Progress in medical genetics. Volume 2 / edited by Arthur G. Steinberg.

Contributors

Steinberg, Arthur Gerald, 1912-

Publication/Creation

New York ; London : Grune & Stratton, 1962.

Persistent URL

https://wellcomecollection.org/works/gg8vyw6z

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

Progress in

MEDICAL GENETICS

VOLUME II

Edited by

ARTHUR G. STEINBERG

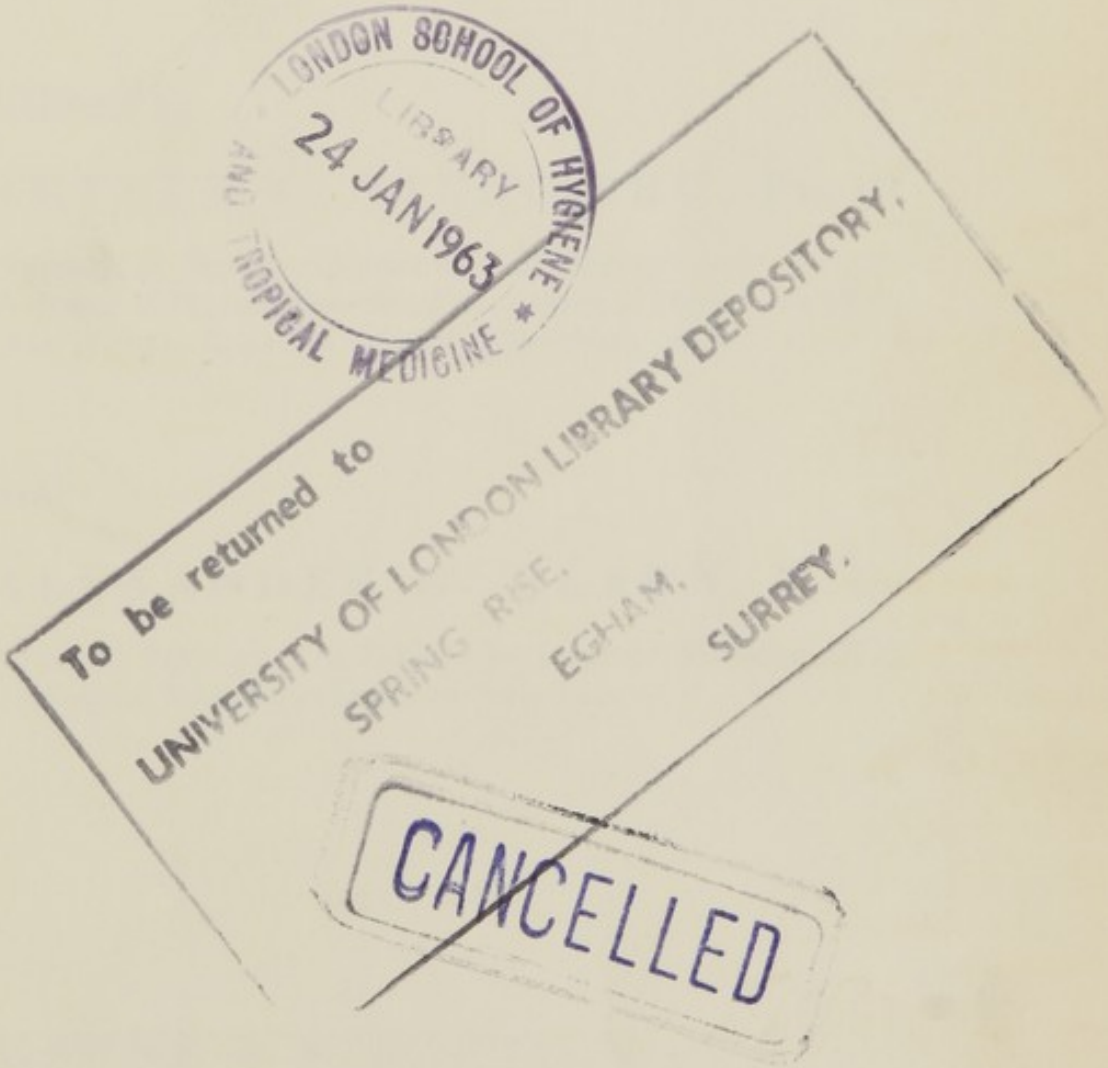
ALEXANDER G. BEARN

22501959226

H. K. LEWIS & CO. LTD.

for 1 month

47



10/-

PROGRESS IN

Medical Genetics

Volume II

Edited by

ARTHUR G. STEINBERG, Ph.D.

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

and

ALEXANDER G. BEARN, M.D.

Associate Professor, The Rockefeller Institute; Physician, Hospital of the Rockefeller Institute, New York

GRUNE & STRATTON

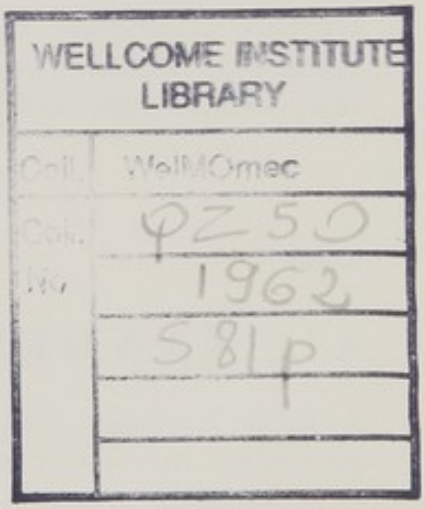
New York and London

1962

331

Library of Congress Catalog Card No. 60–53514

Copyright © 1962
GRUNE & STRATTON, INC.
381 Park Avenue South
New York 16, N.Y.



Printed and bound in U.S.A. (Con–B)

Contents

Foreword iv

1. Progress in the Study of Genetically Determined Human Gamma Globulin Types (The Gm and Inv Groups). *Arthur G. Steinberg* 1

2. The Plasma Transferrins. *E. R. Giblett* 34

3. The Group Specific Component of Serum; Genetic and Chemical Considerations. *Hartwig Cleve and Alexander G. Bearn* 64

4. Hereditary Aminoaciduria. *Charles R. Scriver* 83

5. Antibody Synthesis. *Seikichi Tokuda and Ray D. Owen* 187

6. Clinical and Immunologic Evidence for a Predisposition to Rheumatic Disease in Certain Families. *Halsted R. Holman* 210

7. Chromosome Aberrations in Experimental Mammals. *Liane Brauch Russell* 230

8. Mammalian Genetics in Medicine. *Salome Gluecksohn-Waelsch* 295

9. Progress in Ophthalmic Genetics. *J. François* 331

Author Index 366

Subject Index 373

Foreword

The pattern set in the first volume of *Progress in Medical Genetics* is maintained in this volume. Each chapter deals with a specific subject in the field, and in doing so reflects the increasing scope of medical genetics and the frequent necessity of drawing on the specialized techniques developed by allied disciplines.

Ten years ago hereditary biochemical polymorphic systems were almost restricted to the blood group system. Today, several well defined polymorphic systems have been shown to exist among the plasma proteins. Unfortunately, we are still dismally ignorant of the nature of the forces which maintain the polymorphism. Despite this limitation, information has rapidly accumulated concerning the world-wide distribution of the traits concerned and, in some instances, striking headway is being made toward defining the structural differences between the products of allelic genes (Chapters 1–3).

The introduction of chromatography by Martin and Synge in 1941 has made available to the geneticist a convenient and simple tool to investigate the qualitative and quantitative amino acid distribution in body fluids. The results of the successful exploitation of this and subsequent analytical techniques form the subject of Chapter 4.

The application of immunologic principles to the study of the genetics of antibody production has been fruitful not only of new facts but of new concepts. The genetical aspects of modern immunologic theory are critically evaluated in Chapter 5. A clinical extension of this concept has become apparent from the observation of an increased familial concentration of patients suffering from the so-called auto-immune diseases (Chapter 6).

The application of new cytological techniques to the study of human chromosomes has uncovered a remarkable series of chromosomal abnormalities of clinical importance (see Chapter 8, Volume I, of this series). Similar abnormalities have been observed in the chromosomes of other organisms including various mammals whose genetics have been well studied. This affords the investigator an opportunity to study the mode of origin, of transmission, and of action of development of these abnormalities,

in a manner which cannot be done in man. The data may be helpful in interpreting information derived from the study of human chromosomes. Recent progress made in the study of chromosomal aberrations in experimental mammals is reviewed in Chapter 7. The value of an understanding of microbial genetics to students of medical genetics has been amply and justifiably emphasized in recent years. In contrast, the contribution of mammalian genetics has been frequently overlooked, particularly with respect to gene expression. The review of mammalian genetics in Chapter 8 not only considers established knowledge but also the prospects for future developments in this field.

The field of opthalmology has for many years been one of the most productive of the clinical disciplines in the field of genetics, and recent advances in this area form the subject of Chapter 9.

The editors are extremely fortunate in having been able to persuade the authors to contribute to this enterprise. The chapters in this, the second volume of Progress in Medical Genetics, uphold the high standards set by the contributors to the first volume.

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

Chapter 1

Progress in the Study of Genetically Determined Human Gamma Globulin Types (The Gm and Inv Groups)

Arthur G. Steinberg
Western Reserve University, Cleveland, Ohio

Introduction

Grubb's discovery of the Gm factor (Grubb, 1956) is a remarkable example of serendipity. Grubb wrote, "In the determination of the amount of gamma globulin in sera from six patients with hypogammaglobulinemia by inhibition of the agglutination effect of Coombs' reagent it was observed that one of the sera diluted up to 1:20 in saline agglutinated the red cells coated with 'incomplete' anti-Rh used in this procedure." The serum was from an Rh(+) boy with no anti-Rh, who had acquired hypogammaglobulinemia. As a result of this observation, Grubb ran similar tests on serum samples from several hundred donors (Table 1). Among patients with diseases other than rheumatoid arthritis whose serum agglutinated cells coated with an incomplete anti-D were those with leukemia, cancer, peptic ulcer and the Guillain-Barré syndrome. Grubb classified the agglutinating sera into two broad types: (a) rheumatoid arthritic: these sera yielded firm agglutinates which formed at 18 C. as well as 37 C.,

---

This chapter is dedicated with deepest gratitude, admiration and affection to Professor L. C. Dunn, who introduced me to genetics, guided my studies for the Ph.D. and, unknown to him, served as a model of the kind of person I wanted and want to be. All of us who have the good fortune to know him are richer for that experience.

The author's investigations mentioned in this review were supported in part by PHS research grant RG-7214 from the Division of General Medical Sciences, and PHS research grant H-3708 from the National Heart Institute, Public Health Service.

TABLE 1—*Grubb's Data on the Frequency of Occurrence of the Agglutinating Factor in the Serum of Various Donors*

Serum		Active	
Donor's Health	No.	No.	%
Normal	333	1	0.3
Various Ills	291	13	4.5
Rheumatoid Arthritis	51	8	15.7

and had a titer greater than 1/250; the activity of these sera was inhibited by pooled gamma globulin and by about 60 per cent of sera from normal individuals; (b) nonrheumatoid: these sera yielded less firm agglutinates, had a lower titer, were essentially inactive at 37 C., and their activity was not inhibited by pooled gamma globulin.

All the rheumatoid sera which agglutinated the coated cells were positive (at least weakly) for the Waaler-Rose test for the rheumatoid agglutinating factor (RAF), while the nonrheumatoid sera which agglutinated the sensitized red blood cells (SRBC) were negative, or at most weakly positive, in the Waaler-Rose test (Grubb, 1956). Absorption with streptococcus L or O agglutinins did not inactivate the rheumatoid agglutinators, but treatment with bentonite did (Grubb, 1956).

Grubb and Laurell (1956) showed that the ability of sera to inhibit the agglutinating activity of rheumatoid sera is determined by a dominant gene, present in some 60 per cent of the Swedish population and in about 95 per cent of Eskimos. They demonstrated that the inhibitor is located in the gamma globulin fraction of serum and therefore called inhibiting sera Gm(a+), and noninhibiting sera Gm(a−). Grubb and Laurell showed also that the frequency of Gm(a+) is independent of sex and is not affected by pregnancy. All samples were tested for ABO and D; no association was found between them and Gm. Similarly, no association was found between Gm and the MNS, P, Kell, Le and Lu blood type loci in the 30 samples tested for these factors.

It is interesting to note that two other groups of investigators also reported in 1956 that certain sera could cause coated RBC to agglutinate. Waller and Vaughan (1956) reported that 30 of 173 incomplete anti-D sera could be used to sensitize Rh positive cells so that the coated cells were agglutinated by rheumatoid sera and that one of 76 sera from healthy donors also caused the cells to agglutinate. Milgrom, Dubiski and Wozniczko (1956) reported that 10 of 2,000 sera from healthy donors agglutinated RBC coated with incomplete anti-D sera. The titers were as least 1/8. They reported that the agglutinating factors were not inactivated by exposure to 56 C. for 30 minutes, were absorbable by sensitized RBC, and not inhibited by pooled gamma globulin or individual sera.

Heller, Jacobson, Kolodny and Kammerer (1954) found that certain normal sera when used as a diluent for the RA sera in the Waaler-Rose test, inhibited the test. They showed that the inhibitor was in Cohn's F II (i.e., was 7S gamma globulin often referred to as γ_2-globulin or simply γ-globulin). Unfortunately, neither they nor anyone else appears to have investigated the matter further.

Moullec, Kherumian, Sutton and Espagnon (1956) confirmed Grubb's brilliant observation that the ability to inhibit the agglutination [Gm(a+)] was determined by a dominant gene which was independent of sex and of the Rh blood type. They showed also that about 54 per cent of Parisian French were Gm(a+).

In 1957 Laurell and Grubb showed that the Gm(a) factor is independent of the haptoglobin types and of the Lewis blood groups. Linnet-Jepsen, Galatius-Jensen and Hauge (1958) confirmed the pattern of inheritance of Gm(a+) and reported that about 56 per cent of 1,284 Danes were Gm(a+), and that *Gm*^{a} is not associated with the ABO, MNS, Rh, P, Le, K, Lu or Fy blood types or with the Hp locus. They reported also that 68 pairs of identical twins were concordant for Gm(a), but that 18 of 51 pairs of fraternal twins were discordant.

These papers established the existence of the Gm factor and paved the way for investigations on the nature of the inhibitor (the Gm factor), the agglutinator, and the anti-D sera; on the relation of the factor to disease; on the population frequencies of Gm(a); and for the discovery and analysis of other factors. Some work on these problems appeared before 1959, but intensive work on them seems to have begun then. Recent reviews covering various aspects of the problem have been written by Ropartz (1960a) and Grubb (1959b, 1961).

The Method of Detection of the Hereditary Gamma Globulin Types

The method used to detect the hereditary gamma globulin factors varies slightly from laboratory to laboratory (Ropartz, 1960a), but the basic methodology is the same in all. I will describe the methods usually used in my laboratory.

The reagents commonly used for testing are:

1. Group O red blood cells (RBC) homozygous for the D antigen of the Rh system. We happen to use O R_1R_1 cells; others use with equal success O R_1R_2 or O R_2R_2 cells. Cells heterozygous for the D antigen may be used, but they take up less of the antibody (Masouredis, 1960; Masouredis, Chi and Ferguson, 1960) and therefore give less satisfactory reactions. Unfortunately, the cells from all donors apparently homozygous for the D

antigen are not equally usable, presumably they take up less of the antibody (because of variation in the D antigen?). Only trial can determine whether the cells are adequate.

2. An incomplete anti-D serum of high titer (1/64 or greater) in the Coombs reaction. The anti-D at a concentration of 1/8 should be negative toward cells suspended in saline. Not all incomplete anti-D sera may be used. In all cases thus far reported, the donor of the anti-D is positive for the gamma globulin factor revealed by the use of the anti-D (Grubb, 1957b; Harboe and Lundevall, 1959, and many others), but such positivity does not guarantee that the serum is usable. Here again, the best method for determining the usefulness of a serum is trial. An added complication is the observation that an anti-D which may not be usable with one agglutinating serum (see below) will work excellently with another agglutinating serum (Steinberg, Giles and Stauffer, 1960, and others).

3. An agglutinating serum. These sera are most often found in patients with rheumatoid arthritis (RA), less often in patients with other illnesses and least often in apparently healthy individuals. The reagents from RA patients differ from those from healthy donors in ways which will be discussed in a later section.

In general, the donor of the agglutinating serum is negative for the factor which the serum detects (Grubb, 1958; Laurell and Grubb, 1958; Swahn and Grubb, 1958; Harboe, 1960b), but, as is the case with the anti-D sera, the appropriate Gm or Inv phenotype does not guarantee the usefulness of the serum. Once more only trial can determine this.

The procedure we use to find a system of reagents (agglutinator/anti-D) is to coat the RBC with an anti-D serum (at a 1/10 concentration), and then expose one drop of a 0.3 per cent saline suspension of the washed coated cells to one drop of serum diluted 1/40 if from an RA donor, 1/8 if from a healthy donor. The test is done in a depression of a glass microflocculation test slide. The mixture is shaken (at about 60 r.p.m.) for about five minutes and then placed in a moist chamber (a plastic pan inverted over a wet cloth towel) at room temperature for about 45 minutes. The mixture is then again shaken for about five minutes and the reaction read at 45, 90, or 120× under a dissecting binocular microscope. If the cells are agglutinated, the serum is titrated in serial twofold dilutions against coated cells. Initial trials for gamma globulin typing are done with the agglutinating serum used at a concentration two to four times as great as the minimum concentration giving a three plus agglutination of the cells. Sera of known gamma globulin types diluted 1/4, 1/8, 1/16 and 1/32 are used to test the system. One drop of a given dilution of the test serum is mixed in a well of the slide with one drop of the appropriately diluted agglutinating serum. The mixture is shaken at 60 r.p.m. for about five minutes. One

drop of a 0.3 per cent suspension in normal saline of coated, washed RBC is then added to the mixture. The remainder of the procedure is as described above. Again, trial and error determine the appropriate concentrations of the various reagents (anti-D serum, agglutinating serum, and test sera) to use for a given test.

The tests are performed as follows:

A. Controls: Two types of controls are run with each test. One consists of the addition of one drop of the suspension of coated RBC to one drop of normal saline plus one drop of appropriately diluted agglutinating serum, to establish that the agglutinator is active; if it is, the cells will be agglutinated. In the other, the serum to be tested replaces the agglutinating serum. This is to see if the serum to be tested is an agglutinator. If it is not, the cells will remain unagglutinated and the test has meaning. If the cells agglutinate, a new useful agglutinator may have been discovered.

In addition to the above controls, known positive and negative sera are tested to establish that the entire system is working properly.

B. The typing test: One drop of appropriate dilutions of the serum to be tested is mixed with one drop of the diluted agglutinating serum. The mixture is shaken for approximately five minutes at about 60 r.p.m., after which one drop of the suspension of the SRBC is added. The mixture is shaken for 5 minutes and then allowed to stand in a moist chamber at room temperature for 40 minutes. It is again shaken for 5 to 10 minutes and then read as described above.

If the cells fail to agglutinate in the presence of the mixture of the tested serum and the agglutinating serum, the former is said to be positive; if the cells agglutinate, the tested serum is negative.

The tests may be illustrated as follows:

		Agglutination
	RBC suspended in saline + anti-D → SRBC	−
(a) Control 1	Agglutinator + saline + SRBC	+
(b) Control 2	Test serum + saline + SRBC	−
(c) Test	Agglutinator + negative test serum + SRBC	+
(d) or	Agglutinator + positive test serum + SRBC	−

Table 2 lists some examples of the dilutions at which we use various reagents for the Gm and Inv tests. As can be seen from the table, the anti-D/agglutinator combination together determine the gamma globulin factor which will be detected. This will be discussed in a later section.

The Genetics of the Gm and Inv Factors

Table 3 lists the Gm and Inv factors thus far discovered. Each of the factors has been shown (by the authors first reporting the factors and later

TABLE 2—*Examples of Reagents Used to Test for Various Gamma Globulin Factors*

	Agglutinator	Dilution	Anti-D	Dilution	Dilutions of Tested Sera
Gm(a)	Wils.	1/8	Kim.	1/10	1/8, 1/16
	Rh 7	1/16	Ham.	1/10	1/8, 1/16
	Bowers	1/32	Kim.	1/20	1/16, 1/32
Gm(b)	Bomb.	1/32	Ham.	1/10	1/8, 1/16
	Bomb.	1/128	Greenway	1/10	1/8, 1/16
	A. Berg	1/30	Warren	1/10	1/8, 1/16
Gm(x)	Bowers	1/48	Ham.	1/10	1/16, 1/32
	Bowers	1/64	Mag.	1/10	1/4, 1/8
Gm-like	Carp.	1/16	Warren	1/5	1/16, 1/32
	Bowers	1/16	Warren	1/5	1/8, 1/16
	Bowers	1/16	2,147	1/10	1/8, 1/16
Inv(a)	LeClerc	1/8	Roehm	1/5	1/16, 1/32
Inv(b)	Lucas	1/4	Ham.	1/3	1/16, 1/32

by others) to be inherited as a dominant, or, when properly tested, as a co-dominant.

A. Caucasoids

Among Caucasians, Gm(a) and Gm(b) are inherited as alleles (Harboe, 1959; Ropartz, 1960a; Steinberg, 1962a; and others). Gm(x) is usually produced by an allele Gm^{ax} which also produces Gm(a) (Harboe and Lundevall, 1959, and others). But, as we shall see, exceptions occur to both these generalizations. Gm(r) (Brandtzaeg and Mohr, 1961; Brandtzaeg, Fudenberg and Mohr, 1961) thus far appears to be produced by an allele Gm^{ar} which also produces Gm(a), and by an allele Gm^{arx} which produces Gm(x) as well as Gm(a) and Gm(r). Among 356 Gm(a+) Caucasoids about 90 per cent were Gm(r+) and all of 44 Gm(x+) individuals were Gm(r+) (Mohr and Brandtzaeg, 1961). Gm-like has not been shown to be present in Caucasoids, nor is it known whether this factor is deter-

TABLE 3—*Gm and Inv Factors as of December 1961*

Factor	Reported	Reference
Gm(a)	1956	Grubb and Laurell, 1956
Gm(b)	1959	Harboe, 1959
Gm(x)	1959	Harboe and Lundevall, 1959
Gm-like	1960	Steinberg, Giles, and Stauffer, 1960
Gm(r)	1961	Brandtzaeg, Fudenberg, and Mohr, 1961
Inv(a)	1961	Ropartz, Lenoir, and Rivat, 1961
Inv(b)	1962	Steinberg, Wilson, and Lanset, 1962

TABLE 4—*Phenotypes and Genotypes (Usual Alleles Only) Exhibited by Caucasoids*

A. Gm locus°

Phenotype a	Phenotype x	Phenotype b	Genotype
+	—	—	Gm^a/Gm^a
+	+	—	Gm^{ax}/Gm^{ax}; Gm^{ax}/Gm^a
+	+	+	Gm^{ax}/Gm^b
+	—	+	Gm^a/Gm^b
—	—	+	Gm^b/Gm^b

B. Inv locus

Phenotype a	Phenotype b	Genotype
+	—	Inv^a/Inv^a
+	+	Inv^a/Inv^b
—	+	Inv^b/Inv^b

° Gm(r) has not been included because it has been insufficiently tested and because reagents to test it are not generally available.

mined by an allele of the *Gm* locus or of some other locus (Steinberg, Giles and Stauffer, 1960).

Inv(a) (formerly InV [Ropartz, Lenoir and Rivat, 1961; Ropartz, Rousseau, Rivat and Lenoir, 1961]) was shown on the basis of population studies, to be independent of the Gm locus and of Gm-like (Ropartz, Lenoir and Rivat, 1961; Ropartz, Rousseau, Rivat and Lenoir, 1961). Linkage studies have not yet been done so it can only be said that this factor and Inv(b) (Steinberg, Wilson and Lanset, 1962), are due to alleles at a different locus from the Gm locus and from that causing Gm-like.

The phenotypes and genotypes (common alleles only) among Caucasoids are shown in Table 4. Gm(r) is not shown in the table, because it has been insufficiently tested and because reagents are not generally available to test for this factor.

Table 5 shows the data from 292 white families with 1,724 children tested for Gm(a), Gm(x) and Gm(b) (Steinberg, 1962a, and unpublished data). The data clearly fit the genetic hypothesis outlined above for these three factors.

Henningsen (personal communication) has found a Danish family in which an allele Gm^{xb} seems to be present (Table 6) and Steinberg, Lai, Vos, Singh and Lim (1961) have reported Chinese families in which the allele Gm^{xb} or Gm^{axb} may exist.

Three related families in a religious isolate in the U.S. and a Danish family (Steinberg, 1962a; Nielsen and Henningsen, 1961, respectively)

TABLE 5—*Summary of Gm Data for 292 White Families of a Religious Isolate*°

Mating	No. of Families	Offspring					
			Phenotypes				
		Total	a	b	ab	ax	axb
ab X b	102	597	–	297	300	–	–
ab X a	8	40	21	–	19	–	–
ab X ab	29	200	50	49	101	–	–
ab X axb	12	60	–	19	13	15	13
axb X b	20	139	–	73	–	–	66
axb X axb	3	20	–	6	–	4	10
a X b	15	78	–	–	78	–	–
b X b	91	529	–	529	–	–	–
a X a	4	28	28	–	–	–	–
ab X ax	3	8	3	–	1	2	2
ax X b	3	17	–	–	6	–	11
axb X a	2	8	–	–	3	5	–
Total	292	1,724	102	973	521	26	102

° All sera were tested for Gm(a), Gm(x) and Gm(b). Only the positive reactions are listed in the table.

The data include those published in Steinberg, 1962a.

indicate that an allele which produces neither Gm(a) nor Gm(b) exists at the Gm locus (Table 7).

More recently Ropartz, Rivat and Rousseau (1962) have found an Iranian who is Gm(a– b– x–); Inv(a– b+). His gamma globulin level is normal.

B. Negroids

Moullec, Fine, Henry and Silverie (1959) reported that all of 449 Negroes from Dakar were Gm(a+). Steinberg, Giles and Stauffer (1960) reported that 96 of 98 American Negroes were Gm(a+) and suggested that miscegenation could explain the occurrence of Gm(a–) individuals among American Negroes. These data would lead one to expect essentially all Negroes to be Gm(b–). Steinberg, Stauffer and Boyer (1960) found, however, that about 98 per cent of 623 American Negroes were Gm(b+). Their data for Negroes and whites tested for Gm(a) and Gm(b) are pre-

TABLE 6—*Family Suggesting a* Gm^{bx} *Allele (Henningsen)*

	Mother	Father	Children
Phenotypes	abx	b	3 bx + 3 abx
Genotypes	*ax/bx*	*b/b*	*bx/b* *ax/b*

TABLE 7—*Families Suggesting an Undetected Allele*

Family	Mother	Father	Children
61.10°	a	Dead	3 ab 4 b
68.08°	ab	b	3 b 3 a
151.07†	a	b	4 ab 4 a
Danish‡	a	b	4 ab 2 b

° Steinberg, 1962a.
† Steinberg, unpublished.
‡ Nielsen and Henningsen, 1961.

sented in Table 8. Steinberg, Stauffer and Boyer (1960) concluded that Negroes have an allele Gm^{ab} which produces both Gm factors and that Negroes with no white ancestry are all phenotypically Gm(a+ b+). The latter assumption was confirmed by the observations of Ropartz, Rivat and Lenoir (1960b), and of Boyer and Watson-Williams (1961) that all of 708 African Negroes were Gm(a+ b+). On the basis of these assumptions and using the computed frequency of the Gm^a and Gm^b alleles among American Negroes, it was estimated that American Negroes have approximately 31 per cent of white ancestry (the source of Gm^a and Gm^b alleles) (Steinberg, Stauffer and Boyer, 1960). This estimate is almost identical with that reported in 1953 by Glass and Li (30.6 per cent) using the Rh blood groups.

Steinberg, Giles and Stauffer (1960) reported that one of 75 American Negroes was Gm(x+). These authors believe the Gm(x+) resulted from miscegenation, and that Negroes of unmixed ancestry have no Gm(x). This surmise is contradicted by the report by Ropartz, Rivat and Lenoir (1960b) that two of 399 Negroes from Dakar were Gm(x+); however, in a paper presented at the Second International Conference on Genetics (September, 1961), Ropartz stated that Negroes have no Gm(x) factor (Ropartz, Rousseau et Rivat, 1961b). It is possible that Gm(x) oc-

TABLE 8—*Tests of Serum from U. S. Negroes and U. S. Whites for Gm(a) and Gm(b)*
(Data from Steinberg, Stauffer and Boyer, 1960)

	Gm(a+ b+)		Gm(a+ b—)		Gm(a— b+)	
Total	No.	%	No.	%	No.	%
			A. Negroes			
623	593	95.2	14	2.2	16	2.6
			B. Whites			
249	121	48.6	23	9.2	105	42.2

curs rarely among Negroes of unmixed ancestry, but further testing is desirable.

Gm-like (Steinberg, Giles and Stauffer, 1960) has thus far been found only in Negroes although it has been looked for in whites (ibid and unpublished data), Chinese and Indians (Steinberg, Lai, Vos, Singh and Lim, 1961), Japanese (Ropartz, Rivat, Rousseau et Lenoir, 1961), Eskimos, Amerinds (Steinberg, Stauffer, Blumberg and Fudenberg, 1961), and Australian Aborigines (Steinberg, Vos and Kirk, unpublished data). Its relation to the Gm locus is not clear because Negroes tend to be 100 per cent Gm(a+ b+ x−) and therefore show no segregation at this locus.

Ropartz, Rousseau, Rivat and Lenoir (1961) found 53.6 per cent of 399 Negroes from Dakar to be Inv(a+), and Steinberg, Wilson and Lanset (1962) found 52.8 per cent of 329 American Negroes to be Inv(a+). Steinberg et al. (1962) tested 165 Negroes for Inv(a) and Inv(b). Their data are presented in Table 9. They confirmed Ropartz, Rousseau, Rivat and Lenoir's (1961) finding that the Inv locus is independent of the Gm-like locus.

Negroes appear to have only the allele Gm^{ab} at the Gm locus. [They have not been tested for Gm(r).] They have the Gm-like allele also, but, as stated earlier, it is not known how this is related to the Gm locus. Negroes have the alleles Inv^{a} and Inv^{b}, and possibly an allele Inv^{-} producing neither Inv(a) nor Inv(b) (Steinberg et al., 1962).

C. Mongoloids (Chinese, Japanese, American Indians and Eskimos)

Steinberg, Lai, Vos, Singh and Lim (1961) tested 90 unrelated Chinese from Malaya for Gm(a), Gm(b), Gm(x) and Gm-like. All were Gm-like(−) and Gm(a+). Ninety-three per cent were Gm(b+) and 5.5 per cent were Gm(x+). These data and family data (Steinberg, Lai, Vos, Singh and Lim, 1961) suggest that the Chinese have alleles Gm^{ab}, Gm^{a} and Gm^{ax} with frequencies .741, .231 and .028, respectively. Ropartz (personal communication) has tested over 500 Chinese and has found essentially the same frequencies.

Boyer, Iseki and Mayeda (1961) tested 262 Japanese for Gm(a) and Gm(b) and found all Gm(a+) and 22.9 per cent Gm(b+). Ottensooser (personal communication) tested 58 Japanese for Gm(a), Gm(b) and

TABLE 9—*Data for 165 Sera from Negroes Tested for Inv(a) and Inv(b)*
(Data from Steinberg, Wilson and Lanset, 1962)

	Inv(a+ b−)	Inv(a+ b+)	Inv(a− b+)	Inv(a− b−)	Total
No.	22	74	69	0	165
%	13.3	44.9	41.8	–	100.0

Gm(x), while Ropartz, Rivat, Rousseau and Lenoir (1961) tested 109 Japanese for Gm(a), Gm(b), Gm(x), Gm-like and Inv(a); all were Gm-like(−). Ottensooser, and Ropartz, Rivat, Rousseau and Lenoir (1961) also found all Japanese to be Gm(a+). Yokoyama and Ueno (1961) reported that 1.9 per cent of 952 Japanese were Gm(a−). Ropartz et al. (1961) point out that this percentage corresponds to the frequency of sera which in their experiment agglutinated the coated cells and suggest that this is what occurred in Yokoyama and Ueno's experiment. Since the latter did not run controls, this explanation seems reasonable.

Ottensooser found 22.4 per cent of the Japanese Gm(b+) and 43.1 per cent Gm(x+), while Ropartz, Rivat, Rousseau and Lenoir (1961) found 19.2 per cent Gm(b+), 34.8 per cent Gm(x+) and 50.4 per cent to be Inv(a+). In a study of another 270 Japanese Ropartz et al. (1961) found 47.8 per cent Inv(a+). The Japanese, like the Chinese, have alleles Gm^{ab}, Gm^{a}, and Gm^{ax}. Steinberg, Lai, Vos, Singh and Lim (1961), using Ottensooser's and Ropartz et al.'s data, have estimated the frequencies of these alleles to be .108, .681 and .211, respectively. It is clear that the three alleles occur with markedly different frequencies in the Chinese and Japanese.

Tests of 109 Alaskan Indians, of 34 South American Indians and of 50 Alaskan Eskimos for Gm(a), Gm(b), Gm(x) and Gm-like (Steinberg, Lai, Vos, Singh and Lim, 1961) indicate that these populations all have the alleles Gm^{ab}, Gm^{a} and Gm^{ax} as do the Chinese and Japanese. The estimated frequencies of these alleles among the Alaskan Indians were .009, .787 and .204, respectively, i.e., similar to the frequencies among the Japanese. The sample sizes for the South American Indians and the Eskimos are too small for meaningful estimates to be made.

It appears from the foregoing data that Caucasoid populations are characterized by alleles Gm^{a}, Gm^{b} and Gm^{ax}, and the absence of Gm-like; Negroid populations by allele Gm^{ab}, the absence of Gm(x), and the presence of Gm-like; Mongoloid populations by alleles Gm^{ab}, Gm^{a} and Gm^{ax} and the absence of Gm-like.

The Australian Aborigines from the Western Desert region seem to be unique in that in them alleles producing Gm(b) are absent or very rare. This is not true of the Aborigines from the coastal region (Steinberg, Vos and Kirk, unpublished). The Desert Aborigines appear, on the basis of a sample of about 300, to have alleles Gm^{a} and Gm^{ax} only, while those from the coastal region appear (also on a sample of about 300) to have Gm^{ab}, Gm^{a} and Gm^{ax}, i.e., those present in the Mongoloid populations.

