 29
Syndrome
chromatic positive Klinefelter's, 2
clinical features of Klinefelter's, 2
of deafness with goiter, 92
D-trisomy, 27, 28
Down's, 150
Edwards (trisomy-18), 25, 26, 27, 28
E-trisomy, 26
Gardner, 78, 79
Klinefelter's, 1-6
Maladie du cri du chat, 25, 28, 35
mental retardation and Klinefelter's, 2-3
Patau's (trisomy-13), 25, 26, 27, 28, 29
Pendred's, 93, 94, 95
Peutz-Jeghers, 79-80
polyploid, 39, 44
Sjogren's, 155
testicular feminization, 16
translocation in trisomy-21, 18-19, 21
translocation of, 31-36
triple-21, 8
*See also* Mongolism
trisomy-21, 17, 18, 20, 21, 26
*See also* Mongolism
trisomy G, 24
18 trisomy, 25
Turner's, 9, 13
XO Turner's, 10
Synostosis
radioulnar, 5

Tetrasomy X
in females, 9
Thalassemia, 182
Thyroid
abnormalities, 130-131
antibodies, 128, 129, 150
antibodies in older age groups, 133
autoimmunity, 119, 122, 123, 130, 137-138
carcinoma, 107
chronic lymphocytic, 118-120
disease, 89-110, 118-120
disease and chromosomal aberrations, 104-105
gland, 89
Hashimoto's, 118-120, 123
hormone metabolism, 128
hormones, 89, 90, 108
hyperplasia, 107
metabolism of iodine, 90
pheochromocytoma, 107
subacute, 123
Thyrotoxicosis, 105-107, 121, 127, 128, 129
"secondary," 107
Tolerance
genetics of intestinal carbohydrate, 241-268
Tonsillectomy, 67

Ulcer(s)
antral, 64
bleeding, 64
clinical types of duodenal, 64-65
duodenal, 64
gastric, 64
perforation, 65, 66
secretor character in, 68-69
stomal, 66

"Vesiculation of villi"
in spontaneous abortion, 40
Villi
vescicular chronionic, 40
Von Willebrand's disease, 189-190

Wilson's disease, 189-190

Zygotes
chromosomally abnormal, 39

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE
LIBRARY
-8 APR 1970