The Inv(a) and Inv(b) alleles have been observed in all populations thus far studied. Inv(a) appears to have the lowest frequency in Caucasoids.

The Agglutinating Factors

The initial work on Gm (Grubb, 1956; Grubb and Laurell, 1956; Moullec et al., 1956) was, and most of the subsequent work has been, done using serum from patients with rheumatoid arthritis (RA) as the agglutinator. Grubb initially indicated that nonrheumatoid sera capable of agglutinating sensitized cells are not inhibited by gamma globulin. These sera are similar to those reported by Milgrom et al. (1956).

All RA sera usable for Gm testing (hereinafter called Ragg to contrast with sera from normal donors called SNagg—see below) are positive for one or more of the tests for the rheumatoid factor. However, not all sera positive for the rheumatoid factor may be used for Gm testing (Grubb, 1956, 1957a, 1959a, and others). Grubb (1957a) tested 91 RA sera against RBC coated with an incomplete anti-D serum. Thirty-seven per cent of them caused the cells to agglutinate and all of them occurred among the 83 per cent with a titer of at least 1/32 in the Waaler-Rose test. In a number of cases, both the agglutinator and the substrate (the inhibitor) with which it reacts were present in the serum. These sera showed a prozone when titrated against sensitized RBC (Grubb, 1957a; Laurell and Grubb, 1958; Harboe, 1960b).

A possible explanation of the prozone was provided by the finding (Grubb, 1958) that the inhibition of Ragg serum by Gm(a+) may be overcome by dilution of the Ragg-Gm(a+) mixture with saline. This observation suggests that the prozone shown by RA sera from Gm(a+) donors is due to an association between the Gm(a) factor and the agglutinating factor. Support for this suggestion is derived from the demonstrations that removal of Cohn fraction II from such sera abolished the prozone (Swahn and Grubb, 1958), and that the precipitate obtained by cold distilled water from RA sera showing a prozone agglutinated SRBC without a prozone (Harboe, 1960b). In some cases, the precipitates were active against SRBC which were not agglutinated by the whole serum. Apparently, the agglutinator in the whole serum is completely inactivated by the inhibitor which is also present. Precipitation of the macroglobulins from the serum separates the agglutinator from the inhibitor and permits its detection.

Not all RA sera of high titer in the Waaler-Rose test are usable for the detection of Gm factors. Some cases of failure clearly occur because an inhibitor of high titer is present in the donor's serum, but others may occur because the appropriate incomplete antibody with which to coat the cells is not available. Thus, the system Ragg Bowers/anti-D Kim. tests for Gm(a), the system Ragg Bomb./anti-D Green. tests for Gm(b), but Ragg Bowers does not cause cells coated with anti-D Green. to agglutinate.

The agglutinator in Ragg sera remains active after heating at 56 C., but is destroyed by heating at 63 C. for one-half hour or less (Grubb, 1956, and others). Fractionation of serum by Cohn's method 10 and ultracentrifugation studies of gamma globulins separated from serum by starch block electrophoresis show that the agglutinator in Ragg serum active in the Gm system is a macroglobulin (Swahn and Grubb, 1958) with a sedimentation rate of 19S (Fudenberg, unpublished). Franklin, Kunkel, Müller-Eberhard and Holman (1957) refer to this as 19S globulin, while Grabar and his colleagues call it β_2M (Grabar et Burtin, 1960). Henceforth, the 19S gamma globulin will be referred to as β_2M and the 7S gamma globulin as simply gamma globulin. The so-called rheumatoid agglutinating factor(s) (RAF) detected in rheumatoid serum (Lamont-Havers, 1957) by various agglutinating techniques is (are) also β_2M globulin(s). This poses the question of what is the relationship between the Gm agglutinator (Ragg) and the RAF?

Grubb (1959a) compared the reactions obtained in the Waaler-Rose test which uses rabbit anti-sheep amboceptor with those obtained in the Gm tests. He summarized his results as follows:

Similarities: (1) "Both factors are fairly specific of rheumatoid arthritis"; (2) "Both interact with globulin"; (3) "Both separate out in the same heavy-weight fraction upon ultra-centrifugation."

Differences: (1) "The Waaler-Rose test is positive also with sera heated at 63 C. whereas this treatment abolishes the capacity of the sera to agglutinate coated Rh-positive cells"; (2) "The Waaler-Rose test is more difficult to inhibit with human γ-globulin." (3) "The Waller-Rose test is (not*) more frequently positive in Gm(a+) patients and it is a rare event to find agglutination of the coated Rh-positive cells in sera negative in the Waaler-Rose test." Grubb (1956) had previously reported that absorption with streptococcol L- and O- agglutinins did not inactivate the agglutinator in Ragg sera.

The relation between the Ragg factor and the RAF detected by the F II latex particle test (Hyland Laboratories) has been investigated in my laboratory. Since both tests employ human γ-globulin, it may be expected that the RAF detected by the latex test would show greater similarity to the Ragg factor than would the factor detected by the Waaler-Rose test, and so it does. In addition to the similarities quoted from Grubb's work, serum heated at 63 C. fails to agglutinate the coated latex particles as well as the sensitized red blood cells, although longer heating is required to inactivate the serum against the latex particles. The two factors are not identical, however, because sera from which all detectable anti-Gm ac-

* Added by A. G. Steinberg because it seems clear that it was omitted from the original by error.

TABLE 10—*Absorption Experiments with Ragg Bowers and SNagg Wils.*

Absorbent	Action of Absorbed Serum Against					
	Latex		Waaler-Rose‡		SRBC§	
	R°	S†	R	S	R	S
1. None	+	—	+	—	+	+
2. SRBC	+	—	+	—	—	—
3. Latex + pooled γ-globulin	—	—	—	—	—	—
4. Latex + Gm(a−) γ-globulin	—	—	—	—	—	+
5. Waaler-Rose	+	—	—	—	+	+

° R = Ragg Bowers.
† S = SNagg Wils.
‡ The modified Waaler-Rose test using rabbit anti-human amboceptor.
§ SRBC = sensitized red blood cells.

tivity has been absorbed, by repeated (as many as 10) absorptions with RBC coated with various anti-D sera, still retain an essentially unchanged titer against latex particles coated with F II derived from pooled sera.

A summary of the comparisons of the RAF detected by the modified Waaler-Rose test (which employs rabbit anti-human amboceptor instead of rabbit anti-sheep amboceptor [Jacqueline, Eyquem et Podliachouk, 1957; Podliachouk, Eyquem et Jacqueline, 1958]) with the RAF detected by F II coated latex particles and with the agglutinator detected by the Gm system by means of absorption experiments is presented in Table 10 (Steinberg, unpublished).

The data concerning the effect of the latex particles coated with pooled γ-globulin will be discussed later. The data in the remainder of the table suggest that the β_2M molecules in Ragg sera are of at least three types: (a) one with "latex," "Waaler-Rose," and Gm specificities (line 4, Table 10 in conjunction with lines 2 and 5), (b) one with "latex" and Gm specificity (lines 2 and 4, Table 10) and (c) one with "latex" and "Waaler-Rose" specificity (lines 4 and 5, Table 10). Hence, as far as these experiments are concerned, all β_2M molecules in Ragg sera have activity against coated latex particles, some of these have Gm activity in addition, and others have activity against rabbit amboceptor. Similar observations using rabbit anti-egg albumin-egg albumin precipitates and human diphtheria antitoxin precipitates have been reported by Aho (1961).

Grubb (1961) has recently published a detailed and penetrating review of the relation between Gm activity and rheumatoid arthritis. The reader is referred to that paper for a more extensive discussion of the material reviewed above.

Ropartz and Hurel (1959) demonstrated by titration tests of 10 RA sera

TABLE 11—*Effect of Absorption of a Ragg Serum With a Given Anti-D on the Ability of the Serum to Agglutinate Cells Coated With Another Anti-D*°

	Cells coated with anti-D		
	Kim.	Ham.	Warren
Ragg Bomb.			
Unabsorbed	+	+	+
Absorbed with:			
Kim.	—	—	—
Ham.	+	—	—
Warren	+	+	—
Ragg Bowers			
Unabsorbed	+	+	+
Absorbed with:			
Kim.	—	+	—
Ham.	+	—	—
Warren	+	+	—

° The Ragg serum has a titer of 1,024 or greater against each of the anti-D's used in the experiment. The Ragg serum was diluted 1/128 and then separate aliquots were absorbed 7 times with cells coated with a given anti-D.

against 14 anti-D sera that the titer of agglutination for a given RA serum varied depending upon the anti-D used, and that the usefulness of a given anti-D depended upon the RA serum used. They suggested that the data could be explained by assuming that there are several agglutinating factors or a single multivalent agglutinating factor in a given RA serum. The discovery that Ragg Bomb. and Ragg Bowers each could be used for more than one factor (Steinberg, Giles and Stauffer, 1960; Harboe, 1960a) and the subsequent observation that many of the Ragg sera when properly tested prove to be polyvalent, lend support to this suggestion. Furthermore, absorption experiments with various anti-D sera have shown that these factors must in part, at least, be on different molecules (Harboe, 1960b; Steinberg, 1962b). The results of one series of absorption experiments done in my laboratory are shown in Table 11. Absorption of Bomb. with RBC coated with Kim. removed activity against the other two anti-D's but similar absorption of Bowers left activity against Ham. When more anti-D sera are considered the pattern becomes more complex. Since we have not yet explored this matter in detail, it will not be discussed further at this time, beyond emphasizing that absorption of a Ragg with cells coated with one anti-D does not remove activity against cells coated with any one of several other anti-D sera.

Grubb (1956) and subsequently many others observed that all Gm(a—) sera inhibit anti-Gm(a) Ragg sera, albeit with a low titer. Hence,

Gm(a+) sera are distinguished from Gm(a−) sera by having a higher inhibiting titer. This led to the postulate that the distinction between Gm(a−) and Gm(a+) is quantitative rather than qualitative (Grubb, 1957b, and others). An alternative explanation is that the inhibition of Ragg sera by Gm(−) sera is nonspecific; that a reaction occurs with a non-Gm portion of the macroglobulin molecule and that this reaction inhibits the Gm activity of the Ragg serum by steric hindrance. Further discussion of this hypothesis will be delayed until the agglutinators in serum from healthy donors have been discussed.

Grubb (1957a, b) reported that the agglutinating activity in the serum from a healthy donor could be inhibited by pooled gamma globulin. He did not, however, report on whether this serum showed specificity in tests for the gamma globulin factors. It remained for Ropartz and his colleagues to show that such reagents could be used to test for the Gm factors (Ropartz et Lenoir, 1960a; Ropartz, Lenoir et Rivat, 1960; Ropartz, Rivat et Lenoir, 1960a) and for Inv (Ropartz, Lenoir, Hemet et Rivat, 1960; Ropartz, Lenoir et Rivat, 1961).

As has already been indicated, all Gm(−) sera in high concentration (dilution of ¼ or less) inhibit the agglutinating activity of the RA sera. Ropartz, Lenoir, Hemet and Rivat (1960) and Ropartz, Rousseau et Rivat (1961a) showed that the agglutinating activity in sera from nonrheumatoid donors was not inhibited by Gm(−) sera even when the latter were tested neat. Furthermore, they noted that while the inhibiting titer of Gm(+) sera against Ragg was low (1/16 or 1/32) the same sera had a high titer (1/128 or more) against sera from non-RA donors. They concluded that while the difference between Gm(−) and Gm(+) as detected by Ragg sera is quantitative, as well as qualitative, the difference detected by serum from normal agglutinators is purely qualitative. They suggest that serum from normal donors be called SNagg (Sérum Normal agglutinant). As I have stated elsewhere, the term appeals to me and I have suggested that rheumatoid agglutinating serum be called Ragg. Ropartz et al. (1961a) suggest that the inhibitory reaction of Gm(−) sera against Ragg sera occur because the latter is often (usually?) polyvalent, i.e., may be used to test for more than one Gm factor. All SNagg sera studied thus far have been monovalent.

It is of interest to note that the agglutinating activity in SNagg sera is associated with a β_2 macroglobulin (Audran et Fine, 1961) as it is in Ragg sera (Fudenberg, personal communication). These observations and titration experiments showing that the number of inhibiting units in Gm(a+) sera is the same against Ragg and SNagg sera (Steinberg, 1962b), indicate that the agglutinators in Ragg and SNagg are probably the same.

There remain, however, two apparent differences between Ragg and

TABLE 12—*Mean Titers*° *(Expressed as Last 'Tube' of Serial Twofold Dilutions Showing Inhibition) of Unheated (U) and of Heated (H)*† *Normal Sera Against Ragg and SNagg Agglutinators*‡

		Ragg (1/64) (Bowers)		SNagg (1/8)			
				Wils.		Scol.	
Gm[a]	No.	U.	H.°	U.	H.°	U.	H.°
—	12	2.3	6.4	0	0	0	0
+	13	5.1	9.7	9.8	13.4	10.7	13.7

° Each serum was made up in serial twofold dilutions, the first tube being a 1/2 dilution.

† Heated at 63 C. until visible aggregates formed. The aggregates were not used for the agglutination inhibition tests.

‡ From Steinberg, 1962b.

SNagg agglutinators which require explanation: (1) Gm(−) sera in high concentration inhibit Ragg but not SNagg, although the former have a much higher agglutinating titer than the latter, and (2) Gm(a−) sera which have been heated at 63 C. until visible aggregates form (usually ½ hour) will inhibit Ragg sera even when considerably diluted (1/64) but will not inhibit SNagg sera even when used neat (Steinberg, 1962b; Table 12). In addition to these apparent differences there is a similarity which appears contradictory, namely, the titer of Gm(a+) sera heated at 63 C. is increased to the same extent against Ragg and SNagg sera (Steinberg, 1962b) (Table 12).

As has already been mentioned, the RAF is β_2M as are the agglutinators in Ragg and SNagg sera. The fact that absorption of the Gm agglutinating activity from Ragg sera has little or no effect on the titer of such sera in the latex test for RAF indicates that β_2M globulins which have RAF activity and no Gm agglutinating activity may exist. SNagg sera (which are negative for the RAF tests) clearly have β_2M globulin with Gm activity and no RAF activity. Tests were undertaken to determine if β_2M globulin molecules with Gm agglutinating activity but no RAF activity exist in Ragg sera. The initial experiments were performed with latex particles coated with pooled gamma globulin. (We are grateful to the Hyland Laboratories for the donation of this material.) Absorption of Ragg and SNagg sera with these particles rendered both inactive against SRBC. The latter effect was surprising until we found, at the suggestion of Dr. Henry Kunkel, that the gamma globulin washed off the latex particles. Since pooled gamma globulin was used, Gm(a+) was undoubtedly present and inactivated both the Ragg and the SNagg sera. Accordingly, latex particles were coated with gamma globulin from Gm(a−) donors. Absorption of

Ragg and SNagg sera with these particles removed the Gm agglutinating activity from the Ragg serum but not from the SNagg serum! Hence, it seems likely that molecules with only Gm activity do not exist in Ragg sera, i.e., they have molecules with either RAF activity or with both RAF and Gm agglutinating activity. SNagg sera, on the other hand, have molecules with only Gm agglutinating activity. With these postulates and the knowledge that gamma globulin combines with the β_2M of RA sera, the observations recorded above may be explained.

The gamma globulin in the Gm(−) sera reacts with the RAF activity of the macroglobulin in the Ragg serum (Fudenberg and Kunkel, 1961). The inhibition of Ragg by Gm(−)sera in high concentrations is probably due to steric hindrance resulting from this reaction. The increased titer of heated Gm(−) sera is due to the greater efficiency of heated (aggregated) gamma globulin in combination with the RAF in Ragg (Kunkel, 1957). Since SNagg sera have little or no RAF activity, there is no combination of the gamma globulin in Gm(−) sera with the macroglobulin of SNagg sera.

The increased titer of heated Gm(a+) against Ragg and SNagg agglutinators is probably also due to steric hindrance. It is assumed that the aggregates formed in heated Gm(a+) serum are in large measure composed of gamma globulin with specific Gm activity and of other proteins. (Podliachouk, Eyquem et Jacqueline [1960] found by electrophoretic analysis that Gm activity in heated serum extended from the region of fast albumins to the gamma globulins while it was limited to the region of gamma globulins in unheated serum.) These larger molecules react with the agglutinator via the specific activity of the 7S gamma globulin. The latter's inhibiting capacity is enhanced because each active molecule is combined with one or more other inactive molecules which cover reactive sites in the agglutinator. Podliachouk et al. (1960) and Henry (1960) have also suggested that the inhibitory activity of Gm(a−) sera in high concentration against Ragg agglutinators is nonspecific.

No information is available to explain the frequency of occurrence of agglutinating sera for the gamma globulin factors either among patients with various diseases or among healthy donors. Similarly, it is not known why Ragg sera are usually, if not always, polyvalent, while SNagg sera have thus far been found to be monovalent.

The Inhibitors

(Gm and Inv Factors)

Grubb and Laurell (1956) found that Cohn F II (7S γ-globulin, γ_2-globulin or simply γ-globulin) extracted from pooled serum inhibited Ragg

TABLE 13—*Gm Phenotypes of Mother-Newborn Child Pairs*

A. Negro Mother-Child Pairs

Gm Phenotype				Pairs		Inv Phenotype°		Pairs	
a	b	x	like	No.	%	a	b	No.	%
+	+	—	—	203	64.1	+	—	4	13
+	+	—	+	95	30.0	+	+	15	48
+	+	+	—	5	1.6	—	+	12	39
+	+	+	+	3	0.9	Total		31	100
—	+	—	—	6	1.9				
+	—	—	—	3	0.9				
+	—	+	—	2	0.6				
Total				317	100.0				

B. White Mother-Child Pairs

Gm Phenotype				Pairs	
a	b	x	like	No.	%
+	+	—	—	10	27
+	+	+	—	2	6
—	+	—	—	21	58
+	—	—	—	1	3
+	—	+	—	2	6
Total				36	100

° An additional 134 pairs were tested for Inv(a) only; 68 were Inv(a+) and 66 Inv(a—).

sera. Gamma globulin extracted by paper electrophoresis from seven individual Gm(a+) sera proved to be inhibitory, while similar extracts from Gm(a—) sera were noninhibitory.

Moullec et al. (1956) reasoned that since the only gamma globulin present in the serum of the newborn infant is that derived from its mother, it was likely that the infant's Gm(a) type would be the same as its mother's. Comparison of the Gm factors in mothers and in the cord blood of their newborn infants confirmed this for Gm(a) (Moullec et al., 1956; Brønnestam and Nilsson, 1957; Linnet-Jepsen, 1958; Steinberg, unpublished). Linnet-Jepsen (cited in Linnet-Jepsen et al., 1958) found that the Gm group of the infant does not yet seem to be fully developed at eight months. Studies in my laboratory have extended the observations concerning the identity of the gamma globulin type of the mother and her newborn child to all the other factors except Gm(r). The data are presented in Table 13. It has been demonstrated by isolation experiments that the inhibiting activity of Gm(b), Gm(x), Gm-like (Fudenberg, unpublished

data) and of Inv(a) (Ropartz, Lenoir et Rivat, 1961) and Inv(b) (Steinberg et al., 1962) is carried by gamma globulin.

The Gm(a) substance has been shown by Grubb (1959a) to be stable to heating at 63 C. for one-half hour, to pH 12.3 at 37 C., to treatment with periodate, sulfhydryl compounds and with trypsin. He found that its activity was destroyed by crystalline papain and by pepsin and ficin. From these observations, he concluded that the activity is not included in the carbohydrate part of the molecule and that the Gm(a) activity is lost on the splitting of a very few bonds of the gamma globulin molecule. So far as I am aware, similar analyses have not been made for the other Gm factors or for the Inv factors.

Gm(a) specificity is absent from human milk (Grubb, 1959b; Lawler, 1960) and from human colostrum (Lawler, 1960). Since the gamma globulin content of milk and colostrum is considerably less than that of serum, it would be of interest to repeat these experiments using SNagg agglutinators (agglutinators from normal donors) and concentrates of the gamma globulin from milk and colostrum.

There is no information concerning the biochemical difference between Gm(+) and Gm(−) gamma globulin. It is unlikely, however, that the difference is merely quantitative because no correlation between gamma globulin level (excluding hypogammaglobulinemic individuals whose sera are inactive, as expected) and Gm(a) type appears to exist (Moullec et al., 1959; Grubb, 1959b; Mårtensson, 1961) and because of the absence of activity of Gm(−) and Inv(−) sera against SNagg agglutinators.

Tests of the effect of heat (63 C. for one-half hour or until visible aggregates formed) on the action of Gm(a), Gm(b), Gm(x) and Gm-like against Ragg agglutinators showed that Gm(a+), Gm(b+) and Gm(x+) sera remained positive after heating, while Gm-like (+) became Gm-like (−); and that Gm(a−) and Gm(x−) became positive after heating, while Gm(b−) and Gm-like(−) remained negative after heating (Steinberg and Stauffer, 1960). These data were interpreted as indicating that at least three qualitatively different molecules or active sites must exist for the determination of these factors, one for Gm(a) and Gm(x), a second for Gm(b), and a third for Gm-like.

Mårtensson (1961) also concluded that Gm(a) and Gm(x) are probably carried by a single molecule and that Gm(b) is on another molecule. Mårtensson based his conclusion on his finding that none of M-component gamma globulin extracted from the serum of 16 patients with myeloma was Gm(a+ b+) (on the basis of population data eight Gm(a+ b+) would have been expected to occur), while of the three samples classified for Gm(a), Gm(b) and Gm(x), one was Gm(a+ b− x+); the other two were Gm(a− b+ x−). It is argued that myeloma protein is of a single kind

and is probably the product of a clone derived from a single cell (Martensson, 1961), hence the presence of Gm(a) and Gm(x) in the M-protein of a patient argues that one molecule carries both, while the absence of Gm(a+ b+) protein argues that Gm(a) and Gm(b) are produced by different cells and are on different molecules. These studies were done on sera from white patients. Gm(a+ b+) white individuals are heterozygotes, i.e., Gm^a/Gm^b. If Mårtensson's observations and interpretation are correct, they imply that only one allele is operative in a given cell. The observation of Gm(a+ b− x+) M protein is consistent with this implication, since Gm(a) and Gm(x) are produced by a single allele. It would be of interest to examine the M-component gamma globulin in the serum of Negro patients with myeloma. Such sera should be Gm(a+ b+), because Negroes have an allele which produces Gm(a) and Gm(b) and essentially all Negroes are Gm(a+ b+) (Steinberg, Stauffer and Boyer, 1960). Harboe (personal communication) has confirmed Mårtensson's findings.

The suggestion that in a given cell only a single allele is operative cannot apply in all cases since the RBC of individuals with the sickle cell trait (Hb^A/Hb^S) can all be made to sickle and hence must all carry HbS and at least some must carry HbA, since the greater proportion of the Hb in the cells of such individuals is HbA. Similarly, the vast majority, if not all the RBC of blood group AB individuals have both A and B antigens. An alternate explanation for Mårtensson's observations is that since it has been demonstrated that the M protein γ-globulins have different N-terminal amino acids from the gamma globulin of healthy patients (Putnam, 1959, 1960; Sjöquist and Laurell, 1961), they are abnormal and do not reflect the activity of normal serum proteins.

Mårtensson's observations on the Gm types of the gamma globulin extracted from myeloma serum may be compared with those of Audran, Fine, Moullec et Matte (1961) on the Gm types of the whole serum of 79 patients affected with multiple myeloma (Table 14). The authors point out that three of the phenotypes [Gm(a+ x+ b−), Gm(a+ x− b−), and Gm(a− x− b+)] among the myeloma sera correspond closely in frequency with those among the controls and that of the remaining three, the sum of the deficits in the frequencies of the Gm(a+ x+ b+) and Gm(a+ x− b+) phenotypes as compared to the controls equals the frequency of the Gm(a− x− b−) phenotype which appears among the myeloma sera but not among the control sera. This suggests that Gm(a) and Gm(x) occur with normal frequency in myeloma globulin but that Gm(b) occurs with reduced frequency.

Studies of the five Gm(a− x− b−) myeloma sera showed that four inhibited rabbit anti-human globulin sera (Coombs sera) with titers comparable to those in sera with detectable Gm types. Three of these four sera

TABLE 14—*Gm Phenotypes of the Serum of 79 Myeloma Patients Compared with the Phenotypes of 705 Controls*

(Data from Audran, Fine, Moullec et Matte, 1961)

Gm Phenotypes			Frequencies in Per Cent	
a	x	b	Myeloma Patients	Controls
+	+	+	9	11.9
+	+	—	4	4.7
+	—	+	27	29.9
+	—	—	5	4.3
—	—	+	49	49.2
—	—	—	6	0.0
			100	100.0

appeared, on the basis of immunoelectrophoretic studies, to have β_2A globulin (two had the mobility of β_2 and the third that of β_1), the fourth, γ-globulin with the mobility of β_1. Audran et al. (1961) suggest that the inhibition of the rabbit anti-human γ-globulin is due to a cross reaction; myeloma β globulins having some antigenic groups in common with γ-globulin.

The presence of the Gm(a+ b+) phenotype in 36 per cent of the myeloma sera is in contradiction to Mårtensson's findings. The difference in the findings may result from the fact that Mårtensson studied extracted γ-globulins while Audran et al. studied whole serum. (The myeloma globulin was not γ-globulin in 30 of the 79 sera studied [Fine et Cryssel, 1961]).

It was argued that it is unlikely that the change on heating, of Gm(a—) and Gm(x—) to Gm(a+) and Gm(x+), respectively, is due to a nonspecific interaction of the aggregates with the RAF in the Ragg agglutinator (Steinberg and Stauffer, 1960). I now believe, in the light of experiments discussed in relation to the agglutinators, that this interpretation is wrong. Difficulty remains in explaining the data relative to Gm(b) and Gm-like. It should be noted, however, that the same agglutinator was used to test for Gm(b) and for Gm-like, hence the peculiarity of these factors may be due to the agglutinator (Bomb.) rather than to the factors per se. We plan to repeat the experiments using agglutinators which have since become available.

Podliachouk, Eyquem and Jacqueline (1960) reported that 100 sera left at room temperature for one day and then stored at +4 C. for 10 days were nearly all Gm(a—). After heating at 63 C. for 10 minutes, 51 were Gm(a+) and 49 Gm(a—), i.e., the frequency usually observed in the

French population. They report that such heating does not render Gm(a−), Gm(a+) and recommend that sera be heated before testing. Steinberg and Stauffer (1960) found that Gm(a−) sera diluted (½) do not change on heating. It would appear safer to dilute the serum before heating, if it is planned to heat the serum at all. The Gm factors have been observed to be stable in sera stored at −10 C. (or lower) for as long as five years (Podliachouk, Jacqueline and Eyquem, 1958).

From the foregoing it seems clear that the inhibitors (the Gm and Inv factors) are gamma globulins, that their specificity probably requires essentially the entire molecule and that they are qualitatively different. Their differences may well reside in the primary structure of the gamma globulin molecules, despite the failure of electrophoretic studies to show differences among the Gm types. "Fingerprinting" and analysis of the amino acids in the peptides may reveal the essential differences in these proteins.

Since the above was written (November, 1961) I have learned that Harboe, Osterland and Kunkel (1962), and Fudenberg and Franklin (1962) have shown that while the Gm factors are limited to the 7S γ-globulin, the Inv factors are present in β_2M globulins (19S γ-globulins), β_{2A}-globulins and Bence-Jones proteins. Harboe et al., and Fundenberg split the 7S γ-globulin into subunits by treatment with papain (Porter, 1959; Cebra, Givol, Silman and Katchalski, 1961). The Gm factors were found to be present in a different subunit from that containing the Inv factors. Digestion of γ-globulin with papain yields three fractions of which two are very similar in amino acid composition. These two differ from the third not only in amino acid content, but also in carbohydrate content, in that the latter has most of the carbohydrate of the parent molecule (Porter, 1959). Fudenberg recovered these three moieties. He reports that the Gm activity is associated with the CHO containing moiety, and that the Inv activity is associated with the other two.

Harboe et al. report that after treatment with papain they identified two moieties [slow (S) and fast (F)] by immunoelectrophoresis. These were isolated by starch block electrophoresis. The Gm activity was localized in the F portion and the Inv activity in the S portion of the digested γ-globulin.

Gamma globulin molecules appear to consist of several polypeptide chains linked by disulfide bonds (Edleman and Poulik, 1961), hence it may be that the *Gm* alleles control factors in one polypeptide chain, while the Inv alleles control factors in another polypeptide chain.

The suggestion, made above, that the amino acid sequence in the peptides be analyzed to demonstrate the chemical differences among the Gm types and among the Inv types is made more pertinent and more feasible by these observations.

The Incomplete Anti-serum

Grubb's original discovery of the Gm factor (Grubb, 1956) and his subsequent studies were done with incomplete anti-Rh (anti-D) sera. Presumably for this reason and because such sera are readily available, investigations of the gamma globulin factors have been done with incomplete anti-D sera. It was demonstrated by van de Wiel and Dorfmeijer (1959), however, that other incomplete antibodies may also be used. They found among sera tested that one of three anti-c, one of five anti-K, and one immune anti-A were agglutinated by one or more RA sera. Only one anti-Fy^a serum was tested and it gave negative results. These data demonstrate that the blood group specificity of the incomplete antibody is not of primary importance for the agglutination reaction.

Not all anti-D sera are useful for Gm testing and this remains true if the tests are limited to only high titered sera, all of which show the same blood group specificity (Grubb, 1956). An important factor determining whether the anti-D serum will render the cells agglutinable by anti-Gm sera appears to be the Gm type of the donor (Grubb, 1958a, 1959b; Harboe and Lundevall, 1959; Harboe, 1960b). Harboe (1960c) determined the Gm types of 63 anti-D sera and tested each with two RA sera known to test for Gm(a), two for Gm(x) and one for Gm(b). He found that all anti-D sera which could be used to test for a specific factor were positive for that factor, for example, all 20 anti-D sera which could be used to test for Gm(a) were Gm(a+). The converse is not true, that is, not all anti-D sera from Gm(a+) donors could be used to test for Gm(a). Examples were found in which cells coated with an anti-D from a Gm(+) donor were agglutinated by the appropriate anti-Gm reagents, but could not be used to test for the Gm factor, and still others were found in which the cells were not agglutinated by any of the anti-Gm reagents.

Harboe and Lundevall (1959) showed that absorption of the anti-D activity from a serum used for Gm testing did not detectably reduce the Gm titer of the serum. Nevertheless, there is some association between the anti-D and the Gm reagent because anti-D antibody eluted from sensitized cells can be used to coat other cells to test for Gm (Harboe, 1960c). The elution experiments indicate that some of the gamma globulin has both Gm specificity and anti-D (or other incomplete antibody specificity). The fact that the specificity and titer of the Gm factor in an anti-D serum remains essentially unchanged after the anti-D activity is absorbed indicates that a major portion of the Gm activity is on molecules with no anti-D activity (Grubb, 1956; Harboe and Lundevall, 1959; Harboe, 1960c). The experiments showing that cells coated with some anti-D sera are not agglutinated by anti-Gm sera indicate that gamma globulin with anti-D, but without Gm activity, may exist.

Attempts to combine the anti-D and Gm specificity in vitro (Harboe and Lundevall, 1959) were unsuccessful. The problem of why and how the specificities combine in vivo remains unsolved.

Anti-D sera may have more than one specificity (Harboe and Lundevall, 1959; Steinberg, Giles and Stauffer, 1960). Anti-D Ham., for example, may be used to test for Gm(b), Gm(x), Gm(r) and Inv(b). The factor tested for is determined by the agglutinator used (Steinberg et al., 1960a). It should be noted, however, that the specificity of the agglutinator in turn depends upon the anti-D used with it.

The Relation of the Gamma Globulin Factors to Disease

The only studies which have been done to examine the relation between the genetically determined gamma globulin factors and disease have concerned Gm(a) and these studies have been few. The investigations carried out by Bergman (1957), Grubb (1958), Podliachouk, Jacqueline and Eyquem (1958), Podliachouk and Eyquem (1958), Adinolfi, Marone, Olivelli, Polosa and Siniscalco (1960) and Tiilikainen (1960) have not established significant associations between Gm(a) and: bronchial asthma and related diseases, Besnier's prurigo, urticaria, psoriasis, spondilitis, syphilis, various cancers (unnamed), diseases of the hematopoetic system, diabetes, infectious mononucleosis, thallassemia or rheumatoid arthritis.

Two words of caution are required about these data. First, the samples were in most instances so small that only very large differences could be detected and, second, that while Grubb (1958) and Podliachouk, Jacqueline and Eyquem (1958) found no association between rheumatoid arthritis and Gm(a), Tiilikainen (1960) reported an increased frequency of Gm(a+) among rheumatoid patients. In unpublished data presented at the Conference on Gm held in Rouen in 1960, Tiilikainen reported that the frequency of Gm(a+) was normal in RA patients in whom gamma globulin constituted 25 per cent or less of the total serum protein, but that the frequency of Gm(a+) was considerably increased in patients in whom the gamma globulin constituted more than 25 per cent of the total serum protein. These disagreements can be resolved, as Grubb and Tiilikainen have stated, only by further studies of RA patients and their families.

The observation that Gm(a−) sera inhibit Ragg sera (Grubb and Laurell, 1956, and all subsequent investigators) has led to the suggestion that the difference between Gm(a−) and Gm(a+) may be quantitative and a function of gamma globulin level. The fact that the frequency of Gm(a+) among individuals with diseases in which there is an increase in the level of gamma globulins is not changed (with the possible exception of RA noted above) argues against this view. Similarly, the fact that Gm(a−) individuals have been reported among patients with hypergamma-globuline-

mia (Swahn and Grubb, 1958a; Grubb, 1959b) and patients with lupus erythematosus, and plasma cell hepatitis (Grubb, 1959b) indicates that gamma globulin level does not determine the Gm(a) phenotype.

It is unlikely that the Gm factors play any role in transfusion reactions. It has already been noted that the inhibitor [e.g., Gm(a)] and the agglutinator [e.g., anti-Gm(a)] may be found in the serum of the same donor. Patients with anti-Gm(a) who have been transfused with Gm(a+) serum have manifested no side effects, and the half life of the injected gamma globulin was not reduced (Grubb, 1961).

Relation of the Agglutinating Factor to Disease

Grubb (1956) found the agglutinating factor more often in the serum of RA patients than in patients with other diseases or in healthy individuals. All subsequent investigators have confirmed this observation. About 2 per cent of the undiluted sera from blood donors cause sensitized RBC to agglutinate, but only 1.26 per cent have titers equal to or greater than ¼ (Ropartz et Lenoir, 1960b) and not all of these are useful for the determination of the gamma globulin types. At present there is no explanation for the peculiar distribution of the agglutinator.

Population Studies

Numerous studies of the distribution of the Gm(a) factor among various populations of the world have been reported, but only relatively few studies of the distribution of the other gamma globulin factors are available. Indeed, I know of none for the Gm(r) factor. Only few studies have been made in which all the Gm [except Gm(r)] and Inv factors have been determined. Ropartz (1960a and unpublished) and Ropartz, Rousseau and Rivat (1961b) have summarized the available data. The following brief discussion is based on their work. I am greatly indebted to Dr. Ropartz for sending me a preprint of his material and for permitting me to quote it.

Table 15 presents a general summary of the frequency of the Gm, Gm-like, and Inv(a) factors in various races. The Aborigines from the Western Desert are the only population thus far studied to have no evidence of Gm(b). (The sample [Steinberg, Vos and Kirk, unpublished] consisted of 297 unrelated individuals.) Thirty-one per cent of the Aborigines (268) from the coastal region were Gm(b+). Both populations showed 40 to 45 per cent Gm(x+).

Among European whites the frequency of Gm(a+) varies from 40 to 65 per cent in direct correlation with latitude, as pointed out by Grubb (Ta-

TABLE 15—*General Summary of the Frequency (in Per Cent) of the Gm, Gm-Like, and Inv(a) Factors in Various Races of Man*

	Gm(a)	Gm(x)	Gm(b)	Gm-like	Inv(a)
Whites (Europe)	40–65	20–30	80–90	0	15–20
Negroes	100	0	100	20–100	45–70
Japanese	100	30–45	20–25	0	50
Chinese	100	5–15	90–95	0	50–60
Australian Aborigines					
Western Desert	100	41	0	0	43
Coastal Region	100	45	31	0	37
Eskimos (U. S.)	100	6	20	0	—
Amerinds (U. S.)	100	37	2	0	—

ble 16). If an association between pathogens and latitude, and pathogens and Gm exist (Ceppellini, personal communication), the observation could be explained.

On the basis of population and family data it has been concluded that the alleles present in these populations are as shown in Table 17. The Caucasoids and Negroids are readily distinguishable from each other and from the Mongoloids and the Aborigines on the basis of their alleles. The Mongoloids and Aborigines may be distinguished by the frequencies of the alleles. The Gm, Gm-like, and Inv groups appear to be as satisfactory as the blood groups for distinguishing populations. This remarkable polymorphism implies that these serum factors have some selective value. While, thus far, no such effect has been uncovered, it seems likely that further study of this aspect of the serum factors will be rewarding.

'REPRISE'

The genetically determined gamma globulin factors are clearly widespread in man and equally clearly represent a polymorphic system. The data strongly imply some selective value for the different phenotypes (dif-

TABLE 16—*Distribution of Gm(a+) in Europe*

	N	% Gm(a+)
Italy	125	40.2
France	2,659	52.2
Denmark	1,084	55.6
Sweden	369	59.6
Norway	320	60.6
Finland	477	65.0

TABLE 17—*Alleles at the Gm Locus in Some Races of Man*

Caucasoids	Gm^a, Gm^{ax}, Gm^b
Negroids	Gm^{ab}, possibly *Gm-like*
Mongoloids	Gm^a, Gm^{ax}, Gm^{ab}
Australian Aborigines	
Western Desert	Gm^a, Gm^{ax}
Coastal	Gm^a, Gm^{ax}, Gm^{ab}
Eskimos (U. S.)	Gm^a, Gm^{ax}, Gm^{ab}
Amerinds (U. S.)	Gm^a, Gm^{ax}, Gm^{ab}

fering in the various regions of the world) although no mechanism for this selection has been revealed by the few studies which have been undertaken.

So far as I am aware, this system is the only serum or blood group system in which the same factors are produced by different alleles in the different races of man (i.e., Gm(a) and Gm(b) by separate alleles in Caucasoids and by a single allele in Negroids, and Mongoloids). These serum factors have already been demonstrated to be useful for classification of the races of man and within races for the definition of clines.

Attempts to detect the gamma globulin factors in animals other than man have failed to find Gm(a) in horses, cows, donkeys, pigs, sheep, dogs, cats, rabbits, rats, mice, guinea pigs, chickens, cynocephalous monkeys or rhesus monkeys, but 27/27 chimpanzees were all Gm(a+) (Podliachouk et Eyquem, 1958; Podliachouk, 1959). Boyer and Young (1961) tested 24 chimpanzees, 25 cynomologous, two rhesus, two spider and one red monkey for Gm(a), Gm(b) and Gm(x). All were Gm(x−) and all except the chimpanzees were Gm(a− b−). One chimpanzee was Gm(a+ b−), 11 were Gm(a+ b+), 10 were Gm(a− b+) and two were Gm(a− b−). The data of Boyer and Young on Gm(a+) in chimpanzees frequency disagree with Podliachouk's, but agree with hers in showing the presence of the character. It is not known if the Gm factors are genetically determined in chimpanzees; indeed, it is not conclusively established that the inhibitor present in the serum of these animals is Gm. At any rate, the available data indicate that the Gm factors are probably absent in mammals other than primates and therefore of relatively recent evolutionary origin.

The Gm and Inv loci provide two new markers for linkage studies in man. Although studies of possible linkages with the blood types and the haptoglobins have failed to show linkage, such may eventually be found with certain diseases. Suggestions have been made that the Gm factors be used for paternity testing (Ropartz, 1960h; Harboe and Lundevall, 1961). While many exclusions (confirmed by blood types) have been found in my laboratory, I believe it would be unwise to introduce the Gm system to

paternity testing before adequately standardized reagents become widely available and before more is known about the genetics of this locus.

The role of disease in causing the formation of the agglutinator, indeed the reason for the formation of the agglutinator in any individual, the reason for the occurrence of multiple specificities of Ragg agglutinators and of the single specificity in the SNagg agglutinators remain obscure. Similarly, the mode of association of agglutinator and inhibitor is obscure.

Acknowledgment

The work in my laboratory could not have been done without the efficient and intelligent help of my assistants, Rachel Stauffer and Janet Wilson. It is a pleasure to record and to acknowledge my indebtedness to them.

References

Adinolfi, di M., A. Marone, F. Olivelli, P. Polosa, e M. Siniscalco. 1960. Dati sulla genetica dei gruppi gamma-globulinici nell'Uomo. Accad. Nazionale dei Lincei Serie 8: 28: 390–395.

Aho, K. 1961. The problem of the antibody nature of the rheumatoid factor. Ann. med. exp. et biol. Fenniae (suppl. 7) 7: 1–60.

Audran, R., et J. M. Fine. 1961. Sur le rapport de l'activité anti-Gm présente dans le sérum de certains sujets normaux. Vox Sang. n. s. 6: 469–477.

——, ——, J. Moullec, et C. Matte. 1961. Les facteurs de groups seriques Gm^a, Gm^b et Gm^x chez 79 sujets attients de myelome multiple. Rev. Franç. Études Clin. et Biol. 6: 807–811.

Bergman, S. 1957. Distribution of gamma globulin groups in sera from patients with allergy and psoriasis. Acta allergol. 11: 200–202.

Boyer, S. H., S. Iseki, and A. Mayeda. 1961. Serum gamma globulin groups in Japanese. Ann. Human Genet. 25: 69–74.

——, and E. J. Watson-Williams. 1961. The γ-globulin, Gm^{ab}, in Nigerians. Nature 190: 456.

——, and W. J. Young. 1961. Gamma globulin (Gm group) heterogeneity in chimpanzees. Science 133: 583–584.

Brandtzaeg, B., H. Fudenberg, and J. Mohr. 1961. The Gm(r) serum group. Acta. Genet. 11: 170–177.

——, and J. Mohr. 1961. On the genetics of the Gm serum system. Acta Genet. 11: 111–125.

Brønnestam, R., and S. B. Nilsson. 1957. Gamma globulin groups (Gm) of mothers and their new-born infants. Vox Sang. n. s. 2: 316–319.

Cebra, J. J., D. Givol, H. J. Silman, and E. Katchalski. 1961. A two-stage cleavage of rabbit γ-globulin by a water-insoluble papain preparation followed by cysteine. J. Biol. Chem. 236: 1720–1725.

Edelman, G. M., and M. D. Poulik. 1961. Studies on structural units of the γ-globulins. J. Exper. Med. 113: 861–884.

Fine, J. -M., et R. Cryssel. 1961. Contribution à l'étude immunochimique des "globu-

lines anormales" du sérum au cours du myélome. Rev. Franç. Études Clin. et Biol. 6: 766–772.

Franklin, E. C., H. G. Kunkel, H. J. Müller-Eberhard, and H. R. Holman. 1957. Relation of high molecular weight proteins to the serological reactions in rheumatoid arthritis. Ann. Rheumat. Dis. 16: 315–321.

Fudenberg, H. Unpublished data.

——. 1962. Current status of the hereditary human gamma globulin (Gm) groups. Abstract of a paper presented to the Annual Meeting of the Western Assoc. of Physicians, January, 1962.

——, and H. G. Kunkel. 1961. Specificity of the reaction between rheumatoid factors and gamma globulin. J. Exper. Med. 114: 257–278.

Glass, H. B., and C. C. Li. 1953. The dynamics of racial intermixture. Am. J. Human Genet. 5: 1–20.

Grabar, P., et P. Burtin. 1960. Analyse immuno-électrophorétique. Paris: Masson et Cie, 294 p.

Grubb, R. 1956. Agglutination of erythrocytes coated with "incomplete" anti-Rh by certain rheumatoid arthritic sera and some other sera. The existence of human serum groups. Acta pathet. microbiol. scandinav. 39: 195–197.

——. 1957a. Reaction of rheumatoid arthritic sera with a human antibody gamma globulin. Acta rheumat. scandinav. 3: 55–58.

——. 1957b. A relationship between blood group serology and rheumatoid arthritis serology. Serum protein groups. Vox Sang. n. s. 2: 305–312.

——. 1958. Interactions between rheumatoid arthritic sera and human gamma globulin. Acta haemat. 20: 246–252.

——. 1959a. The relationship between the Gm serum groups and the rheumatoid arthritic serum factor. Immunopathology, 1st International Symposium, Basel/Seelisberg 1958, Basel, Switzerland: Benno Schwabe and Company.

——. 1959b. Hereditary gamma globulin groups in man. p. 264–273. In Wolstenholme, G. E. W., and O'Connor, Cecilia M., Ed.: Biochemistry of human genetics. Boston: Little, Brown and Co.

——. 1961. The Gm groups and their relation to rheumatoid arthritis serology. Arthritis and Rheumat. 4: 195–202.

——, and A. -B. Laurell. 1956. Hereditary serological human serum groups. Acta pathet. et microbiol. scandinav. 39: 390–398.

Harboe, M. 1959. A new haemagglutinating substance in the Gm system, Anti-Gm. Nature 183: 1468–1469.

——. 1960a. Simultaneous occurrence of hemagglutinating substances of different specificities in rheumatoid sera. Acta pathet. microbiol. scandinav. 49: 381–392.

——. 1960b. Relation between Gm types and hemagglutinating substances in rheumatoid sera. Acta pathet. microbiol. scandinav. 50: 89–105.

——. 1960c. Interactions between red cells coated with incomplete anti-D and rheumatoid sera. Acta pathet. microbiol. scandinav. 50: 383–397.

——, and J. Lundevall. 1959. A new type in the Gm system. Acta pathet. microbiol. scandinav. 45: 357–370.

——, and ——. 1961. The application of the Gm system in paternity cases. Vox Sang. n. s. 6: 257–273.

——, C. K. Osterland, and H. G. Kunkel. 1962. Localization of two genetic factors (Gm and Inv) to different areas of 7S γ-globulin molecules. Science 136: 979–980.

Heller, G., A. S. Jacobson, M. H. Kolodny, and W. H. Kammerer. 1954. The hemagglutination test for rheumatoid arthritis. II. The influence of human plasma fraction II (gamma globulin) on the reaction. J. Immunol. 72: 66–78.

Henningsen, K. Unpublished data.

Henry, C. 1960. Considérations techniques sur les méthodes de détermination des groupes de gamma globuline. Thesis. Paris: D. P. Taib.

Jacqueline, F., A. Eyquem, et L. Podliachouk. 1957. Hémagglutination au cours des rheumatismes inflammatoires chroniques. Rev. du Rhumat. 24: 385–398.

Kunkel, H. 1957. Ultra-centrifugation separation of rheumatoid gamma globulin. In Lamont-Havers, R. W., Ed.: Serological reactions of rheumatoid arthritis. New York: Arthritis and Rheumat. Found.

Lamont-Havers, R. W. (Ed.). 1957. Serological reactions of rheumatoid arthritis. New York: Arthritis and Rheumat. Found.

Laurell, A.-B., and R. Grubb. 1957. The Hp and Gm groups and secretor characters of 46 blood donors. Vox Sang. n. s. 2: 312–316.

——, and ——. 1958. Complement, complement components, properdin and agglutination promoting factors in rheumatoid arthritis. Acta pathet. microbiol. scandinav. 43: 310–320.

Lawler, Sylvia D. 1960. A genetical study of the Gm groups in human serum. Immunology 3: 90–94.

Linnet-Jepson, P. 1958. Serum groups in the newborn. Trans. 6th Congr. Europ. Soc. Haemat. 1957. Copenhagen. Basel: S. Karger, pp. 903–905.

——, F. Galatius-Jensen, and M. Hauge. 1958. On the inheritance of the Gm serum group. Acta Genet. 8: 164–196.

Mårtensson, L. 1961. Gm characters of M-components. Acta med. scandinav. (suppl.) 367: 87–93.

Masouredis, S. P. 1960. Rh_o (D) genotype and red cell Rh_o (D) antigen content. Science 131: 1442.

——, Cecile Ann Chi, and Elizabeth Ferguson. 1960. Relationship between Rh_o (D) genotype and quantity of I^{131} anti-Rh_o (D) bound red cells. J. Clin. Invest. 39: 1450–1462.

Milgrom, F., S. Dubiski, and G. Wozniczko. 1956. Human sera with "anti-anti-body." Vox Sang. n. s. 1: 172–183.

Mohr, J., and Brita Brandtzaeg. 1961. Concerning the genetics of the Gm(r) serum groups. Presented at the 2nd Intern. Congr. Human Genet. Rome.

Moullec, J., J. M. Fine, Cl. Henry, et Ch. Silverie. 1959. Le facteur Gm^a et quelques propiétés du serum des noirs africains. Proc. 7th Congr. of the Intern. Soc. of Blood Transfusion, pp. 881–883.

——, R. Kherumian, E. Sutton, and P. Espagnon. 1956. Contribution à l'étude du facteur de groupe Gm^a du plasma humain. Rev. hémat. 11: 512–517.

Nielsen, J. C., and K. Henningsen. 1961. A new allele within the Gm system. Vox Sang. n. s. 6: 634–635.

Ottensooser, F. Personal communication.

Podliachouk, L. 1959. Le facteur sérique Gm^a dans le sérum d'animaux de différentes espèces. Ann. Inst. Pasteur 96: 362–364.

——, et A. Eyquem. 1958. Le facteur sérique Gm^a en pathologie humaine. Proc. 7th Congr. of the Intern. Soc. of Blood Transfusion 869–872.

——, A. Eyquem, et F. Jacqueline. 1958. Le diagnostic de la polyarthrites chronique évolutive par agglutination des globules rouges humains sensibilisés. Ann. Inst. Pasteur 94: 659–662.

——, ——, et ——. 1960. Observations sur certaines proprietes du facteur sérique Gm^a. Ann. Inst. Pasteur 99: 224–240.

——, F. Jacqueline, et A. Eyquem. 1958. Le facteur sérique Gm^a au cours des rheumatismes inflammatoires chroniques. Ann. Inst. Pasteur 94: 590–597.

Porter, R. R. 1959. The hydrolysis of rabbit γ-globulin and antibodies with crystalline papain. Biochem. J. 73: 119–126.

Putnam, F. W. 1959. Plasma-cell myeloma and macroglobulinemia. I. Physiochemical, immunochemical and isotopic turnover studies of the abnormal serum and urinary proteins. New England J. Med. 261: 902–908.

——. 1960. Abnormal serum globulins. In Putnam, F. W., Ed.: The Plasma Proteins, Volume 2. New York: Academic Press.

Ropartz, C. 1960a. Les groupes sériques Gm état actuel de la question. Rev. Franç. Études Clin. et Biol. 5: 933–945.

——. 1960b. Un cas d'exclusion de paternité par le facteur sérique Gmx. Rev. Franç. Études Clin. et Biol. 5: 506.

——. Unpublished summary of population studies.

——, et R. Hurel. 1959. Influences de l'anti-RH et du sérum de polyarthrite chronique évolutive dans le choix du complexe agglutinant destiné a l'exploration du facteur sérique Gm. Rev. Franç. Études Clin. et Biol. 4: 280–282.

——, et J. Lenoir. 1960a. Les antiglobulines humaines presentes dans les serums de sujets normaux. Rev. Franç. Études Clin. et Biol. 5: 40–50.

——, et ——. 1960b. Les antiglobulines humaines presentes dans les serums de sujets normaux. II. Fréquence et caractéristiques sérologiques. Rev. hémat. 15: 40–51.

——. ——, Y. Hemet, and L. Rivat. 1960. Possible origins of the anti-Gm sera. Nature 188: 1120–1121.

——, ——, et L. Rivat. 1960. Identité du facteur Gma et de la propriété inhibitrice In_A (+). Rev. Franç. Études Clin. et Biol. 5: 79–81.

——, ——, and ——. 1961. A new inheritable property of human sera: the InV factor. Nature 189: 586.

——, L. Rivat, et J. Lenoir. 1960a. Identité du facteur Gmx et de la propriété inhibitrice In_B (+). Rev. Franç. Études Clin. et Biol. 5: 604–605.

——, ——, et ——. 1960b. Fréquence des facteurs Gma, Gmb, Gmx, Gm-like et Inv chez quatre cents noirs Africains. Rev. Franç. Études Clin. et Biol. 5: 814–816.

——, ——, et P. -Y. Rousseau. 1962. Mise en evidence d'un allele silencieux au locus Gm. Vox Sang. (in press).

——, ——, ——, et J. Lenoir. 1961. Les facteurs Gma, Gmb, Gmx, Gm-like et Inv chez les Japonais. Rev. Franç. Études Clin. et Biol. 6: 813–816.

——, P. -Y. Rousseau, et L. Rivat. 1961a. Essai d'interpretation quantitative des qualites des reactifs anti-Gm. Rev. Franç. Études Clin. et Biol. 6: 591–596.

——, ——, et ——. 1961b. Donnees actuelles des facteurs sériques Gm dans le monde. Paper read at the 2nd Intern. Congr. Human Genet. Rome, 1961.

——, ——, ——, et J. Lenoir. 1961. Etude genetique du facteur serique Inv frequence dans certaines populations. Rev. Franç. Études Clin. et Biol. 6:374–377.

Sjöquist, J., and C. -B. Laurell. 1961. N-terminal amino acids of isolated M-components. Acta med. scandinav. Suppl. 367: 65–68.

Steinberg, A. G. Unpublished.

——. 1962a. Evidence for a Gm allele negative for both Gm(a) and Gm(b). Vox Sang. 7: 89–92.

——. 1962b. Studies on the Gm factors: Comparison of the agglutinators in serum from patients with rheumatoid arthritis and in serum from healthy donors. Arth. and Rheumat. (In press).

——, Brenda Dawn Giles, and Rachel Stauffer. 1960. A Gm-like factor present in Negroes and rare or absent in whites: its relation to Gm^a and Gm^x. Am. J. Human Genet. 12: 44–51.

——. G. H. Vos, and R. L. Kirk. Unpublished data.

——, L. Y. C. Lai, G. H. Vos, R. B. Singh, and T. W. Lim. 1961. Genetic and population studies of the blood types and serum factors among Indians and Chinese from Malaya. Am. J. Human Genet. 13: 355–371.

——, and Rachel Stauffer. 1960. Effect of heat on the serum factors Gm^a, Gm^b, Gm^x and Gm-like. Nature 187: 791–792.

——, ——, B. S. Blumberg, and H. Fudenberg. 1961. Gm phenotypes and genotypes in U. S. Whites and Negroes; in American Indians and Eskimos; in Africans; and in Micronesians. Am. J. Human Genet. 13: 205–213.

——, ——, and S. H. Boyer. 1960. Evidence for a GM^{a^b} allele in the Gm system of American Negroes. Nature 188: 169–170.

——, Janet Wilson, and Suzanne Lanset. 1962. A new human gamma globulin factor determined by an allele at the Inv locus. Vox Sang. 7: 151–156.

Swahn, B., and R. Grubb. 1958. Purification of hemagglutinating factors in rheumatoid arthritic sera by Cohn's method 10 and ultra-centrifugation. Acta pathet. microbiol. scandinav. 42: 173–180.

Tiilikainen, Anja. 1960. Incidence of the Gm serum groups in collagen diseases. Ann. med. exper. et biol. Fenniae 38: 51–55.

Waller, M. V., and J. H. Vaughan. 1956. Use of anti-Rh sera for demonstrating agglutination activating factors in rheumatoid arthritis. Proc. Soc. Exper. Biol. & Med. 92: 198–200.

Wiel, Th. W. M. van de, and H. Dorfmeijer. 1959. Reumatoide arthritis en erfelijke serumgroepen. Nederl. tijdschr. geneesk. 103: 2314–2319.

Yokoyama, M., and N. Ueno. 1961. The Gm^a factor in Japanese. Nature 190:455–456.

Chapter 2

The Plasma Transferrins

E. R. Giblett
King County Central Blood Bank,
Seattle, Washington

Introduction

Plasma iron is transported to the bone marrow and tissue storage areas by the β-globulin, transferrin (or siderophilin). This is a vital function, since iron is a component of hemoglobin, myoglobin and cytochrome, as well as such enzymes as catalase, succinic dehydrogenase and xanthine oxidase. Although transferrin is capable of combining with other metallic ions in vitro, it is doubtful that such binding occurs under normal physiological conditions (Laurell, 1960). Another in vitro function of the iron-protein complex which may or may not take place in the body is the suppression of viral multiplication (Martin and Jandl, 1960).

Because of the discovery by Smithies (1957) of heritable transferrin variants, numerous investigators have been stimulated to search for additional variants, to study their chemical and biological properties, and to determine their gene frequencies in various populations. The purpose of this paper is to present a brief summary of transferrin structure and function and to discuss in more detail recent biochemical and anthropological data in relation to genetic polymorphism of the iron-binding protein.

Physico-chemical Characteristics

In 1949, Surgenor et al. (1949) prepared the first crystalline transferrin from Cohn's fraction IV-7. The existence of an iron-binding protein in plasma had been previously demonstrated by several investigators, notably

This study was supported in part by Research Grant #H-5780 from the National Heart Institute, United States Public Health Service.

including Holmberg and Laurell (1945) and Schade and Caroline (1946). The latter described a protein with iron-binding properties in Cohn's fraction IV-3, 4. They also showed that addition of iron to the colorless protein results in the development of a salmon-pink color with an absorption peak at 470 mμ which reaches its maximum intensity when the protein is fully saturated. This property was later employed by Rath and Finch (1949) for determining the iron-binding capacity of serum.

Ehrenberg and Laurell (1955) showed that the iron-binding β-1 globulin can combine with two atoms of ferric iron per molecule, and that the bond is ionic, not covalent. With each metal ion, one bicarbonate ion is also bound (Schade, Reinhart and Levy, 1949). The iron-transferrin (FeTf) complex is stable in alkaline solutions, but dissociates at neutral or acid pH, particularly in the presence of iron chelates such as citrate, phosphate or EDTA (Laurell, 1960). That such dissociation occurs under physiological conditions is unlikely, however, since the complex has a stability constant of about 10^{30} (Saltman and Charley, 1960). Both the solubility and resistance to heat denaturation are dependent upon the degree of iron saturation (Inman, Coryell, McCall, Sgouris and Anderson, 1961; Keller and Pennell, 1959).

Crystalline transferrin is a glycoprotein with a carbohydrate content of about 5 per cent, most of which is hexose, hexosamine and sialic acid (Schultze, 1957; Winzler, 1960). It has a molecular weight of about 90,000 and a sedimentation constant ($S_{20,w}$) of 5.0 (Koechlin, 1952; Laurell and Ingelman, 1947). The isoelectric point is pH 5.45 when the protein is fully saturated and pH 5.80 when iron-free (Keller et al., 1959).* Large scale production of purified transferrin from Cohn's IV-7 (Inman, 1956) or IV-4 (Inman et al., 1961) may be accomplished with ethanol and zinc precipitation followed by chromatographic fractionation. Alternatively, whole serum may be treated with Rivanol (2 ethoxy-6, 9 diaminacridine lactate) at pH 8 to 10 with subsequent removal of gamma globulin by alcohol precipitation or DEAE cellulose column fractionation (Kistler, Nitschmann, Wyttenbach, Studer, Niederöst and Mauerhofer, 1960). For the preparation of smaller quantities, the beta globulin can first be isolated by electrophoresis in starch grain, and the transferrin recovered by column chromatography on TEAE cellulose (Parker and Bearn, 1961a).

Physiological Properties

The plasma of normal human subjects has a transferrin content of about 200 to 320 mg. per 100 ml. This quantitation may be performed directly by

*These physical constants apply to Tf C, but not necessarily to the other forms of transferrin.

immunochemical techniques (Jager and Gubler, 1952), but it is usually calculated indirectly and expressed in terms of the total iron-binding capacity of the plasma. For this calculation, it is necessary to know the amount of iron already bound by the transferrin (i.e., the serum iron level) as well as the additional quantity of iron which must be added to achieve full saturation (i.e., the unsaturated iron-binding capacity).

Since each mg. of transferrin can bind about 1.25 μg. of iron (Jager et al., 1952), the total iron-binding capacity of plasma ranges from 250 to 400 μg. per 100 ml. However, the normal concentration of plasma iron is about 80 to 150 μg. per cent, so that transferrin is only one-third saturated, having the capacity to bind another 170 to 250 μg. of iron. (For reviews, see Laurell [1947; 1960] and Wintrobe [1961]).

The transferrin level (and thus, the total iron-binding capacity) is elevated in iron deficiency anemia and pregnancy, but decreased in several disease states, including infection, nephrosis, cirrhosis and malignancy (Wintrobe, 1961). Since binding of iron occurs rapidly, unsaturated transferrin protects the body from acute iron intoxication. Protection is therefore minimal whenever the unsaturated iron-binding capacity is reduced, either through elevation of the serum iron level or depression of β-globulin concentration.

For the synthesis of hemoglobin, the bone marrow normally requires 25 to 30 mg. of iron per day, an amount present in 30 to 40 L. of plasma. Over 30 Gm. of transferrin are needed for this iron delivery. Since the average half-time of the FeTf complex in circulation is less than 2 hours (Huff, Hennessey, Austin, Garcia, Roberts and Lawrence, 1950) and the biological half-life of transferrin is 6 to 12 days (Awai and Brown, 1961; Gitlin, Janeway and Farr, 1956; Katz, 1961), the protein must act as a true carrier, rapidly releasing iron to the tissue acceptors and repeating the process many times before being itself metabolized.

Recent studies with radioiodine-labelled transferrin (Awai et al., 1961; Gitlin et al., 1956) indicate that this protein is distributed almost equally between the vascular and extravascular compartments. Outside of the blood and lymph vessels, transferrin is attached to cells of the liver and reticulo-endothelial system, engaged in the process of iron exchange. The sudden decrease in plasma transferrin levels associated with iron ingestion by iron-deficient subjects (Mitchell, Halden, Jones, Bryan, Stirman and Muirhead, 1960) or mobilization of tissue iron by the newborn infant (Laurell, 1947) suggests that the intravascular compartment can be "tapped" of its transferrin for immediate extravascular function. The studies of Jandl et al. (Jandl, Inman, Simmons and Allen, 1959) indicate that red cell precursors compete with transferrin, but can remove iron efficiently from the protein when it is 30 per cent saturated; whereas much

higher per cent saturation is required for optimal iron removal by liver cells. The mechanism of this transfer is not entirely understood, but it may be accomplished through an energy-requiring process in which iron is directly transferred to receptors on the cell membrane without going through an intermediate stage as "free" iron in the plasma (Jandl et al., 1959; Mazur, Green and Carleton, 1960).

The production of iron-binding protein begins in early fetal life, and transfer of maternal transferrin across the placenta is probably minimal (Dancis, Braverman and Lind, 1957; Giblett and Brooks, unpubl.; Rausen, Gerald and Diamond, 1961). The site of synthesis is not definitely established, although fetal liver slices can incorporate glycine into the production of metal-binding β-globulin (Dancis et al., 1957). Furthermore, the plasma concentration of albumin and transferrin tend to vary in the same direction in a variety of diseases, suggesting that the two proteins may be synthesized in the same organ (Laurell, 1960). Schedl and Bartter (1959) noted that the repeated intravenous infusion of albumin in 4 patients with no demonstrable liver disease was accompanied by a marked drop in plasma transferrin level. One patient with advanced cirrhosis failed to respond in a similar manner. They therefore postulated the existence of a "control" mechanism for transferrin production, presumably located in the liver, which is subject to the stimulus of exogenous albumin. Alternatively, the albumin may not depress synthesis, but increase the flow of transferrin molecules into the extravascular space.

Congenital Atransferrinemia

Absence or near absence of transferrin from the plasma must be an exceedingly rare condition, since only 3 cases have been reported. Two of these (Hitzig, Schmid, Betke and Rothschild, 1960; Riegel and Thomas, 1956) undoubtedly represented acquired hypotransferrinemia, but in the case described by Heilmeyer et al. (1961) the findings were consistent with an inherited defect in transferrin metabolism. The patient, a 7 year old girl, had a history of severe hypochromic, microcytic anemia first detected at 3 months of age. Failure of any response to iron, cortisone and vitamin B_{12} therapy necessitated a transfusion of blood about every 3 months thereafter. Subsequent studies showed extremely low serum iron concentration (9 to 14 μg. per cent) and unsaturated iron-binding capacity (19 μg. per cent) as well as absence of hemosiderin in the bone marrow. The plasma radioiron clearance rate was remarkably rapid (T ½ less than 5 minutes), with localization of large amounts of radioactivity over the liver and greatly impaired bone marrow utilization of iron for hemoglobin synthesis. Absorption of oral iron from the gastrointestinal tract appeared

to be normal, indicating that transferrin need not be present for this function to occur. Electrophoresis of the patient's serum containing added Fe^{59} showed no radioactivity in the β-globulin region, but a discrete "x" band migrating with the γ-globulins contained a significant number of counts above background. Both parents had moderately decreased plasma transferrin levels but were apparently healthy.

The child died suddenly of heart failure. From autopsy findings, death was attributed to severe cardiac muscle damage due to excessive iron deposition and anemic anoxia. Marked hemosiderosis of the liver, splenic trabeculae and pancreas was also noted.

This instructive case supports the existence of a "recessive" gene which in heterozygotes may be associated with moderate depression of transferrin production (or less likely, increased transferrin catabolism) but in homozygotes, the plasma iron-binding capacity is too low to be consistent with adequate hemoglobin production. Iron absorption may be unimpaired, but the absorbed iron either circulates unbound or is loosely attached to nontransferrin proteins which are rapidly relieved of their metal ions by extravascular tissue cells (particularly those in the liver) before they can reach the bone marrow in any appreciable quantity. Alternatively, the marrow erythroblasts may be unable to accept iron unless it is bound specifically to transferrin, so that the (small) amount of synthesized heme could be an indicator of the circulating transferrin level.

On the basis of the above-described case and the well known fact that hemochromatosis is often familial, it has been suggested (Editorial, 1961) that a proportion of cases of iron-storage disease may be due to a genetically determined abnormality in transferrin production. However, since hemochromatosis is rarely associated with anemia (Finch and Finch, 1955), it appears unlikely that transferrin deficiency or structural abnormality is a common basis for the familial form of this disease. Starch-gel electrophoresis of serum obtained from several patients with hemochromatosis has been performed in the author's laboratory. In all instances, the transferrin had the same electrophoretic mobility as that seen in normal individuals and was only moderately decreased in quantity. As expected, the unsaturated iron-binding capacity was very low.

The Inherited Transferrin Types

I. Demonstration of Transferrin Polymorphism

In 1955, Smithies (1955) reported that electrophoresis of serum in starch gel separates the protein components much more effectively than other electrophoretic methods because the "sieving" effect of the gel is added to differentiation due to electrical charge. This quickly led him to the dis-

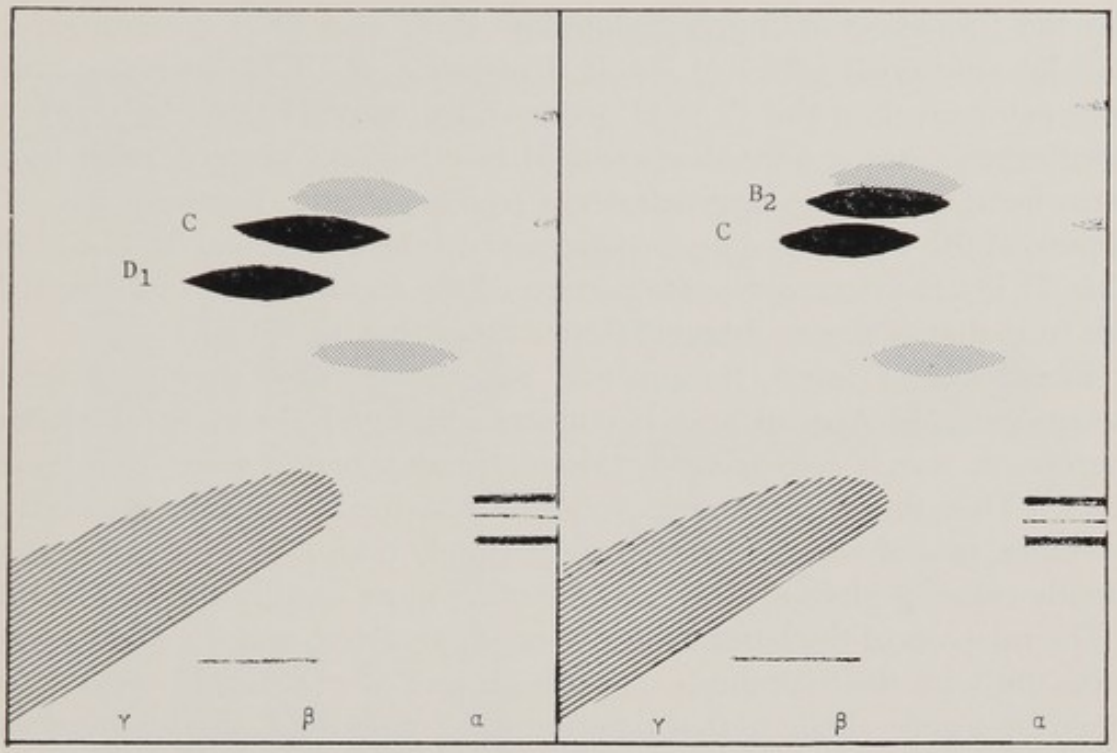


FIG. 1—Two-dimensional electrophoresis: diagram of serum specimens having the transferrin phenotypes CD_1 and B_2C. The fast and slow transferrins were originally named β-globulins D and B, respectively. Albumin and some α-globulins not shown (after Smithies and Hiller, 1959).

covery of the inherited haptoglobin types (Smithies and Walker, 1956), but with the horizontal system then employed, differences among the other proteins were not easily visualized. Subsequently, Poulik and Smithies (1958) performed two-dimensional electrophoresis of serum (i.e., first on filter paper, then in starch gel at right angles), achieving further separation of the protein fractions. They noted that under these conditions, the β-globulin had several components, the most heavily concentrated being third in migration rate, and thus called β-globulin C.

Smithies (Horsfall and Smithies, 1958; 1957; 1958) then studied plasma and serum specimens from individuals of various racial origins by the same method, noting that all contained β-globulin C. However, a small number of Negroes and a larger number of Australian aborigines had a new β-globulin component occurring in about the same concentration as β-globulin C, but migrating more slowly. This protein was called β-globulin D. Whereas this slow-moving component was not seen in the plasma of 425 Canadian subjects, 5 of the latter were found to have, in addition to β-globulin C, a faster moving component which was called β-globulin B. The appearance of these three proteins, as demonstrated by two-dimensional electrophoresis, is shown in Figure 1.

On the basis of family studies, Smithies and Hiller (1959) suggested

that the formation of the 3 β-globulins B, C and D is determined by 3 allelic autosomal genes of equal dominance, the C gene being much more common than the B or D genes. They showed that the serum of heterozygotes has a normal amount of iron-binding protein, each transferrin being present in approximately equal quantity. Furthermore, in a mixture of the sera of two homozygotes (i.e., one containing Tf C and the other Tf D) the electrophoretic pattern of the transferrin is indistinguishable from that of serum obtained from a heterozygote Tf CD.

Harris et al. (Harris, Robson and Siniscalco, 1958) examined serum specimens from African and European subjects by horizontal electrophoresis in starch gel, using the discontinuous buffer system of Poulik (1957). They found the three β-globulins described by Smithies as well as two more, one of which migrated more slowly than β-globulin D (subsequently called β-globulin D_2), and the other more rapidly than β-globulin B. The relatives of the latter subject were then tested, and the results were consistent with the hypothesis that this protein—β-globulin B_1—was determined by a gene allelic to that determining β-globulin C. Thus, there was indirect evidence for a total of five allelic genes in the polymorphism.

Smithies and Hiller (1959) described the independent observations of Poulik, Allison and Sutton (cited as personal communications) which indicated that the genetically controlled β-globulins are, in fact, transferrins, since purified transferrin behaves electrophoretically in the same manner as β-globulin C, and the addition of radioiron to the serum of a CD heterozygote prior to electrophoresis results in the localization of radioactivity in the two β-globulins C and D. For this reason, the symbol *Tf* was suggested for the autosomal locus, with the superscript letters B, C and D, and subscript numerals for relative mobility of their corresponding proteins. The transferrin proteins then became (in order of increasing migration rate) D_2, D_1, C, B_2 and B_1. The known phenotypes at that time were C, B_2C, B_1C, CD_2, CD_1 and D_1.

In 1959, Giblett et al. (Giblett, Hickman and Smithies, 1959) described a simple method of radioautography with Fe^{59} which was used to confirm their finding of three additional transferrins, D_3, D_0 and B_0. The respective phenotypes were CD_3, CD_0 and B_0C. Figure 2, which compares the protein-stained gel slice with its autoradiograph, indicates the relative migration rates of these iron-binding proteins. Electrophoresis was performed with the gel in a vertical position, as described by Smithies (1959a; 1959b).

Shortly thereafter, Harris et al. (1960) reported another genetically distinct form of transferrin found in one English and one Italian family. This protein, migrating between Tf D_1 and D_0 in a borate buffer system, was called Tf D_4. However, the authors appreciated the inconsistency of the

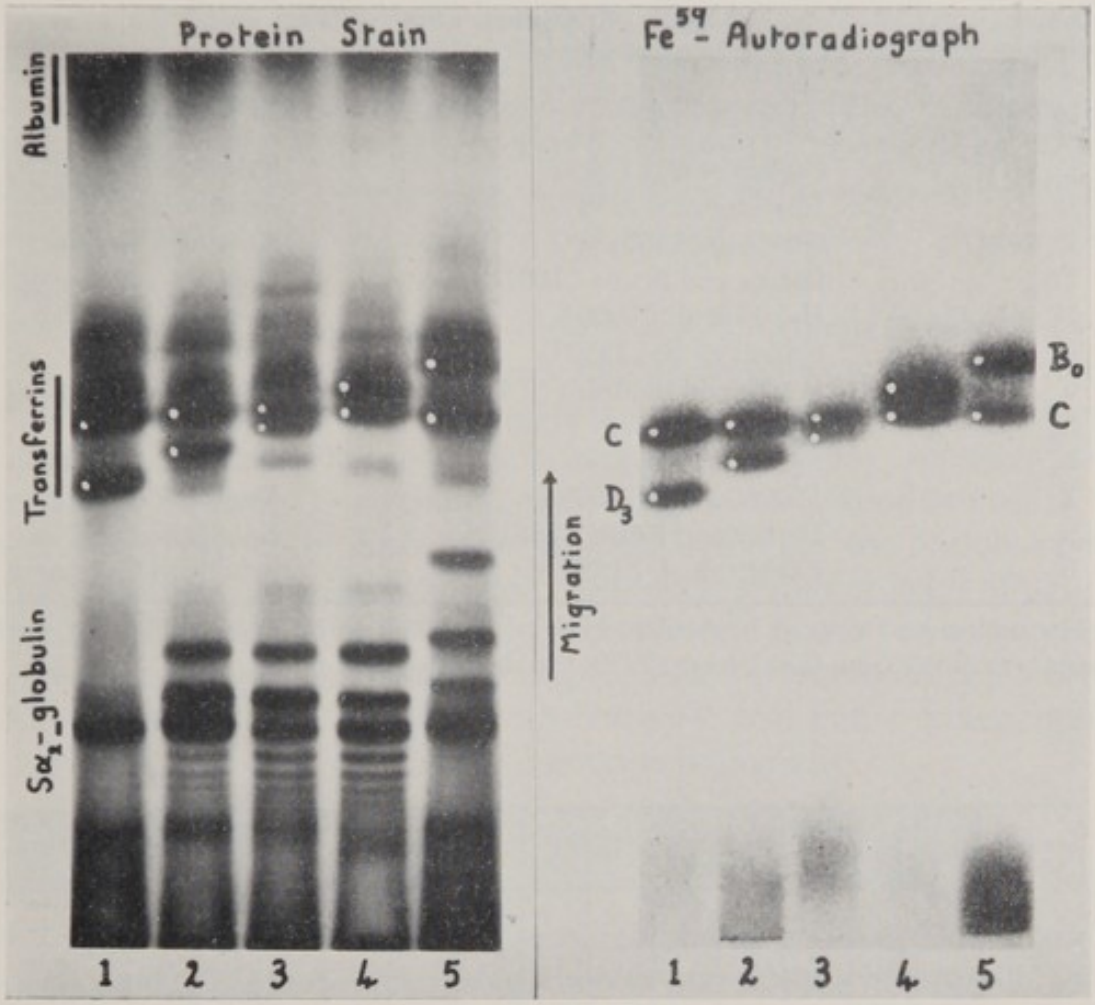


FIG. 2—Starch gel electrophoresis of five serum specimens having the transferrin phenotypes CD_3, CD_1, CD_0, B_2C and B_0C. Fe^{59} was added prior to electrophoresis. One-half of the gel (on the left) was stained with amido black. The other half was applied to x-ray film to make the autoradiograph shown on the right. The white dots indicate the individual transferrins (from Giblett, Hickman and Smithies, 1959).

terminology, since the numerical subscript did not coincide with mobility rate. Parker and Bearn (1961b) have suggested that this protein be called Tf D_{0-1}, and their nomenclature will be used in this paper.

Three additional transferrins have recently been described by Parker and Bearn (1961b; 1961c). The first variant, found in 16 out of 230 Navajo Indians, has an electrophoretic mobility intermediate between B_0 and B_1, and is therefore called B_{0-1}. The other two variants differ only slightly from previously described transferrins under the usual conditions of electrophoresis. However, by increasing the voltage and decreasing the temperature, these authors showed that 7 of 116 Chinese individuals had Tf D_{Chi}, with a mobility faster than D_1 and slower than D_0 or D_{0-1}. Similarly, they found that one serum out of the 46 they obtained from Japanese donors contained Tf B_3, migrating more slowly than Tf B_2.

TABLE 1—*The Human Transferrins*

Transferrin	Described by	Population Source of First Specimen
D_3	Giblett et al. (1959)	American Negro
D_2	Harris et al. (1958)	African Negro
D_1	Smithies (1957)	American Negro
D_{Chi}	Parker and Beam (1961c)	Chinese
D_{0-1} (D_4)	Harris et al. (1960)	English Caucasian
D_0	Giblett et al. (1959)	American Negro
C	Poulik and Smithies (1958)	Canadian Caucasian
B_3	Parker and Beam (1961c)	Japanese
B_2	Smithies (1958)	Canadian Caucasian
B_1	Harris et al. (1958)	English Caucasian
B_{0-1}	Parker and Beam (1961b)	Navajo Indian
B_0	Giblett et al. (1959)	American Caucasian

The molecular forms of human transferrin are listed in order of increasing electrophoretic mobility from the slowest (Tf D_3) to the fastest (Tf B_0).

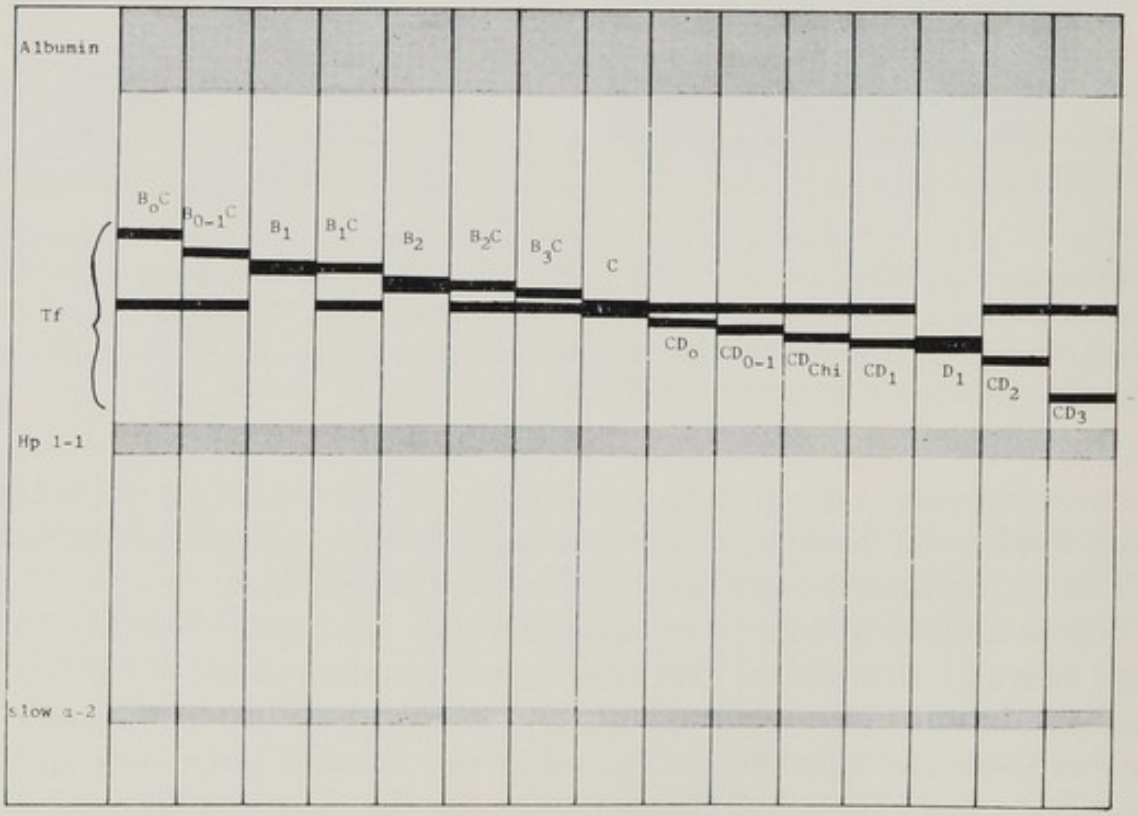


FIG. 3—Diagrammatic representation of the human transferrin phenotypes. Relative electrophoretic migration rates (in starch gel) of the twelve molecular forms of Tf are indicated. The position of D_2 is an approximation, since serum of Tf phenotype CD_2 has not been available to the author. The relative mobility of the transferrins as well as the other serum proteins is dependent on electrophoretic conditions, particularly type of buffer system (including pH and molarity), voltage and temperature.

Table 1 lists the 12 currently known molecular forms of human transferrin in order of increasing electrophoretic mobility. The population source of the first described specimen is also given, because it is, in some instances, indicative of the geographic distribution of the *Tf* genes, which will be discussed in a later section. A diagrammatic representation of the known transferrin phenotypes (with the exception of B_2D_1) is presented in Figure 3.

In addition to the incorporation of radioiron into serum or plasma prior to electrophoresis, two other methods for the identification of transferrin in starch gel have been described. The first is a staining technique developed by Smithies (pers. comm.) which utilizes the highly sensitive salt, Nitroso-R (1-Nitroso-2-napthol-3, 6-disulfonic acid, disodium salt) to detect the presence of iron. The second, described by Sutton (pers. comm.), consists of precipitating out most of the serum proteins at pH 8.4 with Rivanol (2-ethoxy-6, 9 diaminacridine lactate) prior to electrophoresis. The remaining transferrin is readily identified after migration in starch gel. For electrophoresis, Sutton uses a tris-boric acid buffer which, unlike the earlier system of Poulik (1957) is not discontinuous.

II. Inheritance and Linkage Studies

Of the twelve known transferrins, the inheritance of all but two (D_2 and D_0) has been studied in appropriate families (Barnicot, Garlick, Roberts, 1960; Beckman, in press; Giblett and Steinberg, unpub. data; Harris et al., 1960; Harris et al., 1958; Horsfall et al., 1958; Parker and Bearn, pers. comm.; Parker and Bearn, 1961c), and the results have indicated that their corresponding genes are alleles of *Tf*^{c}. Unfortunately, with a single exception, the individual pedigrees contain only two transferrins, one being a variant, and the other being the common Tf C. In the exceptional case (Beckman, 1962) a Tf B_2D_1 mother and Tf C father have two daughters who are Tf B_2C and Tf CD_1, respectively. Whereas a critical mating (e.g., $CD_1 \times B_2C$) has not yet been reported, it is very likely that the discovery of such a family will demonstrate allelism of the three *Tf* genes involved. Most of the variants are rare, so that of the 78 theoretically possible phenotypes, only 16 (12 heterozygous and 4 homozygous) have been found. The fact that no human serum has been conclusively shown to contain 3 transferrins provides some indirect evidence that the 12 transferrins represent a series of 12 alternative dominant alleles at a single locus. The total number of such alleles distributed throughout the world's populations may well be even greater than 12. However, aside from those genes determining Tf B_2 and Tf D_1 (and possibly, Tf B_{0-1} and Tf D_{Chi}), the variant *Tf* genes must have a very low over-all frequency.

Data from three studies (Barnicot, Garlick and Roberts, 1960; Giblett

TABLE 2—*Transferrin Phenotype Segregation*

Population	Mating Type	No.	Offspring Transferrin Phenotypes				
	$C \times CD_1$		C	CD_1	D_1	B_2C	B_2
Nigeria		12	15	18	0		
Am. Negro		14	26	25	0		
Austral. aborigine		6	9	9	0		
Total		32	50	52	0		
	$CD_1 \times CD_1$						
Nigeria		1	0	2	3		
Am. Negro		1	1	2	3		
Total		2	1	4	6		
Austral. aborigine	$C \times D_1$	1	0	6	0		
	$C \times B_2C$						
No. Am. (Hutterite)		47	110			127	0
East. Canada		9	13			14	0
Total		56	123			141	0
No. Am. (Hutterite)	$B_2C \times B_2C$	4	5			15	5

Segregation of transferrin phenotypes in 95 families having one or both parents with transferrin variants. Data taken from Smithies and Hiller (1959), Barnicot et al. (1960) and Giblett and Steinberg (unpubl. data).

et al., unpub. data; Smithies et al., 1959) permit an analysis of the segregation of transferrin types in a total of 13 African Negro, 15 American Negro, 7 Australian aborigines and 60 American Caucasian families. Table 2 summarizes the transferrin types of the 408 children of 5 different mating classes. (Note that the C × C mating type is not included because all children born to such parents had the C phenotype.) In the case of Tf D_1, there is no disturbance of segregation ratios in the children. This is also true of the 9 Canadian families having Tf B_2. The small excess of heterozygotes seen in the Hutterite families is not statistically significant ($p = .2$), and is more likely due to chance than to heterozygote advantage. However, because of the possibility that the transferrins may be involved in some process of natural selection, further data are needed to clarify this important point.

Careful studies of possible genetic linkage between the transferrin genes and those of the other serum or red cell groups have not yet been reported. However, Smithies and Hiller (1959) observed independent segregation of genes at the transferrin locus from those determining ABO, MNSs, P

TABLE 3—*Amino Acid Composition of the Human Transferrins C, B_2 and D_1*
(Adapted from Parker and Bearn (1962))

Amino Acid	Residues per Tf Mol.
Aspartic Acid	82
Glutamic Acid	62
Lysine	60
Leucine	60
Alanine	59
Glycine	51
Valine	47
Serine	42
Half-cystine	40
Proline	36
Threonine	32
Arginine	28
Phenylalanine	28
Tyrosine	26
Histidine	19
Isoleucine	15
Methionine	10
Tryptophan	8
Glucosamine	9
Ammonia	62

and Rh blood groups as well as haptoglobin; thus close genetic linkage with these loci is unlikely.

III. Structure and Function of Transferrin Variants

When Smithies (Smithies and Connell, 1959) investigated serum specimens having the transferrin types CD_1 and B_2C, he noted that the three transferrin proteins had different mobilities in both filter paper and starch gel systems, suggesting that they probably differ in electrical charge, but not necessarily in molecular size. Thus, the molecular weight of all the transferrins is probably about 90,000. This is consistent with the recent report of Parker and Bearn (1962) that ultracentrifugal analysis of purified Tf C and B_2 revealed no difference in sedimentation constant. These authors also determined the amino acid composition of Tf C, B_2 and D_1 (see Table 3). No difference in the number of the respective residues in these three protein molecules could be detected.

Parker and Bearn (1961a; 1962) also investigated the sialic acid content of various transferrins, postulating that their different mobility rates might be due to alterations in the number of sialic acid residues. This was a particularly attractive hypothesis, since they were able to show that treatment of purified transferrin C with neuraminidase (obtained from Vibrio

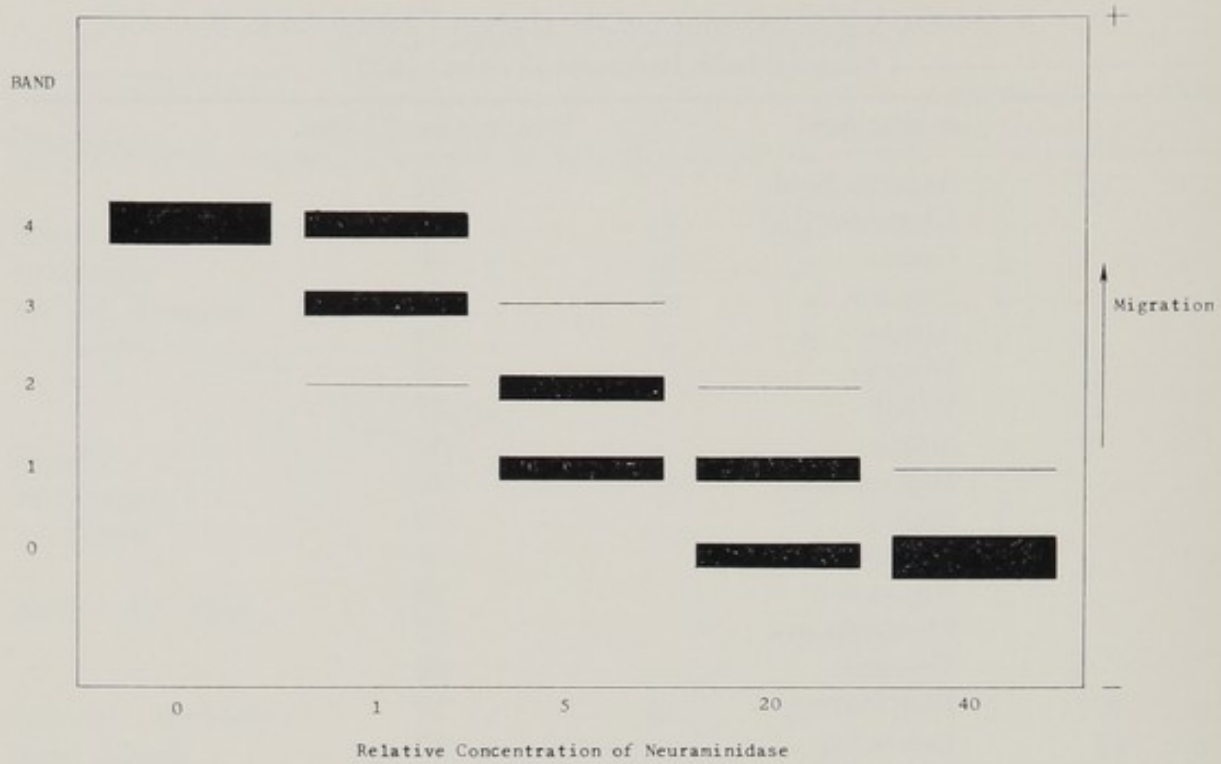


FIG. 4—Diagram of Fe^{59} autoradiograph showing (starch gel) electrophoretic mobility of purified Tf C incubated with various concentrations of neuraminidase. The band numbers correspond to the number of sialic acid residues per molecule of protein (after Parker and Bearn, 1961a).

cholerae) splits the single protein band into four additional slower-moving iron-binding components, the relative concentration of which is dependent upon the amount of neuraminidase used. These components, shown in Figure 4, represent stepwise removal of sialic acid residues, with an associated change in net electrical charge. Thus, the untreated Tf C contains four sialic acid residues, whereas the slowest-moving component contains none. Subsequent analysis of Tf B_0, B_{0-1}, B_1, B_2, D_{Chi}, D_1 and D_3 showed, however, that they, too, contain four sialic residues, and have a similar stepwise pattern of decreasing mobility when subjected to the action of neuraminidase.

In conjunction with this work, Parker and Bearn (1962) studied transferrin from umbilical cord blood and also from cerebrospinal fluid. Electrophoresis of the fetal protein revealed a prominent band in the position of Tf C as well as 4 faint slower-moving bands corresponding to the components seen after neuraminidase treatment of adult transferrin. The spinal fluid transferrin consisted of two principal bands, one migrating in the usual position of Tf C and the other coinciding with sialic acid-free Tf C. In the fetus, these findings suggested incomplete development of an enzyme system for adding sialic-acid residues to transferrin protein. In the

spinal fluid, an early stage in the addition of sialic acid units might be inhibited or enzyme-deficient.

The same authors showed that 6 purified human transferrins, as well as neuraminidase-treated Tf C, were antigenically indistinguishable when tested with antitransferrin by precipitation in agar gel. They observed reactions of only partial identity between Tf C and transferrin obtained from cynomolgus monkeys. From similar studies, it was recently reported by Williams (1961) that chicken transferrin and conalbumin (the avian egg protein which binds iron) are immunologically identical, and that neuraminidase-treated chicken transferrin migrates in starch gel with the same mobility as conalbumin.

Sutton (Sutton and Bowman, 1961) has also investigated the structure of transferrin variants, subjecting tryptic digests of purified Tf C, B_1, D_1 and D_3 to peptide analysis by electrophoresis and chromatography. Whereas Tf B_1 and D_1 could not be differentiated from C, repeatable differences in the peptide "fingerprint" of Tf D_3 were noted. This Tf has the slowest electrophoretic mobility of the known twelve variants, and is thus the most positively charged. Sutton points out that it might be expected to have greater differences in amino acid structure, and reports the presence of 3 peptides in Tf D_3 not seen in the other tested transferrins. On the other hand, he has not yet been able to demonstrate one or more peptides in Tf C which are not present in Tf D_3. The very large number of transferrin peptides and the likelihood that some of the individual spots on the chromatogram represent more than one peptide make the results difficult to interpret. Nevertheless, this technique may be as fruitful in investigating the problem of transferrin fine structure as it has been for the hemoglobins and haptoglobins.

Since the transferrins differ, one from another, in electrical charge, it is reasonable to assume that they might also differ in their ability to bind and transport iron. Turnbull and Giblett (1961) added increments of radioiron to serum containing Tf CD_3 and quantitated the radioactivity in the two transferrins after electrophoresis. They also added a constant (small) amount of radioiron to several portions of serum and stopped the reaction at varying intervals with large amounts of carrier iron, subsequently measuring the Fe^{59} in the two transferrins. The results of these experiments, shown in Figures 5 and 6, indicated that both the amount and rate of iron-binding by the two transferrins were very similar.

The same authors performed in vivo studies in which one transferrin was tagged with Fe^{59} and another with Fe^{55}. The two were mixed and injected intravenously, and the rates of radioiron disappearance from the circulation were measured. Three different transferrins, including the slowest-moving Tf D_3 and the fastest-moving Tf B_0, were compared with Tf C,

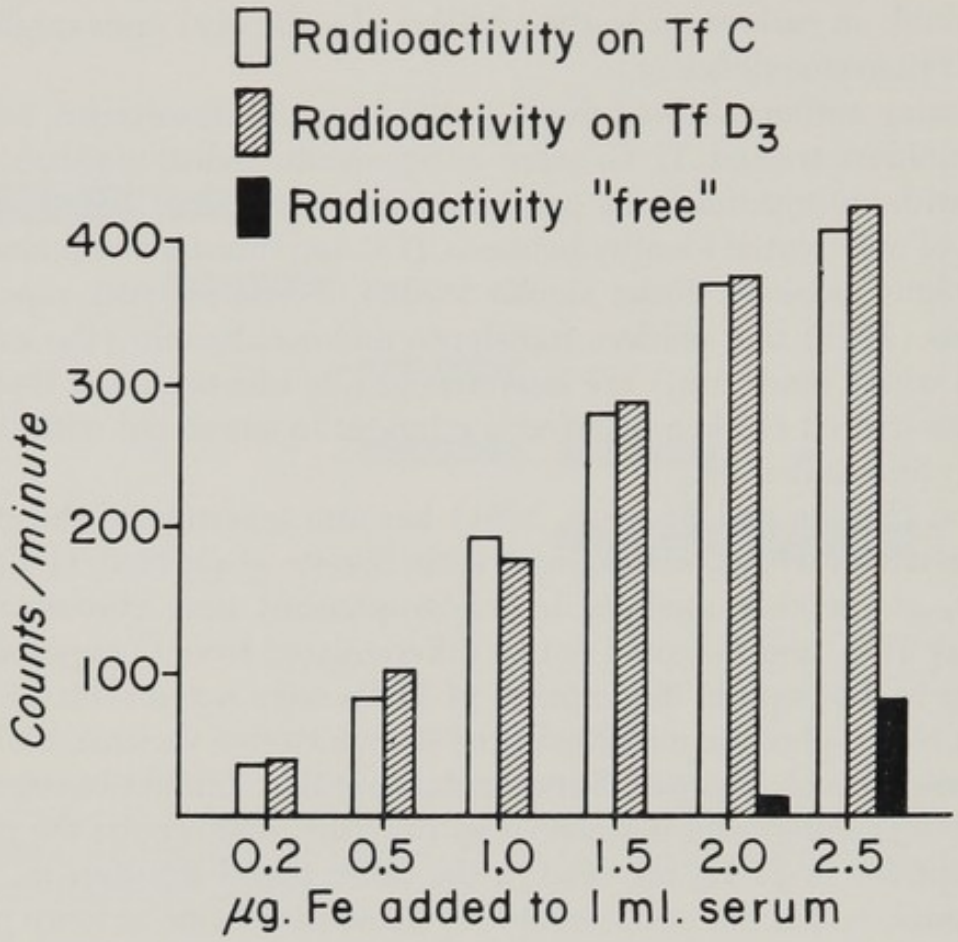


FIG. 5—Quantity of radioiron bound to two transferrins. Radioactivity of the two transferrins of type CD_3 was measured after addition of increments of Fe^{59} to the serum and separation by filter paper electrophoresis (from Turnbull and Giblett, 1961).

each comparison being made in two recipients. In all instances, the rates of iron clearance and the percentage of iron delivered by the two transferrins and utilized by the bone marrow for hemoglobin production were essentially the same. It was therefore concluded that any differences among the transferrins in iron-binding and transport must be of a very small order.

IV. Transferrin Variants in Animals

Transferrin polymorphism has been observed in a wide variety of animal species including cattle (Ashton, 1958a; Ashton, 1959a; Ashton, 1959b; Ashton, 1961; Ashton and McDougall, 1958; Buschmann and Schmid, 1961; Gahne, 1961; Gahne, Rendel and Venge, 1960; Smithies and Hickman, 1958), sheep (Ashton, 1958b; Ashton et al., 1958), goats (Ashton et al., 1958), horses (Ashton, 1958c), monkeys (Beckman, Holmgren, Mäkelä and Lehrovaara, 1961; Blumberg, 1960; Goodman and Poulik, 1961a; Goodman and Poulik, 1961b; Lai and Kirk, 1960; Parker and Bearn, 1961c; Sutton et al., 1961), chimpanzees (Boyer and Young, 1960), mice (Ashton and Braden, 1961), birds (Williams, 1962) and snakes (Dessauer, Fox and

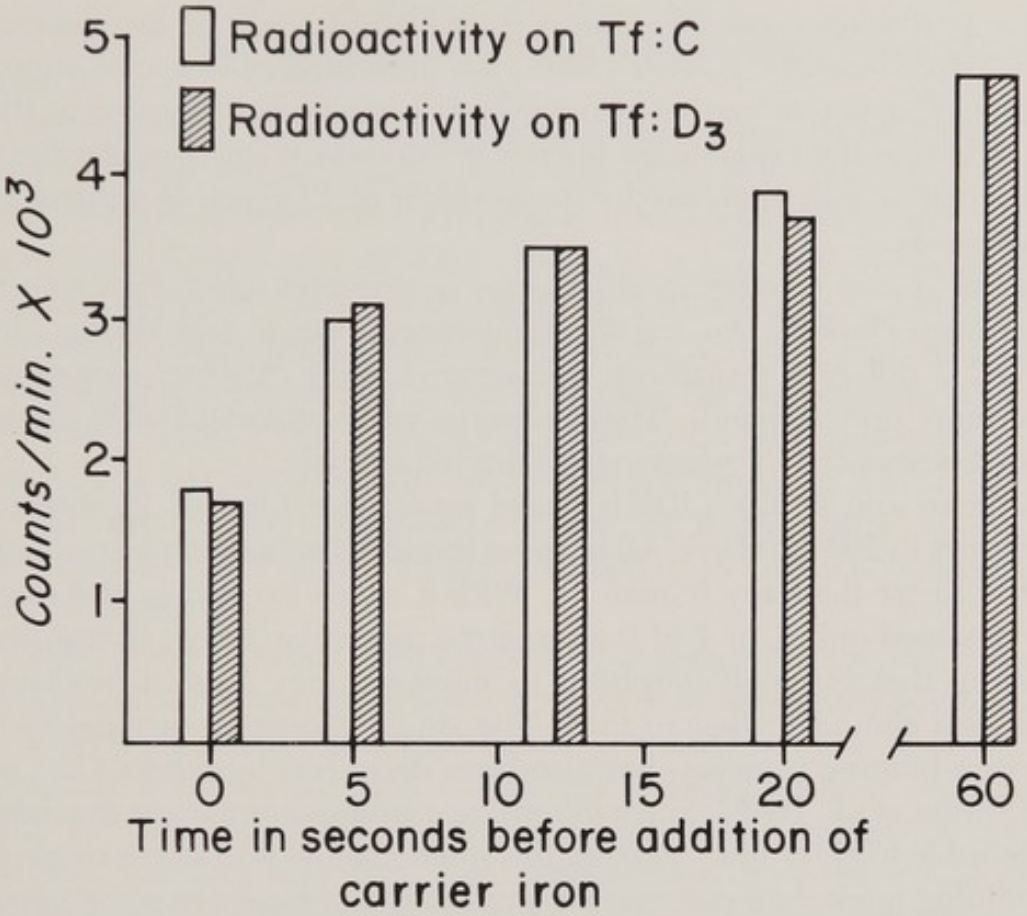


FIG. 6—Rate of iron binding by two transferrins. Radioactivity of the two transferrins of type CD_s was measured after paper electrophoresis of serum to which a constant amount of Fe^{59} had been added and the binding stopped at intervals by addition of carrier iron (from Turnbull and Giblett, 1961).

Hartwig, 1961). In many instances, the *Tf* alleles are associated with more than one protein band when the serum is tested by starch gel electrophoresis, a situation which could be analogous to the haptoglobin polymers found in man. For example, a number of Tf phenotypes have been described in cattle, their inheritance patterns indicating the existence of five allelic genes, which are designated Tf^A, Tf^D, Tf^E, Tf^B *and* Tf^F (in order of their discovery). In each homozygous type, there are four detectable zones, the two with the slowest mobility being most heavily concentrated, and the fastest being very faint. The heterozygote patterns are identical with those obtained by a mixture of the corresponding homozygous sera. In this respect, they differ from the human haptoglobins, in which the Hp 2–1 phenotype does not coincide with a mixture of Hp 1–1 and Hp 2–2.

Ashton (1959a) suggested that the frequency of Tf^E within a breed of cattle may reflect the severity of the climate from which they originated. In addition, he (1959b) reported a deficiency in the number of cattle with the Tf^E gene born to mothers having Tf^E as part of their genotype. Neither

of these postulates received support from Gahn's study of Swedish cattle (1961). More recently, Ashton (1961) has presented evidence of increased breeding efficiency in matings having both partners homozygous at the *Tf* locus. Whereas the explanation for this phenomenon requires clarification, the observation may indicate the involvement of *Tf* genes in natural selection processes.

Mechanisms of transferrin inheritance in primates other than man have not yet been clarified. Among 25 chimpanzees, Boyer and Young (1960) observed 7 different transferrin phenotypes, most of which consisted of two or more protein bands. These patterns were consistent with the existence of 4 postulated *Tf* genes in the sampled animals.

Goodman and Poulik (1961b) have reported a total of 19 transferrin phenotypes in 199 monkeys. All of these transferrins had an electrophoretic mobility faster than any human Tf. With a single exception, the monkey sera contained only 1 or 2 of 9 alternative molecular forms of transferrin, indicating that this polymorphism in monkeys may have a biochemical mechanism similar to that in man. The single exception—a monkey with three iron-binding proteins—has also been described by others (Lai et al., 1960; Sutton et al., 1961). Since inheritance studies are not yet available, it is impossible to determine whether this phenomenon is due to a single gene determining more than one protein band or the presence in some monkeys of more than one *Tf* locus.

Parker and Bearn (1961c) treated the transferrins of chimpanzees, monkeys and cattle with neuraminidase, obtaining two slower-moving components in each case, This result suggested that the iron-binding proteins of these animals contain only two sialic acid residues accessible to the enzyme rather than the four present in human transferrin. The same authors, having observed an immunologic reaction of partial identity between the transferrins of man and cynomolgus monkeys, found that the monkey protein was 20 per cent higher in serine content and 50 per cent lower in glucosamine.

V. *Geographic Distribution of Human Transferrin Variants*

In comparison to the large number of available papers on haptoglobin distribution, relatively few studies on the incidence of the transferrin phenotypes in various populations have been published. Unlike some of the other established polymorphic systems in man, investigation of *Tf* gene frequencies has several handicaps. First of all, with few exceptions, the relative proportion of individuals within a population who have demonstrably aberrant forms of the protein is small. For example, among most Caucasian groups, the slow-moving variants are very rare, and the fast-moving variants are usually found in less than 2 per cent of random sub-

jects. Thus, to establish accurate gene frequency figures, large samples are required.

A second difficulty resides in the fact that phenotype differentiation is based on rather minor differences in electrophoretic mobility. This necessitates careful comparison with control specimens of known phenotype. Since most of such sera are rare, they are not always available for this important purpose. Thus, it is possible that some reports, particularly the earliest ones, have incorrectly designated one phenotype as another. This may be especially important in differentiating the slow-moving Tf D_1 from the slightly faster Tf D_{Chi}, since the latter could be representative of the predominant variant in Asia as opposed to the former in Africa. Since D_{Chi} has been very recently described (Parker and Bearn, 1961c), previous results (such as those of the author) of studies on Oriental populations require re-evaluation.

A third problem arises from the lability of the protein which, in very old or poorly refrigerated specimens, tends to lose its sharp contours during electrophoresis. Bacterial contaminants, presumably because of their neuraminidase activity, can alter transferrin mobility without destroying its ability to bind iron. These serum specimens usually appear to contain 3 Tf bands, but they may have 2 or 4 depending upon the extent of sialic acid depletion (see previous section). Such degraded proteins have not been seen to migrate more rapidly than the common Tf C, but other proteins—particularly ceruloplasmin—may be mistaken for one of the fast-moving transferrins unless suitable techniques are used for transferrin identification.

Table 4 presents a list of Tf phenotype frequencies in various populations of the world. Note that in most areas having no apparent transferrin polymorphism, the samples are relatively small. On the other hand, some ethnic groups appear to be nearly free of aberrant transferrins. This is especially notable of the inhabitants of mid-Pacific Islands, in sharp contrast to the natives of New Guinea and Western Australia, who have the highest incidence of slow-moving transferrin yet reported in any area.

When presented as a unit, the Tf phenotype distribution among the various tribes of a given area can be misleading. For example, the 1,100 inhabitants of Central America tested by Sutton (Sutton, Matson, Robinson and Koucky, 1960; Sutton, unpubl. data) have an over-all Tf CD frequency of .020. However, this figure varies in individual tribes from 0 to .324. The very high proportion was found in the small Rama tribe and undoubtedly reflects genetic drift. Another small tribe, the Lacandon, is especially interesting for its high frequency (.172) of the $B_{0-1}C$ phenotype previously described by Parker and Bearn (1961b) in 7 per cent of a Navajo Indian tribe.

TABLE 4—*Geographical Distribution of Transferrin Phenotypes*

Population and Reference	No.	Tf Phenotype Frequencies								
		Slow-Moving Tf				Tf C	Fast-Moving Tf			
European										
English (Harris, 1959)	139	CD_{0-1}	.002°			.986	B_2C	.007	B_1C	.007
Swedish (Beckman et al., in press)	1173	CD_1	.002			.986	B_2C	.010	B_1C	.002
Lapp (Beckman and Holmgren, 1961)	329	CD_1	.018			.982				
Canadian (Smithies, 1958)	425					.988	B_2C	.012		
U.S. White (Giblett et al., unpubl. data)	471					.987	B_2C	.011	B_0C	.002
American Aborigine										
Alaskan Eskimo (Giblett and Motulsky, unpubl. data)	167					1.000				
Alaskan Indian (Giblet et al., unpubl. data)	49					1.000				
Canadian Eskimo (Parker et al., 1961c)	67					1.000				
No. Am. Navajo (Parker et al., 1961b)	230					.926	B_2C	.004	$B_{0-1}C$	.070
Central American† (Sutton et al., 1960; Sutton et al., unpubl. data)	(1100)‡	CD	.020	D	.004	.958	BC	.018		
Lacandon	58					.828	$B_{0-1}C$	.172		
Itza	86	CD	.024							
Mam	27					1.000				
Quiche	94	CD	.011			.978	BC	.011		
Chol	16					1.000				
Tzotzil	88					.989	BC	.011		
Tzeltal	97					1.000				
Cakchiquel	10					1.000				
Chinanteca	50					.960	BC	.040		
Subtiaba	24					1.000				
Sumo	38					1.000				

Lenca	150	CD	.027			.973				
Kekchi	88	CD	.045			.955				
Jicaque	48	CD	.062			.938				
Rama	37	CD_1	.324	D_1	.108	.568				
Zaboteca	80					.975	BC	.025		
Chipiapaneca	47	CD	.043			.957				
Totonaca	45					1.000				
Mestizo	17					1.000				
South American										
Venezuela (Arends and Gallango, 1960)	(308)	CD_1	.049			.948	B_2C	.003		
Caracas hybrids	102	CD_1	.020			.970	B_2C	.010		
Irapa	89	CD_1	.056			.944				
Paraujano	75	CD_1	.053			.947				
Macoita	42	CD_1	.095			.905				
Peru (Giblett and Best, 1961)	173					.994	B_2C	.006		
Chile (Parker et al., 1961b)	34					1.000				
Asiatic										
Chinese (Parker et al., 1961c)	116	CD_{Ch1}	.060			.940				
Chinese (Malaya) (Kirk et al., 1961)	103	CD_1	.068	D_1	.001	.922				
Chinese (Formosa) (Giblett et al., unpubl. data)	40	CD_1	.025			.950	B_2C	.025		
Japanese (Parker et al., 1961c)	46					.978	B_3C	.022		
Japanese (American) (Giblett et al., unpubl. data)	242	CD_1	.004			.988	B_1C	.004	B_1	.004
Formosan (Giblett et al., unpubl. data)	300	CD_1	.043			.957				
Ceylon (Kirk et al., 1961)	(197)	CD_1	.036	D_1	.005	.959				
Tamils	46					1.000				
Singhalese	87	CD_1	.011			.989				
Veddahs	64	CD_1	.094	D_1	.016	.890				
Malaya (Kirk et al., 1961)	(302)	CD_1	.043			.957				

TABLE 4 (*Cont.*)

Population and Reference	No.	Tf Phenotype Frequencies							
		Slow-Moving Tf				Tf C	Fast-Moving Tf		
Malays	236	CD_1	.047			.953			
Proto-Malays	66	CD_1	.030			.970			
Thailand (Kirk et al., 1961)	(472)	CD_1	.068	CD_0	.002	.928			
Bangkok Thais	274	CD_1	.055	D_1	.004	.941			
Northern Thais	139	CD_1	.101	CD_0	.007	.892			
Maeo	34	CD_1	.029			.971			
Yaeo	25	CD_1	.080			.920			
South India (Kirk et al., 1961)	(345)					1.000			
Tamils	133					1.000			
Todas	89					1.000			
Irulas	74					1.000			
Kurumbas	49					1.000			
North India: Oraons (Kirk et al., 1961)	125	CD_1	.064			.936			
West Pakistan (Kirk et al., 1961)	(392)					.996	B_2C	.002	B_1C .002
Punjabis	207					1.000			
Pathans	185					.990	B_2C	.005	B_1C .005
Oceanic									
W. Australian desert (Kirk and Lai, pers. comm.)	130	CD_1	.400	D_1	.046	.554			
New Guinea (E. highlands) (Bennett, Auricht, Gray, Kirk and Lai, 1961)	80	CD_1	.163	D_1	.025	.812			
New Guinea (W. & E. highlands) (Barnicot and Kariks, 1960)	470	CD_1	.172	D_1	.006	.822			
New Guinea (Papuans) (Barnicot et al., 1960)	48	CD_1	.083			.917			
Marshall Is. (Blumberg and Gentile, 1961)	106					1.000			

Gilbert Is. (Douglas, Jacobs and Sherliker and Staveley, 1961)	236					1.000		
Ellice Is. (Staveley, pers. comm.)	108					1.000		
Tonga Is. (Staveley and Douglas, 1960)	196					1.000		
W. Samoa (Staveley, pers. comm.)	80					1.000		
Fiji (Staveley, pers. comm.)	93	CD_1	.022			.978		
New Hebrides (Staveley, pers. comm.)	110	CD_1	.018			.982		
Philippines (Giblett et al., unpubl. data)	403	CD_1	.010			.990		
African								
Nigeria	(369)	CD_1	.119	D_1	.003	.878		
Fulani (Blumberg et al., 1961)	68	CD_1	.147	D_1	.015	.838		
Fulani (Barnicot et al., 1960)	111	CD_1	.063			.937		
Habe (Barnicot et al., 1960)	120	CD_1	.150			.850		
Ibo (Robson, pers. comm.)	70	CD_1	.129			.871		
Gambia (Robson, pers. comm.)	157	CD_1	.019	CD_2	.006	.975		
Liberia (Neel et al., 1961)	(333)	CD_1	.069			.928	BC	.003
Northwest	179	CD_1	.039			.961		
Central	52	CD_1	.115			.866	BC	.019
Southeast	75	CD_1	.120			.880		
Mandingo	27	CD_1	.037			.963		
Uganda: Baganda (Allison and Barnicot, 1960)	165	CD_1	.030			.970		
Tanganyika: Bondei (Allison et al., 1960)	60	CD_1	.083			.917		
Kenya: Masei (Allison et al., 1960)	50					1.000		
Belgian Congo (Giblett and Motulsky, in prep.)	(753)	CD_1	.061			.939		
Pygmies	121	CD_1	.066			.934		
Bayaka	98	CD_1	.102			.898		
Bwaka	57	CD_1	.105			.895		
Bashi	110	CD_1	.073			.927		

TABLE 4 (*Cont.*)

Population and Reference	No.	Tf Phenotype Frequencies					
		Slow-Moving Tf				Tf C	Fast-Moving Tf
Bahutu	91	CD_1	.022			.978	
Watusi	90	CD_1	.044			.956	
Urban (mixed)	186	CD_1	.043			.957	
South Africa	(859)	CD_1	.056	D_1	.001	.943	
Bushmen (Barnicot, Garlick, Singer and Weiner, 1959)	113	CD_1	.115	D_1	.009	.876	
Zulu (Barnicot et al., 1959)	116	CD_1	.026			.974	
Hottentot (Barnicot et al., 1959)	59	CD_1	.068			.932	
Cape colored (Barnicot et al., 1959)	88	CD_1	.023			.977	
Xhosa (Giblett and Zoutendyk, unpubl. data)	265	CD_1	.023			.977	
Msutu (Giblett et al., unpubl. data)	218	CD_1	.092			.908	
American Negroes							
New York (Parker et al., 1961c)	99	CD_1	.091	D_1	.010	.899	
California (Robson, pers. comm.)	48	CD_1	.104	CD_3	.021	.875	
Seattle and Cleveland (Giblett and Steinberg, unpubl. data)	1362	CD_1	.081	D_1	.001	.912	B_2C .003
		CD_3	.002	CD_0	.001		

° Reported as having 1 in 500 incidence. (Harris et al., 1960.)

† B and D transferrins usually not further identified in these reports.

‡ Figures in parentheses in this column are totals for corresponding area.

From the fairly extensive tests performed on natives of Africa, it appears that the fast-moving transferrins rarely occur there except, perhaps, in untested isolates. This is again reflected in the finding of only 4 individuals with Tf B_2C among 1,362 unrelated Negroes living in Seattle and Cleveland (Giblett et al., unpubl. data). Since about a third of the American Negro gene pool is known to consist of genes obtained from Caucasian admixture (Glass, 1955), it is likely that such admixture is responsible for the B_2C incidence of .003, as compared with about .012 in North American Caucasians.

The incidence of Tf CD_1 in the American Negro is somewhat higher than one might expect from the similar frequencies encountered throughout much of Africa. However, there is some indication of an increased frequency in West Africa, where several tribes have an incidence of 13 to 15 per cent. A comparison of the various Bantu tribes in other areas of Africa reveals no striking differences. For example, individuals from 5 tribes and 2 metropolitan areas comprise the Bantus sampled in the Congo area. In these 7 groups, the CD_1 frequency varied from .002 to .105. Similarly, in South Africa, 4 Bantu tribal groups are represented, having a CD_1 frequency of .023 (Xhosa) to .092 (Msutu). Neel et al. (1961) have suggested that in Liberia, the *Tf* gene distribution may represent a cline parallel to that of the $Hb_\beta{}^S$ gene. However, they also pointed out that the heterogeneity X^2 was just within the range of significance, requiring a larger sample for resolution.

There is considerable heterogeneity in Asia, where the slow-moving (D) variants are confined mainly to the mongoloid populations. Kirk and Lai (1961) suggest that the presence of Tf^D in the Oraons of northeast India and the Veddahs of Ceylon is indicative of mongoloid admixture, because the gene is lacking among the other populations of India as well as Pakistan. They also suggest that this admixture is a fairly recent one in the case of Ceylon, since the Veddoid tribes of south India have the $Hb_\beta{}^S$ but not Tf^D; whereas the Veddahs of Ceylon have $Hb_\beta{}^E$ as well as Tf^D.

Very few reports on the distribution of transferrin variants in Europeans are available. From the combined Swedish, Lappish, English, Canadian and American data, it appears that about 1 to 2 per cent have a fast-moving Tf, usually B_2. As yet, the only Europeans found to have a slow-moving Tf are the 2 with D_{0-1} reported by Harris et al. (1960), plus the 2 out of 1,173 Swedes and 6 of 329 Lapps with D_1 described by Beckman et al. (Beckman, Holmgren and Mårtensson, in press.)

From the available data, it is possible to make a few general statements about *Tf* gene distribution. For example, it is clear that with the exception of certain population isolates, the genes determining fast-moving Tf vari-

ants are rather sparsely distributed; in Africa and the Pacific Islands, they are almost entirely absent. The slow-moving variants have their highest incidence in certain areas of Australia and New Guinea, are fairly prevalent in Africa, and have a variable distribution in Central and South America as well as in the mongoloid peoples of Asia. They are apparently rare among individuals of European or Asiatic Indian origin. Further studies are needed to reinforce these impressions and to add more details by sampling other populations of the world. Additional refinements in technique are also needed so that subtle differences in protein migration rate can be resolved, thus providing a firmer basis for subdivisions within the B (fast-moving) and D (slow-moving) Tf groups. From the latter studies, it is hoped that a new nomenclature will be developed which can be adopted before the present unwieldy system has become too firmly entrenched.

The possible significance of the transferrin polymorphism and its geographic distribution is at present obscure. It is attractive to believe that the variant forms of this protein confer some advantage under appropriate conditions associated with climate, diet or, more likely, disease. Available data provide no evidence that these proteins differ in their ability to find and transport iron. However, the possibility that they may have a variable effect on viral synthesis has not, to our knowledge, been adequately investigated. As pointed out by Bearn, it seems unlikely that viral infections have been as powerful selective forces in the past as bacterial infections. However, resistance to viral disease would prevent the lethal effect of secondary bacterial invaders (Bearn, 1961).

One other area of speculation should be mentioned—namely, that in addition to the forms of transferrin which are distinguishable because of differences in electrical charge, there may be other forms not separable on this basis. Smithies (Smithies et al., 1959) has suggested that the replacement of a valine residue by an isoleucine residue, for example, would alter peptide structure without necessarily changing the over-all charge. Thus, it is possible in the case of transferrin, as well as other protein molecules, that polymorphism not detectable by present methods may exist.

Acknowledgment

In the preparation of this paper, the author has received generous assistance from Dr. A. G. Bearn and Mr. W. C. Parker, as well as from Dr. H. E. Sutton. Many of our own studies reported here would not have been possible without the help of Dr. A. Turnbull and the constructive suggestions of Dr. C. A. Finch and Dr. O. Smithies.

Addendum

Parker and Bearn (pers. comm.) have recently found a "new" transferrin which has an electrophoretic mobility intermediate between D_{Chi} and D_{0-1} (D_4). The propositus is of French and Irish descent, and a family study indicates that his aberrant transferrin is inherited in the same manner as the other twelve molecular forms of this protein. The same authors have also found a variant migrating between B_1 and B_2 in a Venezuelan family. Thus, the number of different molecular species of human transferrin has now reached fourteen.

References

Allison, A. C., and N. A. Barnicot. 1960. Haptoglobins and transferrins in some East Africa peoples. Acta genet. 10: 17.

Arends, T., and M. L. Gallango. 1960. Haptoglobin and transferrin groups in Venezuela. Proc. 8th Inter. Congr. Bl. Transf. Tokyo.

Ashton, G. C. 1958a. Genetics of β-globulin polymorphism in British cattle. Nature 182: 370.

——. 1958b. Further β-globulin phenotypes in sheep. Nature 182: 1101.

——. 1958c. Serum protein variations in horses. Nature 182: 1029.

——. 1959a. β-globulin alleles in some Zebu cattle. Nature 184: 1135.

——. 1959b. β-globulin polymorphism and early foetal mortality in cattle. Nature 183: 404.

——. 1961. β-globulin type and fertility in artificially bred dairy cattle. J. Reprod. & Fertil. 2: 117.

——, and A. W. H. Braden. 1961. Serum β-globulin polymorphism in mice. Austr. J. Biol. Sc. 14: 248.

——, and E. L. McDougall. 1958. β-globulin polymorphism in cattle, sheep and goats. Nature 182: 945.

Awai, M., and E. Brown. 1961. Studies of the metabolism of human transferrin. J. Lab. & Clin. Med. 58: 797.

Barnicot, N. A., J. P. Garlick, and D. F. Roberts. 1960. Haptoglobin and transferrin inheritance in northern Nigerians. Ann. Genet. 24: 171.

——, ——, R. Singer, and J. S. Weiner. 1959. Haptoglobin and transferrin variants in Bushmen and some other South African peoples. Nature 184: 2042.

——, and J. Kariks. 1960. Haptoglobin and transferrin variants in peoples of the New Guinea highlands. M. J. Australia ii: 859.

Bearn, A. G. 1961. Genetical variations in the serum proteins of man. Bull. New York Acad. Med. 37: 593.

Beckman, L., and G. Holmgren. 1961. Transferrin variants in Lapps and Swedes. Acta genet. 11: 106.

——, ——, O. Mäkelä, and R. Lehrovaara. 1961. Serum protein variations in monkeys. Acta genet. 11: 126.

——, ——, and E. H. Mårtensson. Transferrin types in the Swedish population. (In press.)

——. 1962. Slow and fast transferrin variants in the same pedigree. Nature 194: 796.

Bennett, J. H., C. O. Auricht, A. J. Gray, R. L. Kirk, and L. Y. Lai. 1961. Haptoglobin

and transferrin types in the Kuru region of Australian New Guinea. Nature 189: 68.

Blumberg, B. S. 1960. Biochemical polymorphism in animals: haptoglobins and transferrins. Proc. Soc. Exper. Biol. & Med. 104: 25.

——, and Z. Gentile. 1961. Haptoglobins and transferrins of two tropical populations. Nature 189: 897.

Boyer, S. H., and W. J. Young. 1960. β-globulin polymorphism in chimpanzees. Nature 187: 1035.

Buschmann, H. and D. O. Schmid. 1961. Transferrin groups of foetal calf serum. Nature 190: 1209.

Dancis, J., N. Braverman, and J. Lind. 1957. Plasma protein synthesis in the human fetus and placenta. J. Clin. Invest. 36: 398.

Dessauer, H. C., W. Fox, and Q. L. Hartwig. 1961. Transferrins of amphibia and reptilia. Fed. Proc. 20: 72.

Douglas, R., J. Jacobs, J. Sherliker, and J. M. Staveley. 1961. Blood groups, serum genetic factors and haemoglobins in Gilbert Islanders. New Zeal. M. J. 60: 146.

Editorial: 1961. Congenital deficiency of transferrin as a cause of haemosiderosis. Brit. M. J., Oct. 28, 1138.

Ehrenberg, A., and C. B. Laurell. 1955. Magnetic measurements on crystallized Fe-transferrin isolated from the blood plasma of swine. Acta chem. scandinav. 9: 68.

Finch, S. C., and C. A. Finch. 1955. Idiopathic hemochromatosis, an iron storage disease. Medicine 34: 381.

Gahne, B. 1961. Studies of transferrins in serum and milk of Swedish cattle. Animal Prod. 3: 135.

——, J. Rendel, and O. Venge. 1960. Studies on the inheritance of β-globulins in serum and milk from cattle. Nature 186: 907.

Giblett, E. R., and W. R. Best. 1961. Haptoglobin and transferrin types in Peruvian Indians. Nature 192: 1300.

——, and L. Brooks. Unpublished data.

——, C. G. Hickman, and O. Smithies. 1959. Serum transferrins. Nature 183: 1589.

——, and A. G. Motulsky. Blood groups, haptoglobins and transferrins in pygmies and bantus of the Congo region (in preparation).

——, and ——. Unpublished data.

——, and A. G. Steinberg. Unpublished data.

——, and A. Zoutendyk. Unpublished data.

Gitlin, D., C. A. Janeway, and L. E. Farr. 1956. Studies on the metabolism of plasma proteins in the nephrotic syndrome. I. Albumin, γ-globulin and iron-binding globulin. J. Clin. Invest. 35: 44.

Glass, B. 1955. On the unlikelihood of significant admixture of genes from the North American Indians in the present composition of the Negroes of the United States. Am. J. Human Genet. 7: 368.

Goodman, M., and E. Poulik. 1961. Effects of speciation on serum proteins in the genus Macaca with special reference to the polymorphic state of transferrin. Nature 190: 171.

——, and ——. 1961b. Serum transferrins in the genus Macaca: species distribution of nineteen phenotypes. Nature 191: 1407.

Harris, H. 1959. Human Biochemical Genetics. Cambridge University Press, p. 210.

——, D. G. Penington, E. B. Robson, and C. R. Scriver. 1960. A further genetically determined transferrin variant in man. Ann. Human Genet. 24: 327.

——, E. B. Robson, and M. Siniscalco. 1958. β-globulin variants in man. Nature 182: 452.

Heilmeyer, L., W. Keller, O. Vivell, W. Keiderling, K. Betke, F. Wöhler, and H. E. Schultze. 1961. Kongenitale atransferrinämie bei einem sieben Jahre alten Kind. Deutsche med. Zwschr. 86: 1745.

Hitzig, W. H., M. Schmid, K. Betke, and M. Rothschild. 1960. Erythroleukämie mit Hämoglobinopathie und Eisenstoffwechselstörung. Helvet paediat. acta 15:203.

Holmberg, C. G., and C. B. Laurell. 1945. Studies on the capacity of serum to bind iron. A contribution to our knowledge of the regulation mechanism of serum iron. Acta physiol. scandinav. 10: 307.

Horsfall, and O. Smithies. 1958. Genetic control of some human serum β-globulins. Science 128: 35.

Huff, R. L., T. G. Hennessey, R. E. Austin, J. F. Garcia, B. M. Roberts, and J. H. Lawrence. 1950. Plasma and red cell iron turnover in normal subjects and in patients having various hematopoietic disorders. J. Clin. Invest. 29: 1041.

Inman, J. K. 1956. Preparation of crystalline β_1-metal-combining protein. In Proc. 10th Conf. on Plasma Protein and Cellular Elements of the Blood. Cambridge, Mass. Protein Foundation, p. 46.

——, F. C. Coryell, K. B. McCall, J. T. Sgouris, and H. D. Anderson. 1961. A large scale method for the purification of human transferrin. Vox Sang. 6: 34.

Jager, B. V., and C. J. Gubler. 1952. An immunologic study of the iron-binding protein. J. Immunol. 69: 311.

Jandl, J. H., J. K. Inman, R. L. Simmons, and D. W. Allen. 1959. Transfer of iron from serum iron-binding protein to human reticulocytes. J. Clin. Invest. 38: 161.

Katz, J. H. 1961. Iron and protein kinetics studied by means of doubly-labeled crystalline transferrin. J. Clin. Invest. 40: 2143.

Keller, W., and R. B. Pennell. 1959. Sterilization of plasma components by heat. I. β-1 metal combining protein. J. Lab. & Clin. Med. 53: 638.

Kirk, R. L. Personal communication.

——, and L. Y. Lai. 1961. The distribution of haptoglobin and transferrin groups in South and Southeast Asia. Acta genet. 11: 97.

Kistler, P., Hs. Nitschmann, A. Wyttenbach, M. Studer, Ch. Niederöst, and M. Mauerhofer. 1960. Humanes siderophilin: isolierung mittels Rivanol aus blutplasma und plasmafractionen, analytische Bestimmung und Kristallisation. Vox Sang. 5: 403.

Koechlin, B. A. 1952. Preparation and properties of serum and plasma proteins. XXVIII. The β-1 metal-combining protein of human plasma. J. Am. Chem. Soc. 74: 2649.

Lai, L. Y. C. and R. L. Kirk. 1960. β-globulin variants in two species of monkeys. Nature 188: 673.

Laurell, C. B. 1947. Studies on the transportation and metabolism of iron in the body. Acta Physiol. Scand. 14, Suppl. 46.

——, 1960. Metal-binding plasma proteins and cation transport. In F. W. Putnam, Ed.: The Plasma Proteins. New York: Academic Press. p. 349, vol. 1.

——, and B. Ingelman. 1947. The iron-binding protein of swine serum. Acta chem. scandinav. 1: 770.

Martin, C. M., and J. H. Jandl. 1960. Inhibition of virus multiplication by transferrin. In M. J. Seven, and R. A. Johnson, Eds.: Metal Binding in Medicine. Philadelphia: J. B. Lippincott Co., p. 335.

Mazur, A., S. Green, and A. Carleton. 1960. Mechanism of plasma iron incorporation into hepatic ferritin. J. Biol. Chem. 235: 595.

Mitchell, J., E. R. Halden, F. Jones, B. A. Bryan, J. A. Stirman, and E. E. Muirhead. 1960. Lowering of transferrin during iron absorption in iron deficiency states. J. Lab. and Clin. Med. 56: 555.

Neel, J. V., A. R. Robinson, W. W. Zuelzer, F. B. Livingstone, and H. E. Sutton. 1961. The frequency of the A_2 and fetal hemoglobin fractions in the natives of Liberia and adjacent regions, with data on haptoglobin and transferrin types. Am. J. hum. Genet. 13: 262.

Parker, W. C., and A. G. Bearn. Pers. communication.

——, and A. G. Bearn. 1961a. Alterations in sialic acid content of human transferrin. Science 133: 1014.

——, and ——. 1961b. Haptoglobin and transferrin gene frequencies in a Navajo population: a new transferrin variant. Science 134: 106.

——, and ——. 1961c. Haptoglobin and transferrin variations in humans and primates: two new transferrins in Chinese and Japanese populations. Ann. Human Genet. 25: 227.

——, and ——. 1962. Studies on the transferrins of adult serum, cord serum and cerebrospinal fluid: the effect of neuraminidase. J. Exper. Med. 115: 83.

Poulik, M. D. 1957. Starch gel electrophoresis in a discontinuous system of buffers. Nature 180: 1477.

——, and O. Smithies. 1958. Comparison and combination of the starch-gel and filter-paper electrophoretic methods applied to human sera: two dimensional electrophoresis. Biochem. J. 68: 636.

Rath, C. E., and C. A. Finch. 1949. Chemical, clinical and immunological studies on the products of human plasma fractionation. XXXVIII. Serum iron transport. Measurement of iron-binding capacity of serum in man. J. Clin. Invest. 28: 79.

Rausen, A. R., P. S. Gerald, and L. K. Diamond. 1961. Genetical evidence for transferrin synthesis in the foetus. Nature 192: 182.

Riegel, C., and D. Thomas. 1956. Absence of β-globulin fraction in the serum protein of a patient with unexplained anemia. New England J. Med. 255: 434.

Robson, E. B. Personal communication.

Saltman, P., and P. J. Charley. 1960. The regulation of iron metabolism by equilibrium binding and chelation. In M. J. Seven, and L. A. Johnson, Eds.: Metal Binding in Medicine. Philadelphia: J. B. Lippincott Co., p. 241.

Schade, A. L., and L. Caroline. 1946. An iron-binding component in human blood plasma. Science 104: 340.

Schade, A., R. Reinhart, and H. Levy. 1949. Carbon dioxide and oxygen in complex formation with iron and siderophilin, the iron-binding component of human plasma. Arch. Biochem. 20: 170.

Schedl, H. P., and F. C. Bartter. 1959. Serum iron-binding levels after infusion of human serum albumin. Lancet, June 6, 1163.

Schultze, H. E. 1957. Über klinisch interessante Körpereigene Polysaccharidverbindungen. Scand. J. Clin. & Lab. Invest. 10: (suppl. 31) 135.

Smithies, O. 1955. Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults. Biochem. J. 61: 629.

——. 1957. Variations in human serum β-globulins. Nature 180: 1482.

——. 1958. Third allele at the serum β-globulin locus in humans. Nature 181: 1203.

——. 1959a. An improved procedure for starch-gel electrophoresis: further variations in the serum proteins of normal individuals. Biochem. J. 71: 585.

——. 1959b. Zone electrophoresis in starch gels and its application to studies of serum proteins. In C. B. Anfinson, M. L. Anson, K. Bailey, and J. T. Edsall, Eds.: Advances in Protein Chemistry. New York: Academic Press. p. 65, vol. 14.

——. Personal communication.

——, and G. E. Connell. 1959. Biochemical aspects of the inherited variations in human

serum haptoglobins and transferrins. In G. E. W. Wolstenholme, and C. M. O'Connor, Eds.: Boston: Little, Brown, & Co., p. 178.
——, and C. G. Hickman. 1958. Inherited variations in the serum proteins of cattle. Genetics 43: 374.
——, and O. Hiller. 1959. The genetic control of transferrins in humans. Biochem. J. 72: 121.
——, and N. F. Walker. 1956. Notation for serum protein groups and the genes controlling their inheritance. Nature 178: 694.
Staveley, J. M. Personal communication.
——, and R. Douglas. 1960. Transferrins in Tongans. New Zeal. M. J. 59: 546.
Surgenor, D. M., B. A. Koechlin, and L. E. Strong. 1949. Chemical, clinical, and immunologic studies on the products of human plasma fractionation. XXXVII. The metal-combining globulin of human plasma. J. Clin. Invest. 28: 73.
Sutton, H. E. Personal communication.
——, and B. H. Bowman. 1961. Genetic and chemical aspects of the transferrins. Proc. 2nd Intern. Cong. on Human Genet., Rome.
——, G. A. Matson, A. R. Robinson, and R. W. Koucky. 1960. Distribution of haptoglobin, transferrin and hemoglobin types among Indians of southern Mexico and Guatemala. Am. J. Human Genet. 12: 338.
——, and G. A. Matson. Uupublished data.
Turnbull, A., and E. R. Giblett. 1961. The binding and transport of iron by transferrin variants. J. Lab. & Clin. Med. 57: 450.
Williams, J. 1962. A comparison of conalbumin and transferrin in the domestic fowl. Biochem. J. 83: 355.
Wintrobe, M. M. 1961. Clinical Hematology, ed. 5. Philadelphia: Lea and Febiger, p. 147.
Winzler, R. J. 1960. Glycoproteins. In F. W. Putnam, Ed.: The Plasma Proteins. New York: Academic Press, p. 309, vol. 1.

Chapter 3

The Group Specific Component of Serum; Genetic and Chemical Considerations

Hartwig Cleve and Alexander G. Bearn
The Rockefeller Institute, New York, New York

The discovery of genetically determined variations among the serum proteins can be ascribed in large measure to the recent development of separatory techniques of high resolving power. The introduction of starch gel electrophoresis by Smithies and the application of serological micromethods have proved particularly valuable. The electrophoretic separation of serum on a gel of partially hydrolysed starch separates the proteins not only according to their charge, but also their size and shape and permits the recognition of more than 30 protein components (Smithies, 1959a). Using this technique Smithies demonstrated in 1955 the existence of heritable variations in haptoglobins, the hemoglobin-binding α_2 globulin of serum (Smithies and Walker, 1955 and 1956). Two years later Smithies reported genetically determined variations of a β-globulin (Smithies, 1957) which was later identified as transferrin, the serum iron-binding β-globulin (Giblett, Hickman and Smithies, 1959; see Giblett, this volume). Grubb in 1956 found that the agglutination of Rh-antibody coated red cells by certain sera from patients with rheumatoid arthritis was inhibited by some normal human sera and not by others (Grubb, 1956; Grubb and Laurell, 1956) and initiated with this observation the disclosure of a complex inherited polymorphism in normal gamma globulin now known as the Gm and Inv system (see Steinberg, this volume). Recently, Allison and Blumberg found a precipitating isoantibody in the sera of several patients who had received multiple blood transfusions (Allison and Blumberg, 1961; Blumberg and Allison, 1961). The antibody reacted in the gel diffusion test

These studies were supported in part by The National Foundation and the United States Public Health Service A-1542 (C4).

of Ouchterlony (Ouchterlony, 1949) with an antigen occurring in certain normal sera. This system, the Ag-system, is due to heritable variations of the low density β-lipoproteins which serve as antigens. Most of the genetically determined variants of haptoglobins, Gm and Inv groups, and the Ag-system are common, and some of the transferrin variants were found to be frequent in certain populations. Variations in the response of individuals to succinyldicholine (suxamethonium) led to the discovery of an inherited polymorphism of plasma pseudocholinesterase (Forbat, Lehmann and Silk, 1953; Lehmann and Ryan, 1956; Kalow and Staron, 1957; Harris, Whittaker, Lehmann and Silk, 1960). The atypical pseudocholinesterase appears to be quite uncommon; the frequency for the less common allele was found to be 0.02 in Canadians (Kalow and Gunn, 1959). Extremely rare is the heritable variation found in serum albumin, known as bisalbuminemia (Knedel, 1957). Heterogeneity has also been observed in ceruloplasmin (Morell and Scheinberg, 1960; McAllister, Martin and Benditt, 1961) and α_1-acid glycoprotein (orosomucoid; Schmid and Binette, 1961), but it has not yet been clearly established that these variations are under genetical control.

A combination of an electrophoretic procedure with a sensitive serological precipitation test constitutes the immunoelectrophoretic analysis of antigen mixtures, described in 1953 by Grabar and Williams (Grabar and Williams, 1953). A mixture of antigens, for instance human serum, is separated by electrophoresis on agar gel. Following the electrophoretic separation, a gel diffusion test is performed. An antiserum is allowed to diffuse towards the electrophoretically separated antigens which are concurrently diffusing towards the antibodies. The reaction of antigens with their specific antibodies is indicated by the formation of precipitates. During the last several years immunoelectrophoresis has been widely applied to the study of normal and pathological human sera (Grabar and Burtin, 1960). With this technique more than 25 antigenic constituents have been found in a single normal human serum. Although the immunoelectrophoretic patterns of individual sera are very similar, slight variations in the position and shape of α_2 globulin precipitation lines have been observed since 1955 (Williams and Grabar, 1955). Detailed systematic studies on the reproducibility of the immunoelectrophoretic pattern of normal human sera were performed by Hirschfeld, who recognized characteristic differences in the electrophoretic mobilities of the haptoglobin types. In 1959 he described a systematic variation in the electrophoretic mobility of a previously unrecognized α_2-globulin (Hirschfeld, 1959b). This protein was clearly distinguishable from the already characterized and identified globulins of the α_2 region. The different electrophoretic positions and shapes of the precipitate enabled the recognition of three distinct phenotypes in normal human sera. Hirschfeld called this protein the "Group specific

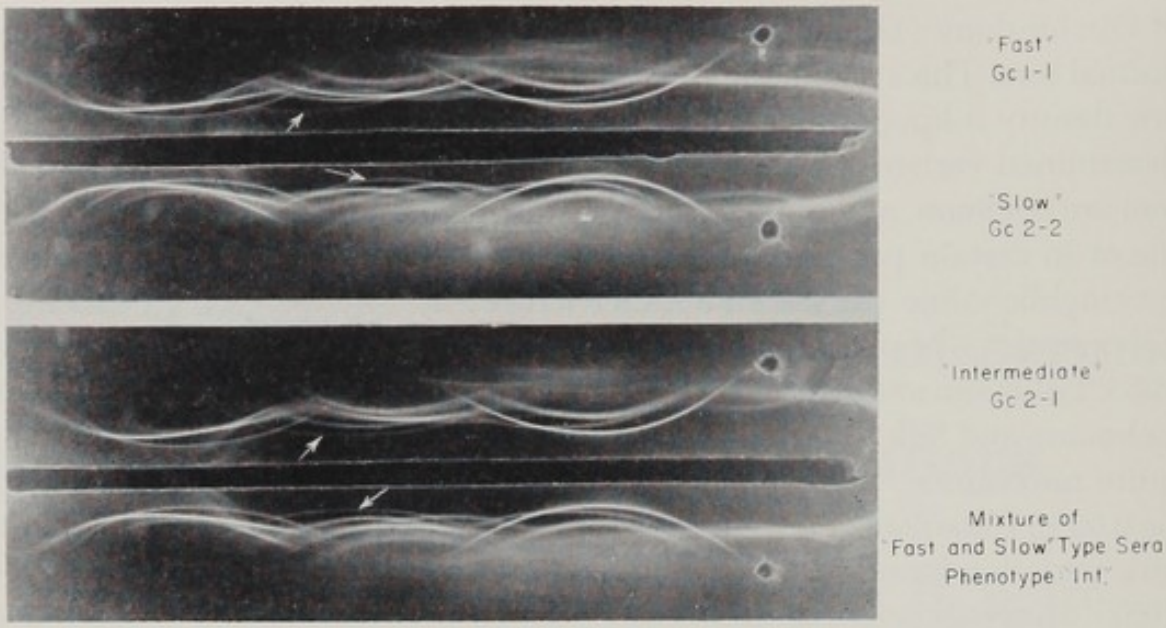


FIG. 1—Comparative immunoelectrophoretic analysis of 3 normal human sera demonstrating the 3 common phenotypes of the group-specific component. The immunoelectrophoretic pattern of a mixture of equal amounts of "fast" and "slow"-type sera is also illustrated. The Gc is indicated by arrows. The separation has been carried out from right (cathode) to left (anode).

component" and presented evidence that the variants of this system were under genetical control (Hirschfeld, Jonsson and Rasmuson, 1960). This polymorphic system is referred to as the Gc system.

THE COMMON PHENOTYPES OF THE GC SYSTEM

The immunoelectrophoretic analysis of several normal human sera is illustrated in Figure 1. With an antiserum from a horse immunized with pooled normal human sera 19 precipitation lines have been seen, each line representing one of the 19 different antigenic constituents: albumin, 4 α_1-globulins, 8 (or 9) α_2-globulins, 5 β-globulins and γ-globulin. With the aid of specific staining methods, absorption of the antiserum with purified proteins and the use of specific antisera 15 of the 19 antigens can be identified: albumin, α_1-lipoprotein, α_1-glycoprotein, α_2-macroglobulin, haptoglobin, ceruloplasmin, the barium α_2-glycoprotein and the zinc α_2-glycoprotein, α_2-lipoprotein, pseudocholinesterase, transferrin, β_1-heme-binding-globulin, β_1 A- and β_1 C-globulin and γ-globulin. In Fig. 1 the group specific component is indicated by an arrow; the precipitate is located close to the antiserum basin and occupies different electrophoretic positions in the α_2-globulin region. In some sera a fast-moving component appears, in others a slow-moving component. In a third group of sera a long, stretched

precipitation line occurs, which occupies an intermediate position. Under optimal resolving conditions this precipitate forms two peaks, where the positions of the maxima correspond to the fast and slowly migrating components. An in vitro mixture of equal amounts of a serum with a fast-moving component and a serum with a slow-moving component is indistinguishable from the intermediate-phenotype and does not reveal immunological differences between the fast- and slow-moving components. Approximately 5,000 normal and several hundred pathological human sera have been studied in different laboratories. Except in a few cases, which will be discussed later, every serum could be classified into one of these three groups.

Methodological Considerations

For the routine typing of Gc in human sera immunoelectrophoresis is performed on glass slides according to the technique of Scheidegger (Scheidegger, 1955). To obtain clear-cut reproducible results, distinct resolution of the α-globulin region is required and can be achieved by slight modifications of the original Scheidegger procedure which consists primarily of a longer separation time (Hirschfeld, 1959a). The serum is separated for 100 to 120 minutes employing a voltage gradient of approximately 7 volts/cm. at pH 8.6 in a barbital buffer to which calcium lactate has been added (Laurell, Laurell and Skoog, 1956, modified by Hirschfeld, 1959a). Although the original buffer system of Grabar and Williams can be used, the calcium lactate barbital buffer results in a slight but definite improvement in the resolution of the α-globulin region. Antihuman immune sera containing antibodies against the group specific component have been prepared in rabbits, horses, mules, sheep, monkeys and mice (Hirschfeld, 1962). Suitable antisera are now commercially available and include the antihuman horse serum no. 13411 of the Pasteur Institute, France, the antihuman horse serum no. 3438 and the antihuman rabbit serum no 3421 of the Behringwerke, Germany.

The persistence and reproducibility of the classification of Gc types have been studied by Hirschfeld as well as by ourselves (Hirschfeld, 1962; Cleve and Bearn, 1961b). No alteration of the Gc phenotype, characteristic of an individual, has been observed. It was also established that storage at –20 C. and repeated freezing and thawing did not alter the Gc-precipitates, and the classification of the Gc types was unaffected.

The Mode of Inheritance

On the basis of family studies, it has been established that the variations of the Gc are controlled by two co-dominant autosomal alleles, where the

TABLE 1—*Gc-Phenotypes in 73 Families with 180 Offspring*

Parental Phenotypes	No. of Families	Offspring			Total
		Fast	Int.	Slow	
Fast–Fast	34	85	—	—	85
Fast–Int.	27	38	35	—	73
Fast–Slow	5	—	7	—	7
Int.–Int.	6	3	6	5	14
Int.–Slow	1	—	1	—	1
Slow–Slow	0	—	—	—	0
	73	126	49	5	180

fast- and slow-moving components represent the two homozygotic Gc types and the intermediate phenotype corresponds to the heterozygous Gc type (Hirschfeld, Jonsson and Rasmuson, 1960; Cleve and Bearn, 1961a). In a fashion analogous to the haptoglobins the notation Gc 1–1, Gc 2–1, Gc 2–2, has been proposed for the Fast, Intermediate and Slow types respectively and Gc^1 and Gc^2 for the two alleles (Hirschfeld and Beckman, 1960). Thus far a total of 115 families with 317 offspring have been studied (Hirschfeld, 1962; Cleve and Bearn, 1961c). In Table 1, our findings in 73 families with 180 offspring are summarized. No deviations from the postulated genetic mechanism have been found. In addition, a total of 647 mother-child combinations have been tested and were consistent with the hypothesis (Hirschfeld, 1962; Cleve and Bearn, 1961c). In 17 monozygotic twins, the Gc type was concordant; in 17 dizygotic twins, 13 pairs were concordant and 4 pairs had discordant Gc types (Cleve and Bearn, 1961a). Gc was present in 68 cord sera obtained from newborn infants. Comparative studies on maternal and cord sera disclosed discordant Gc types in 23 instances, while the Gc types in 42 pairs were concordant (Hirschfeld, 1962; Cleve and Bearn, 1961c). Exceptions to the suggested mode of inheritance were not found. The discordance of Gc types in maternal and cord sera indicates that the presence of the Gc in the sera of newborn infants is not due to placental transfer of the protein. This possibility was considered because density gradient ultracentrifugations had shown that the Gc belonged to the group of low molecular weight serum proteins with an S-rate of approximately 4S (Cleve and Bearn, (1961b). The fetal synthesis of the Gc was investigated further by immunoelectrophoretic analysis of fetal sera from miscarriages and premature births (Cleve and Bearn, 1961c). Gc was absent in sera obtained from miscarriages of 17, 24 and 28 weeks but present in samples obtained from premature births of 33 and 36 weeks.

Evidence for Further Gc Variants

The detection of slight but distinct variations in relative electrophoretic mobilities by immunoelectrophoretic analysis on microscopic slides is technically more difficult than comparable studies by starch gel electrophoresis. However, in a few individuals, variations distinguishable from the main phenotypes, Gc 1–1, 2–1 and 2–2, were observed. Out of 2,000 normal human sera, Hirschfeld found 2 with unusual Gc types (Hirschfeld, 1962). In one serum from a Swedish individual, a Gc precipitate was observed, which appeared to be more extended and slower migrating than the common Gc 1–1. The observation was technically reproducible. On repeated examinations the precipitate was occasionally two-peaked. In this instance, the fast peak occupied the same electrophoretic position as the Gc 1–1 precipitate, but the slower peak was situated in the intermediate a_2-globulin region and not in the slow a_2-globulin position characteristic of Gc 2–2. This serum type was designated Gc 1–X. An extended or two-peaked precipitate was also found in a serum from an African Negro, but in this case the slower peak corresponded to the Gc 1-1 precipitate and the faster peak occupied a position of a component migrating faster than Gc 1–1. This type was described as Gc 1–Y. In one patient with an abnormal paper electrophoretic pattern an unusually extended two-peaked Gc type was found. Although all these variations appeared to be technically reproducible, it was unfortunately not possible to examine a second sample of serum or to study family members. Variations from the usual Gc 1–1 pattern were occasionally seen in other sera but did not prove to be reproducible.

In our laboratory, 6 of 2,000 sera examined revealed unusual Gc 2–1 phenotypes. Four of these sera were found during a study of the Gc gene frequency in a sample of 159 Chippewa Indians (Cleve, Schacht, Kleinman and Bearn) and one was found in a Chinese in New York City. With the collaboration of L. E. Schacht and H. Kleinman, we were able to secure a second sample of blood from all 4 individuals and in addition, to obtain samples from family members. The Gc 2–1 variation (Gc 2–1v) is illustrated in Figure 2. The two peaks appear to be more prominent and distinctly separated than in the common Gc 2–1 sera. The position of the two peaks does not correspond precisely to the position of the Gc 1–1 and Gc 2–2 precipitates. The faster peak migrates more rapidly than Gc 1–1. The slower peak moves more slowly than Gc 2–2, but the difference is only small. Using several different antisera, the Gc 2–1 variations were technically reproducible on repeated examinations. The second sample obtained after several months from the Gc 2–1 variant individuals disclosed identical patterns. Three of the 4 samples originally found in the Chip-

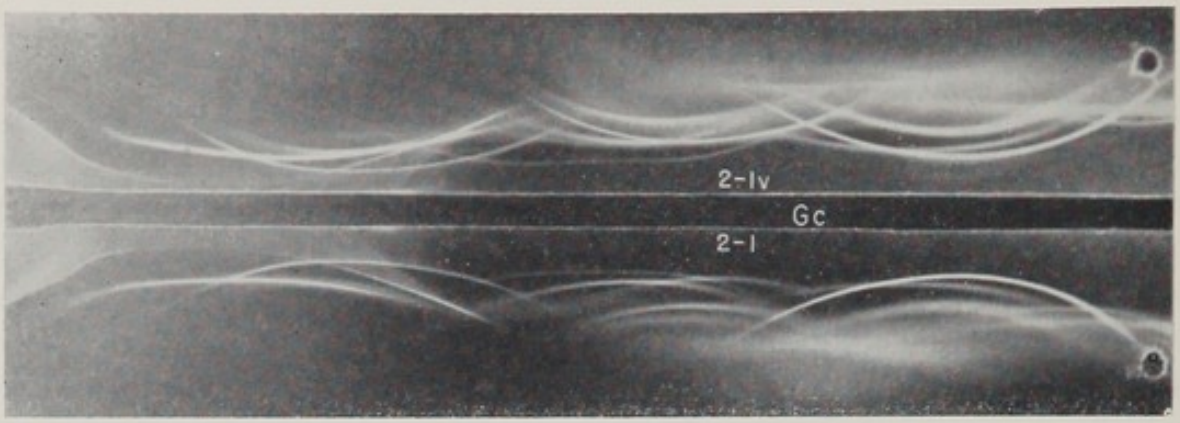


FIG. 2—Gc 2-1 variant found in Chippewa Indians (Gc 2-1v). The common heterozygous type Gc 2-1 is also illustrated.

pewa Indian population were members of one family. Samples of other members of the family were obtained and one additional case with a Gc 2–1 variant (Gc 2–1v) was found. Among the available relatives of the other case of Gc 2-1v no additional cases were revealed. The pedigree of the Chippewa Indian family, containing 4 individuals with an unusual Gc 2–1 type, is illustrated in Figure 3. An attempt to demonstrate an unusual pattern of the Gc 1–1 (II–6) or Gc 2–2 (IV–2) precipitates in the 2 members of the family with homozygotic Gc types failed. It is as yet not possible to give a final interpretation of the findings illustrated in the pedigree. If we assume a genetic mechanism, at least two possibilities have to be considered. The variation could be due to a modified Gc^1 allele, which is detectable in the presence of Gc^2 but obscure when present in combina-

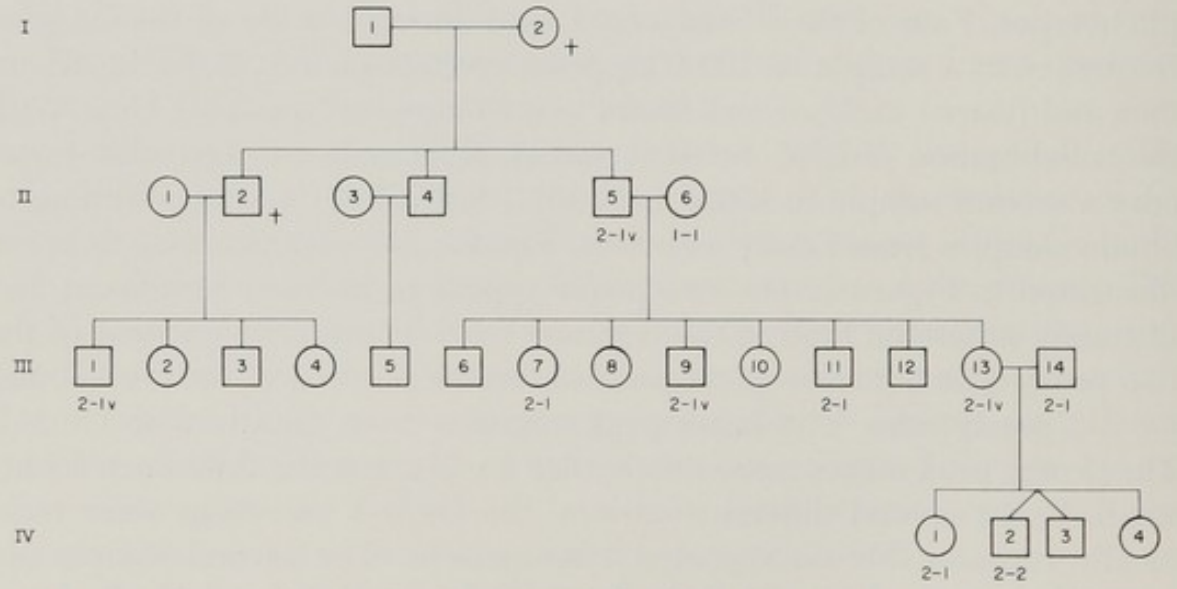


FIG. 3—Pedigree of a family of apparently healthy Chippewa Indians where several of the members studied thus far possessed an unusual heterozygous Gc-phenotype, indicated by the symbol Gc 2-1v.

tion with Gc^1. On this hypothesis, we have to assume that the variation is transmitted from the Gc 1–1 mother (II–6) to the offspring (III–9 and III–13) and not from the father. An alternative explanation would ascribe the variation to the action of a modifying gene at another locus that differentially effects the rate of synthesis of the products of the Gc^1 and Gc^2 alleles. The common heterozygous Gc type appears to contain the two group specific components in equal amounts (Fig. 1). A precipitate similar to that seen in Gc 2–1v can be obtained when Gc 1–1 and Gc 2–2 sera are mixed in a 2:1 ratio. A theoretical highly unlikely third possibility is the presence of trisomy in the individuals carrying the variation. The normality of the individuals, however, speaks against the possibility.

The disclosure of variations in the Gc system would be facilitated by the introduction of other techniques for the demonstration of the group specific component. The localization of Gc on vertical starch gel electrophoresis using the conventional borate buffer system was investigated in our laboratory by re-examination of fractions from the starch gel by immunoelectrophoresis on agar gel (Cleve, Sabatini and Bearn). The fractions were obtained either by punching out small pieces from the unstained and unfixed gel and placing them in the agar gel for immunoelectrophoretic analysis, or the gels were frozen and cut into 1 cm. segments and the fluid eluted by disruption of the frozen segments by means of a glass rod. The fluid obtained was concentrated and the fractions examined by immunoelectrophoresis. Gc was found at the cathodal edge of the broad albumin band and in the postalbumin region. With our conditions usually two, occasionally three postalbumin bands can be seen, but it was not possible to identify with certainty the Gc proteins with these bands. This was in part due to the fact that other α_1-globulins and the barium-α_2-glycoprotein* migrate in this region. Schultze et al. used the Tris-Borate buffer system employing a high voltage gradient of 11v/cm. and succeeded in resolving the postalbumins of normal human sera into three clearly separated bands (Schultze, Biel, Haupt and Heide, 1962a). The product of the Gc^1 allele corresponded to the second and that of the Gc^2 allele to the third band. Different intensities of these bands were found in Gc 1–1, 2–1 and 2–2 sera and it was suggested that these variations in the starch gel electrophoresis pattern of sera with different Gc-types are probably identical with the postalbumin variations described by Smithies (1959b). However, in their system also other globulins migrated with the second and third band of the postalbumins; the barium-α_2-glycoprotein, for instance, was found in the third band. Similar variations in the postalbumin region have been recog-

* Purified preparations of barium-α_2-glycoprotein and zinc-α_2-glycoprotein were kindly supplied by Dr. K. Schmid, Massachusetts General Hospital, Boston, Mass.

nized in our laboratory when sera of known Gc types were examined by horizontal starch gel electrophoresis with the discontinuous Tris-borate buffer system (Poulik, 1957). Thus far, it has been found difficult to achieve a reproducibility which would permit the routine classification of sera of unknown Gc types based on a single examination in the starch gel system. Further studies focused on the modification of the starch gel procedure are currently being performed in an effort to define more precisely the conditions under which the Gc type can be reliably determined.

Distribution of the Gc Alleles in Different Ethnic Groups

The variation in the distribution of Gc genotypes has been studied in samples from different geographical areas and ethnic groups (Cleve and Bearn, 1961a, 1961b, 1961c; Hirschfeld, 1962). Table 2 summarizes the gene frequencies obtained from 30 populations. Gc^1 is the more common allele in all populations studied thus far. The highest frequency of Gc^2 was found in Ashkenazi Jews (0.34) and the lowest in Navajo Indians (0.02). In the various European populations studied similar Gc gene frequencies were found varying between 0.29 in England and 0.23 in Denmark. Heterogeneity-χ^2-tests did not reveal significant differences within the samples from Caucasians. In African Negroes, significantly lower frequencies of Gc^2 were found, from 0.10 in Bondei to 0.05 in Fulani. Among the African Negro populations studied, however, no significant differences could be found by heterogeneity-χ^2-tests. The American Negroes have a slightly higher Gc^2 frequency of 0.11, though the sample was too small to establish a significant difference. The geographical variation of gene frequencies of several genetic traits has been used to calculate the proportion of non-Negro ancestry in the American Negro population. The estimates of Glass and Li on the basis of R^0 frequencies (Glass and Li, 1953), 30.6 per cent, and Steinberg et al. obtained from Gm group frequencies, 31.0 per cent, (Steinberg, Stauffer and Boyer, 1960) agreed remarkably well with the suggestion of anthropologists that approximately one third of the ancestry of the American Negro is Caucasian or Indian. Slightly higher values were obtained by Neel and Schull from sickle-cell frequencies, 34 per cent (Neel and Schull, 1954). Calculations on the basis of haptoglobin frequencies, however, led to figures which were, in some instances, considerably higher. The estimated non-Negro ancestry in Negroes from California, Ann Arbor, New York and Seattle is 34, 41, 41 and 56 per cent respectively (Parker and Bearn, 1961). It has been suggested that these elevated admixture estimates for the U. S. Negro reflect conditions of unstable gene frequencies, in which selection is operating against the high frequency of the Negro Hp^1 allele in the North American environment. The admixture

TABLE 2—*Gc Gene Frequencies in Various Populations*

Population	Total	Gc^2	s′	Reference
Arabs	48	0.260	0.045	Cleve, Ramot and Bearn, 1962
Australian Aborigines	49	0.112	0.032	Cleve and Bearn, 1961c
England	49	0.286		Hirschfeld, 1962
Norway	383	0.278		Reinskou, 1962
USA (New York City)	148	0.274	0.026	Present study
Germany	208	0.264		Baitsch and Jenssen, 1962
Sweden	1744	0.257		Hirschfeld, 1962
Denmark	126	0.230		Mansa-Olsen, 1962
Eskimos	67	0.298	0.040	Cleve and Bearn, 1961b
American Indians				
Chippewa	159	0.223	0.023	Cleve et al.
Alabama-Coushatta	65	0.150	0.040	Cleve and Bearn, 1961c
Navajo	245	0.023	0.008	Cleve and Bearn, 1961b
Indians (Bombay)	90	0.306	0.034	Cleve and Bearn, 1961c
Israelis (Jews)				
Ashkenazi	99	0.338	0.034	Cleve et al., 1962
North African	64	0.297	0.040	Cleve et al., 1962
Persian	49	0.245	0.043	Cleve et al., 1962
Iraqi	85	0.241	0.033	Cleve et al., 1962
Yemenite	49	0.194	0.040	Cleve et al., 1962
Kurdish	42	0.190	0.043	Cleve et al., 1962
Lapps (Sweden)	79	0.127		Hirschfeld, 1962
Negroes				
USA (New York City)	144	0.108	0.018	Cleve and Bearn, 1961b
Africa				
Bondei	57	0.096		Hirschfeld, 1962
Congo (Bantu)	100	0.095		Hirschfeld and Sonnet, 1961
Baganda	81	0.093		Hirschfeld, 1962
Nigerian Habe	103	0.073	0.018	Cleve and Bearn, 1961b
Masai	50	0.070		Hirschfeld, 1962
Nigerian Fulani	100	0.050	0.015	Cleve and Bearn, 1961b
Chinese (New York City)	117	0.231	0.028	Cleve and Bearn, 1961b
Japanese	108	0.227	0.028	Present study

Gc^2: gene frequency of Gc^2.
s′: standard error of the gene frequency estimates.

figure obtained using the Gc frequencies in West African Negroes, the American Negro and American White population is considerably lower (17.4 per cent). Definite admixture calculations on the basis of Gc gene frequencies have to await the collection of more extensive data in the American and African Negro population, especially in view of the rela-

FIG. 4—Geographic distribution of the frequency of the Gc^2 allele.

tively small differences in Gc gene frequencies. If, as seems likely, the different gene frequencies observed for the Gc system in various parts of the world reflect the influence of selective forces, admixture calculations will be treacherous until these forces are defined. Anthropological interpretations must similarly await a more complete understanding of the Gc system. Striking differences in gene frequencies were observed in three North American Indian tribes. In Chippewas, Gc^2 was found to be 0.22, in Alabama-Coushatta 0.15, whereas in Navajos the lowest incidence of Gc^2 was observed, 0.02. Although different amounts of admixture of genes of non-Indian ancestry have to be considered, the observed differences between the Indian tribes are more easily explained by the occurrence of genetic drift. The highest incidence of Gc^2 was observed in Ashkenazi Jews of Eastern European origin, in non–Ashkenazi Jews from different geographical areas lower Gc^2 frequencies were found. The Gc^2 allele seems to be less common in Eastern Asiatics than in Europeans. The distribution of the Gc alleles in 26 populations is illustrated in Figure 4.

The observed distribution of the Gc genotypes within a population was generally in good agreement with the expected distribution based on the Hardy-Weinberg equilibrium. The Navajo population, however, was not in equilibrium but the relatively small sample with respect to the rareness of the Gc^2 allele in this population did not permit meaningful equilibrium

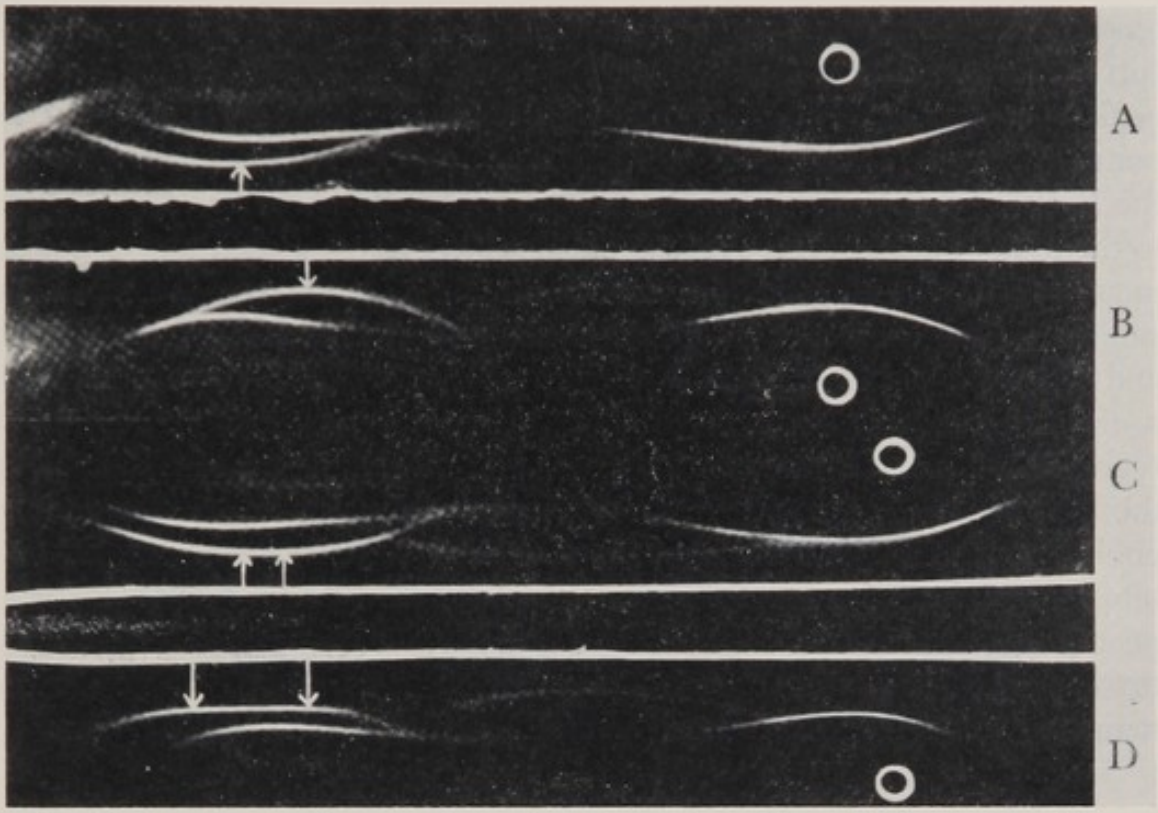


FIG. 5—Comparative immunoelectrophoretic analysis of sera of Macaca irus. Demonstration of 4 different phenotypes. (Adapted from Hirschfeld, J., 1962. By courtesy of Dr. J. Hirschfeld and S. Karger, Basel/New York).

calculations. For two other inherited traits, haptoglobins and transferrins, this population was found to be in equilibrium (Parker and Bearn, 1961).

PHYLOGENETIC ASPECTS

Variations in relative electrophoretic mobilities in a_2-globulins have been shown by immunoelectrophoretic analysis in species other than man. In Macaca irus, a system of variants was observed, which appeared to be strikingly similar to the Gc-system in man (Hirschfeld, 1962). By suitable mixing experiments of human sera with sera of these monkeys the precipitates of the human group specific component revealed an immunological reaction of identity or partial identity, depending on the antiserum used, with the precipitates of the variable a_2-globulin system of Macaca irus, suggesting a close immunological relationship between these proteins. The 4 phenotypes observed in a sample of 36 monkeys are illustrated in Figure 5. In some sera a fast-moving component occurs, type 1 (A), in others a slow-migrating component, type 2 (B). The third type is characterized by an extended precipitate with an intermediate electrophoretic position (C). This type corresponds to the phenotype obtained from an in vitro mixture of type 1 and type 2 sera. The fourth type similar to type 3, reveals an ex-

tended, but clearly two-peaked precipitate (D) where the slower peak corresponds to the position of the slow migrating component and the fast peak appears to be situated closer to the anode than the fast-moving component of type 1. Compared to the relative electrophoretic mobilities of the human Gc the slow-migrating component in Macaca irus corresponds to the human Gc 1–1 type. Family studies have not yet been performed in monkeys, and the mode of inheritance remains to be studied. In swine sera, similar a_2-globulin variations have been observed (Brummerstedt-Hansen and Hirschfeld, 1962). Three main patterns were observed, but in addition to the fast- and slow-migrating component and the extended, two-peaked type, other intermediate types were noticed. The relation of the porcine to the human Gc system is not yet known, but it is of interest that certain pooled antiswine immune sera prepared in rabbits also gave strong precipitate lines with the human Gc system. This cross reaction suggests an unexpected immunologic relationship which needs confirmation. In addition, the relation of the variations of a_2-globulins observed in rabbits to the human Gc system has not yet been investigated (Hirschfeld, 1959a).

On the Nature of the Group-Specific Component

Preliminary information on the nature of this protein was first obtained by studies on whole serum or isolated a_2-globulin fractions prepared by zone electrophoresis (Cleve and Bearn, 1961a, 1961c). The solubility of the Gc in whole serum was studied by various classic precipitation techniques. Gc is soluble in 40 per cent ammonium sulfate but is precipitated at 60 per cent saturation. Rivanol at 0.4 and 0.3 per cent concentration precipitates the Gc when 1.2 ml. rivanol is added to 1 ml. of serum. Gc is not soluble in 0.6M perchloric acid, the concentrated supernatant of perchloric acid precipitated normal serum does not contain Gc. Gc is not soluble in 5 per cent trichloracetic acid. Because the Gc is not precipitated by phosphate at pH 5.4 or 5.8 at a concentration of 0.02M or 0.005M it does not belong to the euglobin fraction (Cleve and Bearn, 1961a).

Heating of serum to 37 C. and 56 C. for 24 hours does not affect the position or shape of the precipitate of the group specific component. Incubation of serum with neuraminidase of Vibrio cholerae at 37 C. for 12 hours does not alter the relative electrophoretic mobilities of the Gc 1–1 or Gc 2–2 precipitates, indicating that the Gc does not contain sialic acid accessible to the enzyme. The variations in electrophoretic mobilities of Gc 1–1 and Gc 2–2 are therefore not due to differences in sialic acid content.

The different relative electrophoretic mobilities of the 3 Gc types can be demonstrated by starch block electrophoresis as well as by agar gel immunoelectrophoresis. In barbital buffer 0.1 M at pH 8.6 Gc 1–1 migrates

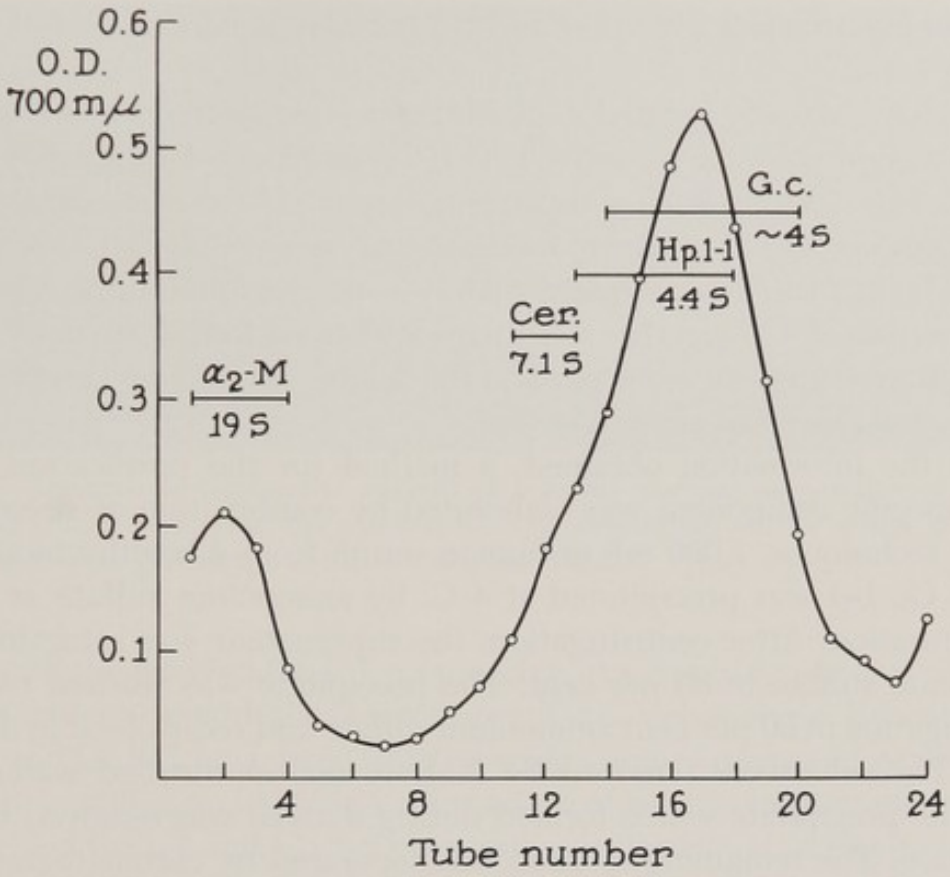


FIG. 6—Density gradient ultracentrifugation of α_2 globulin fraction indicating the distribution and calculated S-rate of the Gc. (The position and S-rates of the α_2 macroglobulin, ceruloplasmin and haptoglobin 1-1 are also illustrated.)

as a fast α_2-globulin, located between the α_1- and α_2-globulin fraction. Gc 2–2 has the mobility of an α_2-globulin, situated in the slower migrating part of this fraction. Gc 2–1 is distributed from the end of the α_1- to the end of the α_2-globulin fraction. Starch block electrophoresis at pH 4.5 using an acetate buffer separates the α_2-globulins into two distinct fractions. Gc 1–1 is found in the second, slower migrating fraction, corresponding to the MP-2-fraction (Cleve and Bearn, 1961c).

Density gradient ultracentrifugation of α_2-globulin fractions obtained by starch block electrophoresis was performed using a sucrose gradient of 10 to 40 per cent. The distribution of α_2-macroglobulin, ceruloplasmin, haptoglobin 1–1, Gc 1–1 after density gradient ultracentrifugation is illustrated in Figure 6. Specific staining techniques were used to identify haptoglobin and ceruloplasmin and a specific antiserum for the recognition of α_2-macroglobulin. Using the reported sedimentation rates for ceruloplasmin and haptoglobin, the sedimentation rate of Gc was calculated to be approximately 4S (Cleve and Bearn, 1961c). Gc therefore belongs to the group of low-molecular weight proteins of human serum. Differences in

the sedimentation rate of Gc 1–1 and Gc 2–2 were not found with this technique.

Electrophoretically isolated a_2-globulins were separated by ion exchange chromatography on TEAE and DEAE (Tri-or Di-ethylaminoethyl) cellulose columns. Eluting with phosphate buffer at pH 6.0 and increasing salt concentrations the a_2-globulin fraction was separated into three major peaks. The first fraction contained mainly a_2-macroglobulin, the second the major portion of Gc together with haptoglobin and other unidentified a_2- and contaminating a_1-globulins. The third peak formed by ceruloplasmin contained only a small amount of Gc.

With the information obtained, a method for the purification of the group specific component was elaborated by combination of several separatory techniques. 1,000 ml. of human serum from 4 healthy individuals of type Gc 1–1 was precipitated at 4 C. by ammonium sulfate at 40 per cent saturation. After centrifugation, the supernatant was saturated with ammonium sulfate to 60 per cent. The precipitate was washed twice by centrifugation in 60 per cent ammonium sulfate and redissolved in distilled water. The salts were removed by dialysis against distilled water for 3 days. The precipitate which formed during dialysis was removed by centrifugation. The remaining solution was separated by chromatography on a TEAE cellulose column after equilibration against 0.05M PO_4-buffer at pH 7.2. An elution gradient of increasing salt concentration was utilized. Gc appeared in the second major peak, the fractions containing Gc were pooled, concentrated by ultrafiltration and dialysed against barbital buffer 0.1M, pH 8.6. Hemoglobin-C solution obtained from a patient homozygous for hemoglobin C,* and purified by starch block electrophoresis was added in concentrations sufficient to saturate the haptoglobin present in the Gc preparation. The solution was separated by starch block electrophoresis at pH 8.6, 0.1M barbital buffer. The fractions containing the Gc were pooled and concentrated and were found to be free of haptoglobin and albumin. Further purification was achieved by rechromatography on TEAE cellulose with PO_4-buffer applying a gradient of increasing salt concentration and decreasing pH. The preparation of the group-specific component obtained was tested at various concentrations by agar gel diffusion test with several antisera, traces of two contaminating a_2-globulins were found, the electrophoretic analysis on polyacrylamide gel revealed a high degree of purification and the amount of contamination was estimated to be approximately 10 per cent. In Figure 7, the immunoelectrophoretic analysis of the highly purified Gc 1–1 preparation is illustrated and a single component can be seen. The purified protein is compared with a mixture of a Gc 2–2

* Hemoglobin C was kindly supplied by Dr. Helen Ranney, Albert Einstein College of Medicine, New York, N.Y.

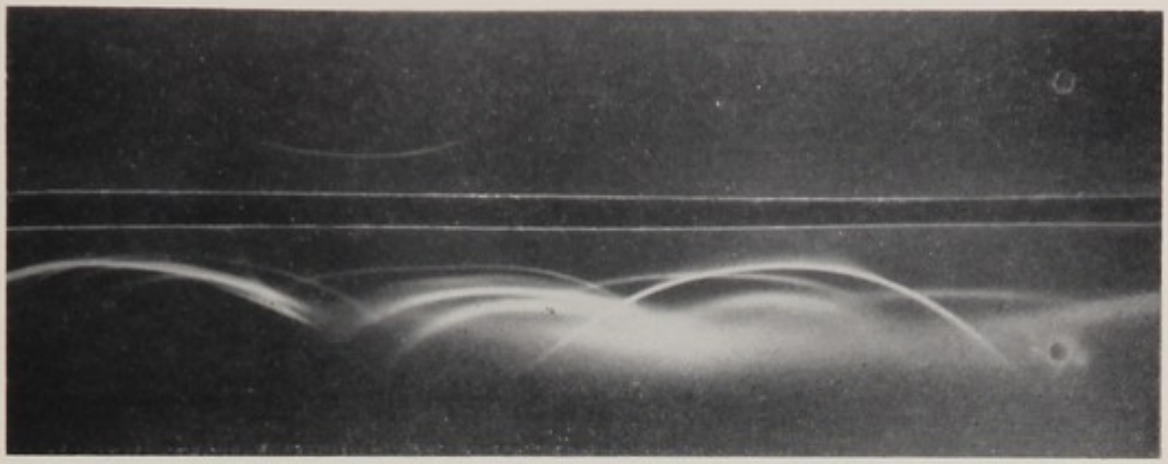


FIG. 7—Immunoelectrophoretic analysis of a highly purified preparation of Gc 1-1, compared with a mixture of the purified Gc 1-1 preparation with a serum of Gc 2-2 type revealing the phenotype of the heterozygote Gc 2-1.

serum with the purified Gc 1–1 preparation, the resulting phenotype in the mixture is identical with the heterozygous Gc 2–1 phenotype. Recently both Gc 1–1 and Gc 2–2 have been isolated with a higher degree of purity using a slightly modified technique. A comparison of the electrophoretic mobility of a purified Gc 1–1 and Gc 2–2 preparation is illustrated in Figure 8. Comparative studies on the chemical properties of the two chemically isolated gene products are under way.

Schultze et al. have reported the isolation of a purified preparation of Gc 2–1 from a fraction of their combined ammonium sulfate-aluminium hydroxide plasma fractionation procedure. The purification was achieved by combined fractionated ammonium sulfate precipitation, precipitation with rivanol, preparative zone electrophoresis at pH 8.6 and subsequently at pH 4.4. (Schultze, Biel, Haupt and Heide, 1962b).

Thus far, no known biological activity is associated with the group-specific component. Immunoelectrophoretic analysis using specific enzyme stains did not reveal evidence for esterase or cholinesterase activity. Autoradiographs of immunoelectrophoretically separated sera tagged with radioactive zinc failed to demonstrate zinc binding capacity of Gc. Gc, which is present in plasma as well as in serum, does not seem to have clotting activity. In the plasma of patients with various clotting factor deficiencies, the group-specific component was present, and the shape of the precipitate did not indicate decreased concentrations.

Identity with or close association of the Gc system to the blood groups ABO, Rh, MNS, P, K, Le or Fy and the inherited serum groups haptoglobin, Gm (a), Ag (a), transferrins or cholinesterase types has been excluded (Hirschfeld and Beckman, 1960). Exact analysis of family material for linkage of the Gc system to blood groups and other genetically controlled serum groups has not been reported.

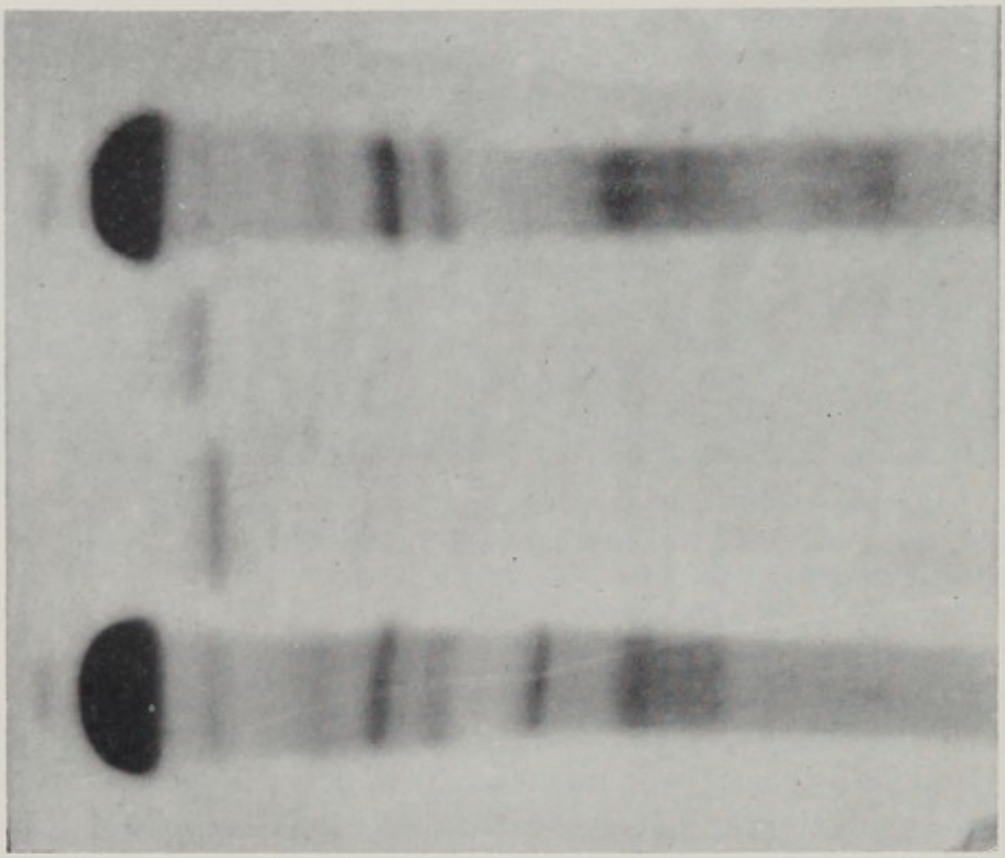


FIG. 8—Horizontal starch gel electrophoresis in tris-borate buffer. (*Top*) Electrophoretic separation of serum from individual of Group 1–1; immediately below is the purified Gc 1–1 preparation. (*Bottom*) Electrophoretic separation from individual of Group 2–2; immediately above is the purified Gc 2–2 preparation. Note the clear cut difference in mobility of the purified preparations and the absence of detectable contaminants.

Thus far, studies on the Gc system have not extended much beyond the recognition of the principal phenotypes and their distribution in different populations. The isolation of the group-specific component has been achieved recently, and at the moment attempts are being made to characterize the proteins which are responsible for the phenotypic differences in the Gc system. However, the isolation and characterization of the Gc proteins, even at the molecular level, will not permit us to understand the nature of the selective factors operating on this polymorphic system. Only when the physiological significance of these proteins has been elucidated will it be possible to understand more fully this example of a human biochemical polymorphism.

REFERENCES

Allison, A. C., and B. S. Blumberg. 1961. An isoprecipitation reaction distinguishing human serum protein types. Lancet 1: 634–637.

Baitsch, H., and W. Jenssen. 1962. Zur Populationsgenetik des Gc Systems: Allelenhäufigkeit in einer Stichprobe bayerischer Blutspender (in press).

Blumberg, B. S., and A. C. Allison. 1961. Studies on the isoprecipitin determined human serum polymorphism. Two new phenotypes. Proc. 2nd Int. Conf. Hum. Genetics, Rome (in press).

Brummerstedt-Hansen, E., and J. Hirschfeld. Data to be published; cited by Hirschfeld, J., 1962.

Cleve, H., and A. G. Bearn. 1961a. Inherited variations in human serum proteins: studies on the group-specific components. Ann. New York Acad. Sc. 94: 218–224.

——, and ——. 1961b. Studies on the group-specific component of human serum. Gene frequencies in several populations. Am. J. Human Genet. 13: 372–378.

——, and ——. 1961c. Genetic and chemical aspects of the group specific component. Proc. 2nd Int. Conf. Hum. Genetics, Rome (in press).

——, B. Ramot, and A. G. Bearn. 1962. Distribution of the serum group specific components (Gc) in Israel. Nature 195: 86–87.

——, Z. Sabatini, and A. G. Bearn. Unpublished results.

——, L. E. Schacht, H. Kleinmann, and A. G. Bearn. Data to be published.

Forbat, A., H. Lehmann, and E. Silk. 1953. Prolonged apnoea following injection of succinyldicholine. Lancet 2: 1067–1068.

Giblett, E. R., C. G. Hickman, and O. Smithies. 1959. Serum transferrins. Nature 183: 1589–1590.

Glass, B., and C. C. Li. 1953. The dynamics of racial intermixture; an analysis based on the American Negro. Am. J. Human Genet. 5: 1–20.

Grabar, P., et P. Burtin. 1960. Analyse immuno-électrophorétique. Paris, Masson.

——, et C. A. Williams. 1953. Méthode permettant l'étude congugée des propriétes électrophorétiques et immunochimiques d'un mélange de proteines. Application au sérum sanguin. Biochim. Biophys. Acta. 10: 193–194.

Grubb, R. 1956. Agglutination of erythrocytes coated with incomplete anti-Rh by certain rheumatoid arthritic sera and some other sera; the existence of human serum groups. Acta. path. et microbiol. scandinav. 39: 195–197.

——, and A. B. Laurell. 1956. Hereditary serological human serum groups. Acta. path. et microbiol. scandinav. 39: 390–398.

Harris, H., M. Whittaker, H. Lehmann, and E. Silk. 1960. The pseudocholinesterase variants. Esterase levels and dibucaine numbers in families selected through suxamethonium sensitive individuals. Acta Genet. et Stat. Med. 10: 1–16.

Hirschfeld, J. 1959a. Individual precipitation patterns of normal rabbit sera. Acta. path. et microbiol. scandinav. 46: 224–238.

——. 1959b. Immune-electrophoretic demonstration of qualitative differences in human sera and their relation to the haptoglobins. Acta. path. et microbiol. scandinav. 47: 160–168.

——. 1962. The Gc system. In Progr. Allergy. Karger, Basel/New York, vol. 6.

——, and L. Beckman. 1960. A new group-specific serum system (Gc-groups) in relation to blood and serum groups. Acta. Genet. Stat. Med. 10: 48–53.

——, B. Jonsson, and M. Rasmuson. 1960. Inheritance of a new group specific system demonstrated in normal human sera by means of an immuno-electrophoretic technique. Nature 185: 931–932.

——, and J. Sonnet. 1961. Distribution of group specific components (Gc) in the sera of native Africans. Nature 192: 766.

Kalow, W., and D. R. Gunn. 1959. Some statistical data on atypical cholinesterase of human serum. Ann. Human Genet. 23: 239–250.

——, and N. Staron. 1957. On distribution and inheritance of atypical forms of human serum cholinesterase, as indicated by dibucaine numbers. Canad. J. Biochem. Physiol. 35 (2): 1305–1320.

Knedel, M. 1957. Die Doppel-Albuminämie eine neue erbliche Proteinanomalie. Blut 3: 129–134.

Laurell, C. B., S. Laurell, and N. Skoog. 1956. Buffer compositions in paper electrophoresis; considerations on its influence, with special reference to the interaction between small ions and proteins. Clin. Chem. New York 2: 99–111.

Lehmann, H., and E. Ryan. 1956. The familial incidence of low pseudocholinesterase level. Lancet 2: 124.

Mansa-Olsen. Personal comm. to Hirschfeld, J.; cited by Hirschfeld, J., 1962.

McAllister, R., G. M. Martin, and E. P. Benditt. 1961. Evidence for multiple coeruloplasmin components in human serum. Nature 190: 927–929.

Morell, A. G., and I. H. Scheinberg. 1960. Heterogeneity of human ceruloplasmin. Science 131: 930–932.

Neel, J. V., and W. J. Schull. 1954. Human Heredity. Chicago: University of Chicago Press, p. 255.

Ouchterlony, Ö. 1949. Antigen-antibody reactions in gels. Acta. path. et microbiol. scandinav. 26: 507–515.

Parker, W.C., and A. G. Bearn. 1961. Haptoglobin and transferrin variation in humans and primates: Two new transferrins in Chinese and Japanese populations. Ann. Human. Genet. 25: 227–241.

Poulik, M. D. 1957. Starch gel electrophoresis in a discontinuous system of buffers. Nature 180: 1477–1479.

Reinskou, T. Personal comm. to Hirschfeld, J.; cited by Hirschfeld, J., 1962.

Scheidegger, J. J. 1955. Une micro-méthode de l'immunoélectrophorèse. Internat. Arch. Allergy 7: 103–110.

Schmid, K., and J. P. Binette. 1961. Polymorphism of a_1-acid glycoprotein. Nature 190: 630–631.

Schultze, H. E., H. Biel, H. Haupt, and K. Heide. 1962a. Über die Gc-Komponenten von Hirschfeld. I. Lage im Stärkegel-Elektrophoresebild. Naturwissensch. 49: 16–17.

——, ——, ——, and K. Heide. 1962b. Über die Gc-Komponenten von Hirschfeld. II. Darstellung und Eigenschaften. Naturwissensch. 49: 108.

Smithies, O. 1957. Variations in human serum β-globulins. Nature 180: 1482–1483.

——. 1959a. Zone electrophoresis in starch gels and its application to studies of serum proteins. Adv. in Protein Chemistry. 14: 65–113.

——. 1959b. An improved procedure for starch-gel electrophoresis: Further variations in the serum proteins of normal individuals. Biochem. J. 71: 585–587.

——, and N. F. Walker. 1955. Genetic control of some serum proteins in normal humans. Nature 176: 1265–1266.

——, and ——. 1956. Notation for serum protein groups and the genes controlling their inheritance. Nature 178: 694–695.

Steinberg, A. G., R. Stauffer, and S. Boyer. 1960. Evidence for a Gm^{ab} allele in the Gm system of American Negroes. Nature 188: 169–170.

Williams, C. A., Jr., and P. Grabar. 1955. Immunoelectrophoretic studies on serum proteins. I. The antigens of human serum. J. Immunol. 74: 158–168.

Chapter 4

Hereditary Aminoaciduria

Charles R. Scriver
Montreal Children's Hospital, Montreal, Canada

> If (genetics) should ever disappear as a separate discipline, it will only be because all theoretical biology has been bound together into a single field, to a large extent through its efforts. (Wright, 1959)

This quotation comes from the conclusion of Sewall Wright's address to the Tenth International Congress of Genetics and it will be familiar to many readers. Although the subject of his address was biology in general, it is evident that the perspectives stressed by Wright are quite applicable to a topic such as "hereditary aminoaciduria." The following comments try to correlate knowledge now available, concerning genetics and cytological structure and function, with metabolism and cellular transport of amino acids.

Human aminoaciduria has long been a clinical topic of genetic interest (Garrod, 1908). Contemporary approaches to aminoacidurias have steadily widened the interpretation of these phenomena, exemplifying Beecher's premise (1960) that clinical medicine and biophysical sciences are becoming inextricably wed. Dent (1957a) recently summarized possible mechanisms by which genetic disorders can interfere with substrate metabolism, and further consideration indicates that hereditary aminoacidurias, in all instances, must represent altered interaction between a specific molecule (amino acid or related substance) and a macromolecule whose configuration is genetically determined and which functions either as a transport or an apoenzyme site for its particular substrate.

Descriptive aspects of aminoaciduria began with the origins of amino acid chemistry (Wollaston, 1810); since then, a formidable literature on

This study was carried out with the support of grants from the Medical Research Council (Canada) and the National Institutes of Health (U.S.A.).

the topic of aminoaciduria has accumulated and several reviews have appeared in recent years (Bigwood, Crokaert, Schram, Soupart and Vis, 1959; Chisolm, and Harrison, 1960; Ghadimi and Shwachman, 1960; Holt and Snyderman, 1960; McKusick, 1961). This has made the present task easier in some ways, but raises the problem of justifying yet another review (Rutstein, 1961). The present endeavor follows.

A. Definitions and Problems

"*Amino Acid*"

An amino acid is "a chemical compound which possesses amino and acid groups" (Meister, 1957). The general configuration of an amino acid is represented by $R–\underset{\displaystyle NH_2}{\overset{\displaystyle H}{\overset{|}{\underset{|}{C}}}}–COOH$, the reactive amino and carboxyl groups being attached to the alpha carbon; other positions of amino-group attachment may occur, however, or there may be more than one amino group; imino groups also occur. Amino acids with multiple carboxyl groups may also exist. Finally, variations in the structure of the side chain (R-group), in combination with the previous variations, are responsible for a large number of amino acids which can be classified into groups, on the basis of their molecular characteristics.

The presence of at least one asymmetric carbon atom in all amino acids except glycine confers stereoisomerism, D- or L- optically active forms being present; generally speaking, the D-enantiomorphs of amino acids are of minor importance in human and metazoan metabolism. The size, position and shape of the side chain in relation to the reactive amino and carboxyl groups also confer important characteristics on the molecule.

In physiological solutions, the carboxyl and amino groups of amino acids are both capable of donating or accepting a proton and hence amino acids are dipolar ions, or zwitterions. The charged nature of these compounds is exploited in certain analytical methods; this character also prevents simple diffusion through "lipoid" cell membranes, a feature to be considered further in the discussion of cellular transport. Exhaustive discussion of chemical and physicochemical properties, and metabolic reactions of amino acids are available elsewhere (Cohn and Edsall, 1943; Greenstein and Winitz, 1961; Meister, 1957) and the field is reviewed at regular intervals in the Annual Reviews of Biochemistry.

"*Aminoaciduria*"

The term has broad or narrow implications, according to the individual using it. Strict usage limits the definition to "the occurrence of amino acids

in urine," a normal occurrence in itself. However, popular interpretation has broadened usage to suggest that aminoaciduria has pathological significance.

Certain terrain must first be evaluated before hereditary aminoacidurias can be described. Accurate description of pathological aminoaciduria is dependent on properly defined methodology, and so a discussion of limitations and objectives of various available methods is included in this review; also to be considered are valid reference coefficients for quantitative data. Even more important to define are the nonhereditary or environmental influences on aminoaciduria. It may seem at the end of these preliminary discussions that study of urinary amino acid content is indeed a cumbersome approach to a genetic problem. Let it be remembered, however, that urine still remains the easiest and often the first route of access into the majority of human genetic disorders affecting free-amino acid metabolism.

B. Methods

1. "Total Amino Nitrogen" Methods

These techniques are frequently used for quantitative determinations of aminoaciduria. Several disadvantages are inherent in the various methods. In general, they are laborious and often are insufficiently specific, measuring compounds other than free amino acids. Even a relatively simple and specific method may be incapable of detecting significant variations in the excretion of individual components of the total urinary amino acid excretion. Also, because of the effect of changing composition of amino acid mixtures in different samples on the final result obtained with each individual method (Table 1), comparison of results for "total α-amino nitrogen" obtained by different methods is quite inappropriate.

Total amino-nitrogen methods fall into three groups: gasometric, titration and colorimetric.

a. The gasometric-ninhydrin method of Van Slyke (Van Slyke, Dillon, MacFayden and Hamilton, 1941), when meticulously applied (Sobel, Henry, Chiamori and Segalove, 1957) is considered to be stoichiometric and the most specific for urinary α-amino acid nitrogen determination. Other gasometric methods using nitrous acid (Van Slyke and Kirk, 1933) and N-bromosuccinimide (Chapelle and Luck, 1957) react with a wide spectrum of amino groups, other than free α-amino acids.

b. Formol titration, by Northrop's modification (1926), is hampered by relatively low specificity.

c. Color reactions such as that employing β-naphthoquinone-4-sulfonate (Frame, Russell and Wilhelmi, 1943) have been developed and they are easier than gasometric methods. Specificity is sacrificed, however, and color

TABLE 1—*Comparison of α-Amino Nitrogen Levels in Urine Determined by Three Different Methods*°

Subject	Ninhydrin Method† μg. α-amino N/ml.	Ninhydrin Method × 100 / N-bromosuccinimide Method‡ per cent	Ninhydrin Method × 100 / Napthoquinone Method§ per cent
1	213	65	120
2	18	26	129
3	95	71.5	138
4	105	40	328
5	106	92.5	282
6	207	51	148
7	211	81	135

° Taken from Khachadurian, Knox and Cullen (1960).
† Khachadurian, Knox and Cullen, 1960.
‡ Chapelle and Luck, 1957.
§ Frame, Russell and Wilhelmi, 1943.

reactions with urea, ammonia, proteins, peptides and other amino acid conjugates make their application to urine unsatisfactory. Sobel and coworkers' copper phosphate photometric method (1957) achieved higher specificity by first separating and concentrating amino acids from urine on ion exchange columns. Meyer (1957) has described a method which is not contaminated by urea.

More recently, Khachadurian and associates (Khachadurian, Knox and Cullen, 1960) presented the best compromise to date; their method has the simplicity of the colorimetric ninhydrin reaction, good precision and high specificity. Large numbers of determinations could be performed in a working day. Approximately 80 per cent of the total free α-amino nitrogen present in the sample could be detected with relatively little variation, regardless of composition of the amino acid mixture present in urine.

2. Individual Amino Acid Determinations

Investigation of aminoaciduria usually demands identification of the individual amino acids and for this purpose there are many available methods. These techniques fall into four broad categories: chemical, microbiological, chromatographic and electrophoretic.

a. Chemical. These methods have seniority. At the time when Napoleon was contemplating invasion of Russia, Wollaston (1810) used the available chemical techniques to identify a compound which he called "cystic oxide" in a urinary bladder calculus from a 5 year old boy, and also in calculus no. 47 from the Guy's hospital collection of urinary calculi. (This was the beginning of the history of cystinuria [Knox, 1958a and b]).

Chemical methods are of most value when studying the urinary excretion behavior of a specific amino acid. Examples of suitable adaptations of a chemical technique are to be found later in the discussions of idiopathic hyperglycinemia (Childs, Nyhan, Borden, Bard and Cooke, 1961), hyperhistidinemia (Ghadimi, Partington and Hunter, 1961) and cystinuria.

Chemical reactions of further interest are those producing color reactions with specific amino acids, following chromatographic separation of the latter. Many examples of these color reactions are described in appropriate texts (Block, Durrum, Zweig, 1958; Hais, Macek, 1958; Smith, 1960). The scope and significance of chemical reactions of amino acids in general, are discussed elsewhere (Greenstein and Winitz, 1961; Meister, 1957).

Under the present heading may also be mentioned such techniques as polarography (Reed, 1942) and the use of isotope-labelled amino acids or precurser compounds. Isotope dilution techniques have been used to define the circumstances underlying certain disorders related to aminoaciduria (Crawhall, Scowen and Watts, 1959; Watts and Crawhall, 1959).

b. Microbiological methods have three subdivisions. The first group of methods employs mutant strains of microorganisms whose growth requires certain amino acids in the nutrient media. Under proper conditions growth rate becomes proportional to concentration of the specific amino acid in the nutrient medium, thereby providing a correlation which can be adapted to quantitative analysis.

Enzymatic decarboxylation by specific microorganisms, under appropriate conditions, provides quantitative measurement for certain amino acids.

A third approach, the "inhibition assay," has recently been developed by Guthrie (1961; Guthrie and Tieckelmann, 1960) in which principles of molecular antagonism and inhibition (Harris and Morrison, 1961) have been applied to a microbial assay system. A suitable microorganism is innoculated into a culture medium containing an antimetabolite; growth inhibition occurs, unless the appropriate molecular compound is introduced which will reverse the inhibition. By using urine or blood-impregnated filter paper discs it is possible to induce microorganism growth, provided the biological fluid contains sufficient quantity of the specific molecular configuration which will be a growth promoting analogue of the inhibitor.

The combination of B. Subtilis, strain ATCC-6051, and the inhibitor β-2-Thienylalanine, can be used to screen for phenylalanine, phenylpyruvic acid and proline; tyrosine and hydroxyproline will not reverse the thienylalanine inhibition. The number of assay system combinations seems unlimited and 5-fluorouracil has already been studied as an inhibitor in both B. subtilis and yeast assays.

c. Filter paper partition chromatography and related techniques. 1. Qualitative Filter Paper Techniques. Recognition and investigation of

aminoaciduria received its greatest impetus when Dent (1946; 1947; 1948) recognized the extraordinary potential in partition chromatography on filter paper (Consden, Gorden and Martin, 1944). The principal advantages of this technique are its specificity for amino acids and related compounds, avoidance of interfering effects from other substances once desalting has been accomplished, detection of all anticipated as well as unexpected amino acids present in sufficient concentration to be identified with the location reagent, simplicity of technique and reliability of results. These features have led to the widespread acceptance of filter paper partition chromatography in the investigation of aminoaciduria.

Many variations and adaptations of the original method (Consden, Gorden, and Martin, 1944; Dent, 1947) have evolved; several comprehensive discussions of amino acid chromatography have been referred to in the valuable review by Bigwood and co-workers (Bigwood et al., 1959); other important references include those by Berry (Berry, Sutton, Cain and Berry, 1951), Smith (1960), and Hais and Macek (1958). The merits of unidimensional, bidimensional and other chromatographic systems, the many solvents for development of chromatograms, and the location reagents which may be used, cannot be discussed here and these points are well covered in the publications mentioned above. The inclusion of reference maps for the position of some 60 amino acids in various two-dimensional systems has lent extra value to other publications discussing specific chromatographic systems (Berry et al., 1951; Bowden, 1959; Dent, 1948; Parry, 1957; Smith, 1960).

The handling of a large number of samples is easier with a "universal" frame (Datta, Dent and Harris, 1950; Smith, 1960) which is capable of carrying several chromatograms at once. The uniformity of conditions pertaining to each batch allows reliable comparison between the separate samples in the same run, and handling of individual chromatograms is diminished considerably.

Recently, two-dimensional paper chromatography of amino acids has been developed on ion exchange cellulose papers (Knight, 1959) and sulfonated polystyrene resin-loaded papers (Knight, 1960), offering certain advantages for some problems.

The advantages of paper chromatography are such that it is usually the method of choice in genetic field work on hereditary aminoacidurias. However, the method should not be considered to be a diagnostic panacea, and careful selection of a particular chromatographic technique is necessary for each individual problem. (The recent report on the effect of phenol solvent on glycine chromatography (Huggins and Moses, 1961) is an instructive example.)

2. Quantitative Methods on Filter Paper. Several approaches to quanti-

tative chromatography were discussed at a recent colloquium (Eastoe, Buckle, Partridge, Esden, Harris, Whitehead and Hartley, 1961). The adaptability and technical simplicity of paper chromatography has encouraged many modifications of the simpler qualitative techniques in attempts to obtain quantitative results. Unfortunately, these modifications, if they achieved reliable quantitative analytical accuracy, usually greatly increased the complexity of the original method. Recent descriptions of successful quantitation, which also cite pertinent literature in more detail, have been presented by Filteau and Martel (1956), Haynes and co-workers (Connell, Dixon and Hanes, 1955; Hanes, 1961), McMenamy and co-workers (McMenamy, Lund, Neville and Wallach, 1960), Berry (1960) and by McEvoy-Bowe and Lugg (1961). A relatively rapid and simple method, employing combined displacement preparative column chromatography and quantitation of separated components of effluent fractions, by means of two-dimensional paper chromatography, was recently described (Lundquist and Galatius-Jensen, 1960).

d. Ion exchange resin column methods. The demands for greater resolution (Westall, 1952; Westall, 1955), coupled with quantitative requirements, led to the development of ion exchange column chromatography (Westall, 1961).

Ion exchange columns are used principally for removal of interfering ions (Drèze, Moore and Bigwood, 1954; Jagenburg, 1959), for preparative separation of a particular amino acid (Sörbo, 1961), or an unknown compound, and most commonly for quantitative analysis. Moore and Stein, Bigwood and their respective collaborators (Moore and Stein, 1951; Moore and Stein, 1954a and b; Schram and Bigwood, 1953; Schram, Dustin, Moore and Bigwood, 1953) have developed techniques providing excellent quantitative analysis of most ninhydrin-reacting compounds in protein hydrolizates and biological fluids; the principal disadvantage is the week-long analysis time. Recent modifications (Moore, Spackman and Stein, 1958; Piez and Morris, 1960; Spackman, Stein and Moore, 1958) have accelerated the procedure while providing continuous photometric measurement of the automated color reaction in conjunction with automatic recording. The problem has now become one of keeping up with the data; to cope with them, an integrated logarithmic plotter has been designed (Cope, 1961); easy conversion of the chromatogram into numerical data is claimed, but improvements are still required in the accuracy and speed of the integrator (Cope, F. W., personal communication).

e. Gas-liquid chromatography. Successful adaptation of gas-liquid chromatography (Keller, Stewart and Giddings, 1960) to the separation of amino acid mixtures has been reported by Meister's group (Johnson, Scott and Meister, 1961). N-acetyl amino acid N-amylester derivatives were first

prepared and then run on two or eight foot columns with Chromosorb W. coated with 0.5 per cent polyethylene glycol. The advantages of this gas-liquid chromatographic method are short analysis time (two hours), and great sensitivity (10^{-10} Moles). Unfortunately, it suffers from the disadvantage that it is not yet possible to identify all the amino acids by this method.

f. Electrophoretic techniques. The amphoteric nature of amino acids has encouraged several authors to exploit this property to separate them by ionophoresis at high or low voltages in various buffer systems. The procedure has additional advantage in that desalting is not required before analysis. Filter paper is used as the supporting medium which upon drying may then be stained with appropriate location reagents.

MacDonald and co-workers (McDonald, Urbin and Williamson, 1951) have described the behavior of several amino acids under electrophoretic conditions. Evered (1959) developed a low-voltage technique requiring several buffer systems and separate runs to achieve separation of acidic neutral and basic amino acids; the technique is not generally useful for aminoaciduria studies because of the sequential analyses required to obtain identification of all amino acids.

A very successful method has been described by Efron (1959), extending the original suggestions for separation of ampholytes by high voltage electrophoresis (Werner, 1954) and by combined ionophoresis and chromatography (Durrum, 1951). Efron employed partition chromatography in a second dimension after high voltage ionophoretic separation had been achieved in the first dimension. The method is applicable with ease to most biological fluids; resolution is excellent and sensitivity of the method is considerably greater than with conventional two-dimensional partition chromatography.

Gross has applied high voltage electrophoresis in both dimensions to the two-dimensional separation of amino acids in biological solutions (Gross, 1959), achieving better separation than in earlier experiments by Durrum with low voltage systems (Durrum, 1951).

g. Identification of D- and L- enantiomorphs. Occasionally, it is necessary to determine whether an aminoaciduria is composed solely of L-amino acids, since misinterpretation could exist in the presence of D-enantiomorphs (see section C–1).

Two groups of methods can readily determine which optical configuration predominates. Microbiological preparations can be found which will distinguish between D- and L- forms of specific amino acids. An enzymatic method with reasonably wide spectrum has been described by Bonetti and Dent (1954). Biological fluid treated with sheep's kidney cortex D-amino acid oxidase, or snake venom L-amino acid oxidase, is chromatographed on filter paper in the usual manner for comparison with an untreated control

sample. This method identifies the optical configuration of alanine, histidine, isoleucine, leucine, phenylalanine, proline, tryptophan, tyrosine and valine.

3. *"Screening" Programs*

Systematic programs screening large numbers of samples for the detection of metabolic abnormalities in individual patients, have become an integral part of many public health and research projects. Large "open-end" programs such as those carried out by Shaw and co-workers in California (Shaw, Perry, Jacobs, Gutenstein, Walker and Koch, 1961) and Berry and her group at Cincinnati (Berry, 1959b; Berry, Sutherland and Guest, 1960) screen for a great variety of metabolites including amino acids, keto acids, indolic compounds, phenols, proteins, reducing substances and mucopolysaccharides in urine. Urine is the raw material most commonly processed and whereas collections of urine in bulk are often difficult, the Cincinnati group has devised an ingenious method of collection onto filter paper, which is then mailed to the laboratory for processing (Berry, 1959b; Berry, Sutherland, Guest and Warkany, 1958).

Screening programs can provide a broad spectrum of detection with accuracy and simplicity. However, false positive and false negative results are occasionally the forfeit for substitution of a simple screening method for more specific analysis. The problems involved in detection of phenylketonuria, particularly in the newborn, offer an excellent example of this drawback. The addition of ferric chloride reagent to fresh urine may be used for recognition of the disease phenylketonuria; this procedure implies that phenylpyruvic acid is the limiting compound for detection of the disease. False negatives could occur for several reasons: small amounts of phenylpyruvate might be missed since ferric chloride is less sensitive than 2, 4-dinitrophenylhydrazine for detection of the keto acid (Armstrong and Binkley, 1956); phenylpyruvate excretion is associated only with a plasma phenylalanine accumulation above approximately 15 mg. per cent (Armstrong and Low, 1957; Kretchmer, 1959); the keto acid cannot be synthesized endogenously for several weeks after birth although phenylalanine accumulation begins at birth in phenylketonuria (Armstrong and Binkley, 1956; Kretchmer, 1959).

False positive color reactions with Fe CI_3 may occur in the presence of drugs or a variety of metabolites other than phenylpyruvic acid (Heffelfinger and LaGro, 1960; Menkes and Jervis, 1961).

Certain screening procedures sacrifice precision in detection of aminoaciduria itself, and the case against total amino nitrogen techniques has already been stated. Criticism may also be levelled at the "pie-plate" chromatographic method recently proposed as a screening process (Ghadimi and Shwachman, 1959; Ghadimi, Clarke and Shwachman, 1961) which,

despite its rapidity, cannot replace a two-dimensional chromatogram capable of revealing even small variation in excretion patterns or the presence of unsuspected compounds.

The screening programs previously mentioned (Berry et al., 1960; Shaw et al., 1961), and others known to the author, employ carefully standardized two-dimensional partition paper chromatographic systems as the simplest and most reliable method for detection of unusual amino acid excretion. Even then, the individual investigator must interpret the results carefully; Hillcoat (1960b) noted that attempts to classify two-dimensional amino acid chromatograms by "summing" the intensities of spots, in lieu of interpreting the amino acid chromatographic *pattern*, could lead to neglect of pathological aminoaciduria. Dent has expressed for many people the feeling about the worth of a two-dimensional chromatogram with this comment: "The chromatographic picture has a value in its own right for expressing amino acid composition and is sometimes better than a row of figures. The picture can be remembered and subsequently recognized like somebody's face" (1957b).

Summary: Choice of Method

To summarize, the choice of a method for investigation of aminoaciduria largely depends on the objectives of the investigation. The study may be well served by a selective analytical method which accurately identifies the compound in question, but relatively ignores other ampholytes. More often, however, one is interested in the relation of the various urinary ampholytes to each other; furthermore, detection of unsuspected compounds is often a critical and informative part of the investigation. Methods which utilize variable mobilities of different ampholytes in solvent or buffer systems across a supporting medium, which can then be stained for identification of the spacially separated ampholytes, have served the purpose more effectively than most other methods. Two-dimensional systems not only provide a pattern of characteristic relationships of one compound to another, but also offer immediate identification of most compounds; they may also define some of the characteristics of unknown compounds. Elution or displacement chromatography on ion-exchange resin columns provides a means of isolation in bulk of unknown compounds for further identification; excellent separation and quantitative analysis of many of the ampholytes in urine is also possible by such methods.

4. Quantitative Amino Acid Excretion Coefficients or Indices

A valid excretion coefficient is necessary for interpretation and definition of aminoaciduria, and without which, comparisons of data from heterogeneous populations are difficult. In the attempts to employ a reliable coefficient, the solution may be so cumbersome that it inhibits the study of

aminoaciduria in any but carefully supervised patients. An excretion coefficient, therefore, must be established which will be satisfactory to the physiologist and biochemist, as well as to the geneticist, and which will suit the requirements of the investigations.

The *unit* expressing amino acid content in urine is important. A unit of weight, such as milligrams, is the most frequently used. However, some recognition of stoichiometric metabolic relationships between the individual components of aminoaciduria is often desirable and "molar" concentrations would be more acceptable.

Since aminoaciduria is actually a reflection of amino acid metabolism, the excretion coefficient should represent an equivalent of this metabolism; (the problem has been faced in other metabolic problems [Butler and Richie, 1960; Forbes, 1959]). The following coefficients are in use.

a. Amino acid unit/urine volume. This obviously is unsatisfactory, even when volume is multiplied by 100! Urine concentration fluctuates temporally in a single subject and between different individuals. Volume of urine also bears no relation to metabolism of amino acids.

b. Amino acid unit/24 hours. Three disadvantages are inherent in this type of excretion ratio. First, the ratio gradually increases with age from birth until somatic growth has stabilized; provided standards are available (see Fig. 2 in Ghadimi and Shwachman, 1960). Second, there is the influence of diurnal food intake on amino nitrogen excretion, especially when renal tubular function is disturbed (see section C, 2, b). The third disadvantage is the difficulty of accurate 24 hour urine collections in infants and younger children. The reliability of sample collection is always doubtful when the patient is not in hospital; pedigree and population studies could not therefore be served satisfactorily by this excretion coefficient.

c. Amino acid unit/body weight/24 hours. This index has the advantage of being valid for all age groups. Inherent diurnal variations are reduced by the temporal clause. The disadvantages are the need for timed urinary collection, and discrepancies introduced by anomalies in body weight due to disease states.

d. Amino acid unit/creatinine excretion. Many investigators have used this ratio on the assumption that creatinine estimation was easy and that its urinary excretion was constant. Creatinine excretion, however, may not be constant. Vestergaard and Leverett (1958) showed that creatinine excretion in the majority of adults could vary diurnally and that it was unsafe to assume constancy of creatinine excretion throughout short-term collections, unless the excretion characteristics of the subject had been previously determined. The picture is further complicated by Jagenburg's observation (1959) that amino nitrogen excretion, expressed as a creatinine coefficient, varies with the sex of the subject.

Creatinine excretion during infancy and early childhood is a contro-

versial and complicated phenomenon. Stearns and co-workers (1956) considered creatinine excretion, as a coefficient of body weight, to be relatively constant in later infancy and childhood. Other workers (Winberg, 1959; Woolf and Norman, 1957) state that creatinine excretion varies considerably during infancy. In another study (Clarke, 1961), creatinine excretions expressed as a coefficient of body weight in childhood were not comparable with adult values, a finding at variance with the earlier study (Stearns, 1956). Variability of creatinine excretion is particularly marked in the premature and newborn population, both in long-term and in short-term collections in single individuals and between individuals in the total group (Bergstedt, O'Brien and Lubchenco, 1960; O'Brien, Bergstedt, Butterfield, Ibbot and Lubchenco, 1960). The recent work (Berglund, 1961) showing that creatinine is both actively secreted and reabsorbed by the renal tubules may account for the observed variations in its excretion.

Whereas inherent variations of creatinine excretion in healthy individuals may be quite significant, they are even greater in many disease states; renal disease, however, has the converse effect and depresses the degree of variation occurring in diurnal creatinine excretion rates (Krawczynski, Kujawa, Tyburczyk and Zelazowska, 1961).

Finally, an accurate method is essential for creatinine determination. Lloyd's reagent improves the specificity of the color reaction (Hare, 1950), but this maneuver, added to what is essentially a rapid and simple determination, still encourages many to use simpler methods (Clarke, 1961) which may be subject to greater error.

e. Amino acid unit/total nitrogen excretion. This coefficient has proven to be a valuable index for all age groups (Bergstedt et al., 1960; Jagenburg, 1959), and has the advantage of being a metabolic parameter closely related to amino acid metabolism. However, total urinary nitrogen can vary under certain conditions. Diet will affect total nitrogen more than amino nitrogen (see section C, 2, a) and the effect is exaggerated in renal tubule dysfunction. This alimentary effect is probably the explanation for the poor correlation of the indices for amino nitrogen excretion with total urinary nitrogen excretion, and 24 hour coefficients (Ghadimi and Shwachman, 1960). Total nitrogen is readily determined by the micro-kjeldahl technique (Ma and Zuazago, 1942).

f. Amino acid excretion expressed as endogenous amino acid renal clearances. This approach has great value in the investigation of aminoacidurias. Procedures and data from investigation of normal infants (O'Brien, Butterfield, Walker, Ibbott and Lubchenco, 1961), normal adult subjects (Cusworth and Dent, 1960a) and certain aminoacidurias (Cusworth and Dent, 1960a) have been published. Disadvantages include technical complexity and pitfalls of endogenous renal clearance investigation. However, even relatively casual clearance techniques (Evered, 1956) can still pro-

vide valuable data, capable of giving insight into the pathogenesis of an aminoaciduria.

g. A coefficient for qualitative work. Standardized amounts of urine should be applied to filter paper chromatograms of urinary amino acids if valid comparisons are to be made between different chromatograms. Four standardizations are in common use: (i) A fixed volume of urine may be chosen. For reasons given earlier, this procedure does not provide any standardization. (ii) A timed urine volume may be used, but this is rarely practical in genetic fieldwork. (iii) A urine volume equivalent to a certain amount of creatinine may be used. Criticisms of this have already been presented; however, some workers (Woolf and Norman, 1957) have constructed relatively elaborate ways to handle the variables. (iv) Useful and reliable chromatograms are obtained when a urine volume equivalent to 250 μg. of total nitrogen is used. Certain drawbacks again exist, as discussed earlier, and understatement of the chromatogram results in the face of heavy aminoaciduria.

h. Summary. A satisfactory excretion coefficient for urinary amino acids, which is applicable to all patients in all situations, does not exist. Several workers (Bergstedt et al., 1960; Ghadimi and Shwachman, 1960; Jagenburg, 1959) have pointed out the considerable inherent variation in certain coefficients within the single subject or between individuals; the interrelation between *different* coefficients is even more complex, quite low correlation often being apparent.

Standardization of qualitative urine amino acid studies is advisable and a urine volume containing 250 μg. of total nitrogen is usually satisfactory for this purpose.

Urinary amino acid quantitative data is probably best expressed as a ratio of amino acid to total urinary nitrogen, or to body weight per 24 hours, although the latter coefficient usually requires special precautions for sample collection. The maximum information concerning the pathogenesis of the aminoaciduria is obtained if filtered and reabsorbed amounts of individual amino acids can be calculated. Interrelations of individual amino acid excretion rates are most satisfactorily interpreted when data are expressed in molar concentrations of amino acids. Since data in any study may need reassessment later, it is recommended that body weight, urine volume and total nitrogen output also be stated.

C. Factors Other than Inherited Disease Influencing Urinary Amino Acid Excretion

There is probably no factor influencing aminoaciduria which is completely free of genetic control. Nevertheless, there are several predominantly environmental influences affecting aminoaciduria, many of which

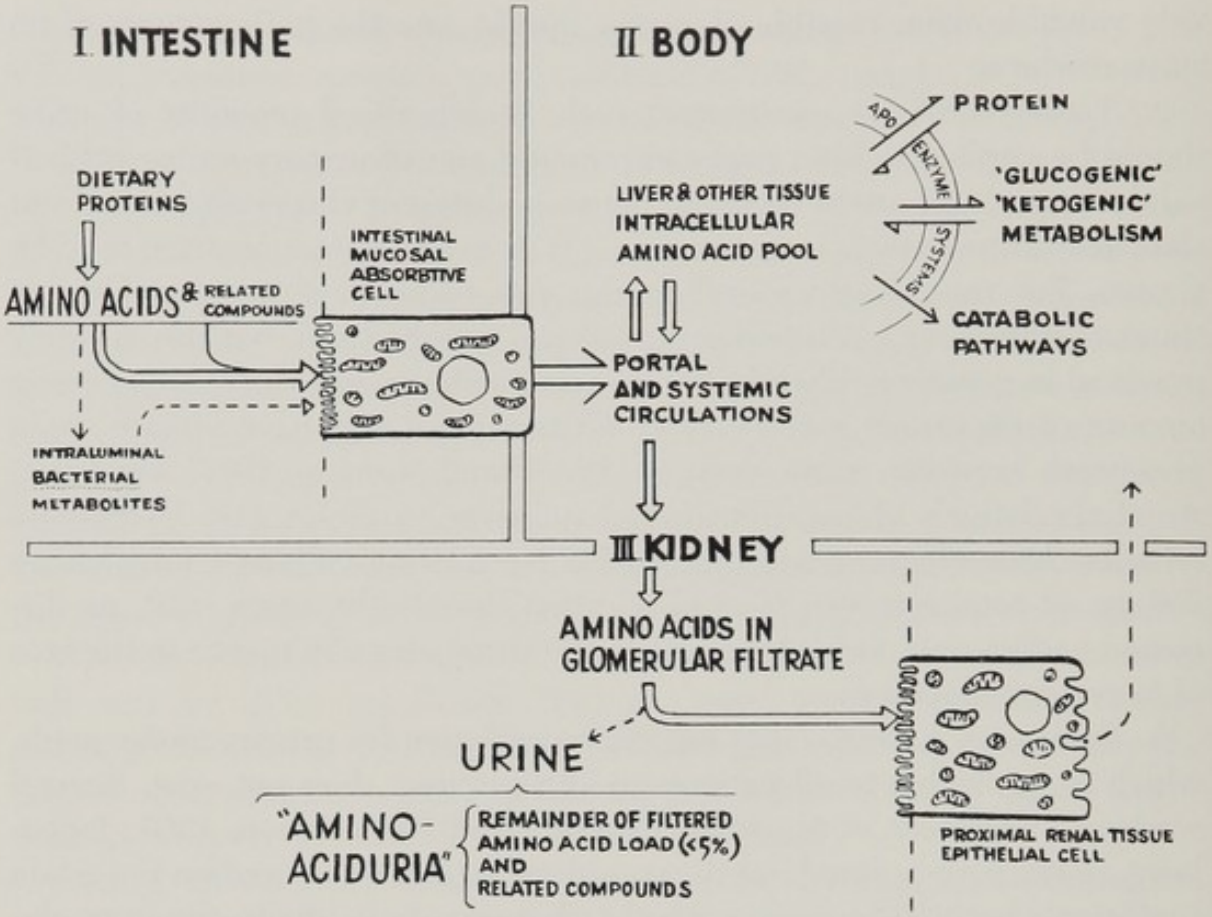


FIG. 1—Scheme indicating factors ultimately influencing aminoaciduria.

are implied in the scheme found in Figure 1. Furthermore, recognition of a special type of genetic control, namely normal developmental phenomena, is indicated before aberrant genetic determinants of aminoaciduria can be segregated. The following sections discuss these factors with particular indebtedness to the studies and discussions by Jagenburg (1959) and by Bigwood and co-authors (Bigwood et al., 1959).

Dietary Influence

A more or less discreet isolation of alimentary influence on aminoaciduria is possible in only a few instances, and many other factors operate in most situations. In the following situations, dietary influence on aminoaciduria is of some importance.

a. Dietary protein intake with normal renal tubular function. Studies in normal adults (Eckhardt and Davidson, 1949; McMenamy et al., 1960; Rosenberg et al., 1961) and children (Huisman, 1957) have shown that ingestion of protein, within the usual limits of intake, has only minimal influence on total amino acid excretion. Protein restriction may diminish the normal aminoaciduria (Nyhan et al., 1961). McMenamy and co-work-

ers (McMenamy et al., 1960) noted only 60 per cent increment in post-absorptive amino acid excretion rates above the control fasting rates; an earlier study (Eckhardt and Davidson, 1949), using different levels of protein intake, noted similar results. Huisman (1957) studied 72 children, from 6 months to 13 years of age, observing that a tenfold increase in protein intake resulted in only a two or threefold increase in free amino nitrogen excretion and a slightly greater increase of hydrolyzable total urinary amino nitrogen excretion; the majority of the increment in free amino nitrogen excretion was accounted for by those amino acids with naturally high excretion rates. Excretion ratios (mg. amino nitrogen/Kg./24 hours) were relatively constant in all age groups studied by Huisman.

b. Effect of diet with defective tubular amino acid reabsorption. The excretion of amino acids is greatly increased by protein ingestion in patients with defective renal tubular amino acid transport (Rosenberg, Mueller and Watkin, 1961; Stein, Bearn and Moore, 1954). The elevated amino acid excretion ratios in newborns and prematures (Bergstedt et al., 1960; Ghadimi and Shwachman, 1960; Jagenburg, 1959; O'Brien et al., 1960) may be partly related to high dietary protein intake, in association with diminished tubular reabsorption (Jonxis, 1951); however, careful choice of excretion coefficients is important in the neonatal group and varying interpretations have been presented (Ghadimi and Shwachman, 1960; Jagenburg, 1959; O'Brien et al., 1960) concerning possible effect of diet on neonatal aminoaciduria.

c. Qualitative effects of dietary protein. Certain amino acids are particularly affected by protein ingestion, a correlation having been demonstrated between increased 1-methylhistidine excretion and meat ingestion (Datta and Harris, 1951; Stein, 1953; Stein et al., 1954). Dietary meat protein similarly affects histidine excretion (Eckhardt and Davidson, 1949; Fowler, Norton, Cheung and Pratt, 1957) and taurine excretion (Stein, 1953).

Beta-aminoisobutyric acid, taurine and ethanolamine excretion increases in infants fed human milk in place of cows' milk (Jagenburg, 1959), a finding analogous to the response to protein malnutrition (Edozien, Phillips and Collis, 1960).

When synthetic amino acid mixtures are substituted for whole protein sources of amino acids, urinary amino acid excretion patterns are altered (Fowler et al., 1957). D-amino acids, when present in supplement mixtures, are relatively unmetabolized and poorly reabsorbed by the renal tubule, and will appear in the urine (Schendel, Antonis and Hansen, 1959); they are indistinguishable, except by special analytical methods (Bonetti and Dent, 1954), from the corresponding L-enantiomorphs and therefore may be interpreted as an apparent L-aminoaciduria.

d. Effect of nutritional state of individual. Beta-aminoisobutyric acid ex-

cretion is increased by starvation and fasting in some subjects (Robson and Rose, 1957; Sandler and Pare, 1954) and in protein malnutrition states (Dubois et al., 1959; Edozien et al., 1960) (see also section E, Ib, 2). Ethanolamine (Edozien et al., 1960) and taurine (Dubois et al., 1959) are reported to be similarly affected by malnutrition. More generalized aminoaciduria has been reported in some patients with kwashiorkor (Dubois, Vis, Loeb and Vincent, 1959; Schendel et al., 1959), a condition in which low or normal plasma amino acid levels occur (Dubois et al., 1959; Westall, Roitman, de la Peña, Rasmussen, Cravioto, Gomez and Holt, Jr., 1958). Hyperaminoaciduria may be even more apparent during the early recovery state of kwashiokor following high protein diet intake (Dubois et al., 1959). These findings may reflect impaired cellular metabolic activity in protein depletion (Kaplansky, 1959; Westall et al., 1958).

e. Effects of unusual or deficient dietary components. Unusual ampholytes in urine may occasionally be of dietary or medicinal origin. The excretion of 5-hydroxypipecolic acid following ingestion of dates (Gartler and Dobzhansky, 1954) is one example. Gamma-aminobutyric acid has its only endogenous origin in brain, where it is rapidly metabolized (Roberts, 1960), and when found in urine is probably of dietary origin (Westall, 1950).

Generalized aminoacidurias have been described with dietary deficiency of vitamin D (Visakorpi, 1960), and of ascorbic acid (Chadwick, Fawcett and Warburton, 1959); the former condition has been demonstrated to impair renal tubular reabsorption of affected amino acids (Jonxis, 1961). Tyrosyluria, reflecting specific neonatal impairment of tyrosine metabolism (see section C, 2) has been noted in association with relative ascorbic acid deficiency in the neonatal period (Berry and Sutherland, 1960; Bloxam, Day, Gibbs and Woolf, 1960). Vitamin B_6 deficiency may affect human cystathionine excretion (Scriver, Hutchison and Coursin, 1961).

There are many toxic substances whose ingestion may impair renal tubular function, resulting in aminoaciduria; comment may be found on these aminoacidurias in the previously mentioned general reviews (Bigwood et al., 1959; Ghadimi and Shwachman, 1960; Rowley et al., 1961; Snyderman and Holt, Jr., 1960). Galactosemia is of particular interest in this context, however, because of its genetic basis. In galactosemic patients, renal tubular dysfunction with aminoaciduria is directly correlated with galactose ingestion; a careful study of this problem has been reported by Cusworth and co-workers Cusworth, Dent and Flynn, 1955). Similarly, in the newly described hereditary disorder associated with defective intestinal carbohydrate digestion (Darling, Mortensen and Søndergaard, 1960), a correlation between carbohydrate ingestion and generalized aminoaciduria has been noted.

Finally, reference should be made to the effects of intestinal bacterial metabolism. Unusual urinary excretion patterns of metabolites closely related to amino acids have been observed when impairment of intestinal amino acid transport exists and the affected amino acids accumulate intraluminally; intestinal bacteria can convert these amino acids to products which are then absorbed from the intestine and excreted in the urine. Such observations have been described in Hartnup disease (Scriver and Shaw, in prep; Shaw, Redlich, Wright and Jepson, 1960) and in cystinuria (Milne, Asatoor, Edwards and Loughridge, 1961).

2. *Effects of Age*

Exaggerated aminoaciduria is well known in earliest postnatal life (Anonymous, 1961) and it is presumed that regulatory phenomena affecting cell and enzyme activity underlie this circumstance. Timing of genetic activity determining cell differentiation and activity (Beerman, 1956; Paigen and Noell, 1961), and genetic regulator mechanisms controlling other genes (Jacob and Monod, 1961), have been described in biological systems, increasing the possibility that such events may also exist in human biology. Future investigation and interpretation of age correlation and activity of amino acid transport systems in the young, such as are now being reported (Wilson and Lin, 1960), may provide further insight into aminoaciduria. These data would supplement the valuable information already available concerning age correlations and apoenzyme activity in amino acid metabolism (Driscoll and Hsia, 1958; Kretchmer, 1959).

Studies of large groups of newborns (Berry, 1960a; Berry, 1960c; Woolf and Norman, 1957) show characteristic aminoaciduria patterns (Fig. 2) suggesting some consistent biological sequence at work. Variations within the over-all pattern are compatible with interaction between environmental and individual developmental patterns.

a. Age-effect on quantitative aminoaciduria. Previous discussions (Anonymous, 1961; Dustin, Moore and Bigwood, 1955; Ghadimi and Shwachman, 1960; Jagenburg, 1959; Sereni, McNamara, Shibuya, Kretchmer and Barnett, 1955) have reviewed much of the earlier literature on this topic. Existence of relatively greater aminoaciduria in the neonatal period was suggested over 50 years ago (Simon, 1911). Regardless of choice of amino acid excretion coefficient in the newborn period (Bergstedt et al., 1960; Dustin et al., 1955; O'Brien et al., 1960) or of the method used for analysis of urinary amino acids, there is general agreement that full-term newborn infants have greater aminoaciduria than older infants and children (Berry, 1960a; Berry, 1960c; Childs, 1952; Dustin et al., 1955; Ghadimi and Shwachman, 1960; Huisman, 1957; Jagenburg, 1959; Simon, 1911; Woolf and Norman, 1957). Premature infants also exhibit this increase (Berg-

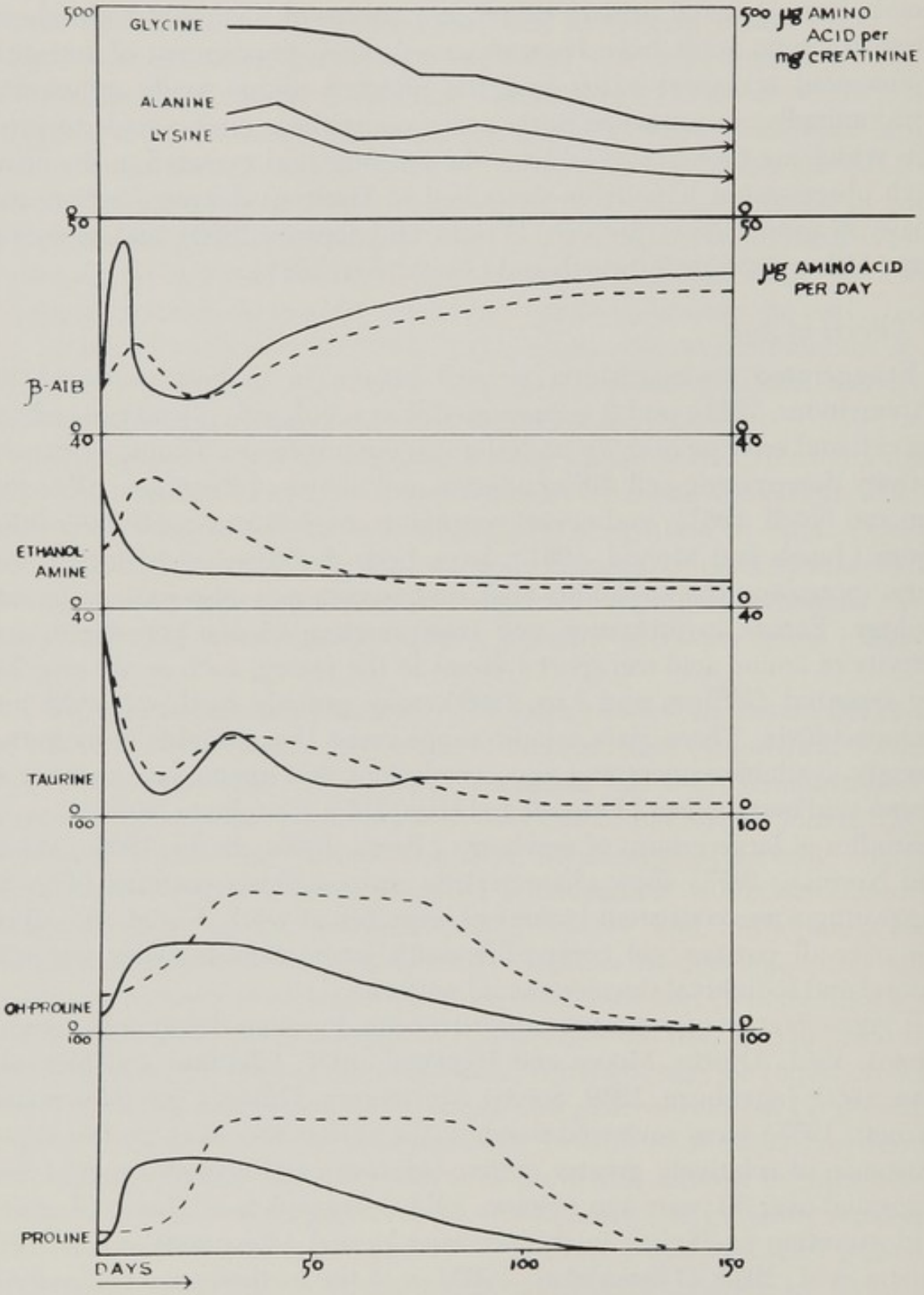


FIG. 2—Figure adapted and redrawn from data by Woolf and Norman (1957), and Berry (1960c), presenting changes in excretion of certain amino acids in newborn period. (——, premature infant; ——, full term infant).

stedt et al., 1960; Jagenburg, 1959; Norton, Pratt and Hasselmeyer, 1954; O'Brien et al., 1960; Sereni et al., 1955). Hyperaminoaciduria in full-term infants does not persist, however, much beyond the first few weeks or months of life (Dustin et al., 1955; Fowler et al., 1957; Huisman, 1957; Jagenburg, 1959; Woolf and Norman, 1957). Unfortunately, gestational age is rarely stated in reports dealing with premature infants, so that interpretation of factors responsible for aminoaciduria is made more difficult.

Several factors may contribute to the exaggerated amino acid excretion of the newborn. These may include higher blood amino acid levels (Glendening, Margolis and Page, 1961) and a relatively high protein intake (Jagenburg, 1959; Norton et al., 1954). It is unlikely, however, that these can be considered important causes for an hyperaminoaciduria which has both remarkable qualitative characteristics (Fig. 2) and complex interrelationships between its various components (Woolf and Norman, 1957). More important considerations are the effects of postnatal development of apoenzyme systems with subsequent effects on endogenous amino acid metabolism, and the development of renal tubular amino acid transport systems. Neonatal hyperaminoaciduria has no constant relation to the mellituria of the same age group (Bickel, 1961; Woolf and Norman, 1957).

Sereni and co-workers (1955), using relatively insensitive quantitative methods, recognized that neonatal aminoaciduria probably resulted largely from diminished renal tubular amino acid reabsorption. The Denver group (O'Brien, 1961; O'Brien et al., 1961) investigated this problem again with ion exchange column chromatographic techniques and have confirmed the renal origin of many components of neonatal aminoaciduria. Other components appear, however, to be of prerenal origin.

b. Qualitative features of aminoaciduria in the newborn. i. Tyrosine. The earlier work of Levine and co-workers (1947) and later that of Bloxam et al. (1960) described the not infrequent occurrence of excessive excretion of tyrosine, p-hydroxyphenylpyruvic acid (p-HPPA) and p-hydroxyphenyllactic acid in the urine of newborn infants. Increased protein intake, prematurity, or insufficient vitamin C supplement appeared to predispose to "tyrosyluria" and hydroxyphenyluria. However, Bloxam and co-workers (1960) noted that apparently normal full-term infants receiving average amounts of vitamin C and protein could also have temporary tyrosyluria and hydroxyphenyluria, which disappeared spontaneously. Berry and co-workers (Berry and Sutherland, 1960) made a similar observation in another infant.

The basis of the tyrosyluria and hydroxyphenyluria appears to be temporary inactivity of the hepatic tyrosine oxidizing system (Kretchmer, 1960; Kretchmer, Levine and McNamara, 1957; Kretchmer, Levine, McNamara and Barnett, 1956). This reaction is believed to occur as follows:

$$\text{TYROSINE} \xrightarrow{\text{Transaminase}} \text{p-HPPA} \xrightarrow[\text{Ascorbic Acid + "Quinone"}]{\text{Oxidase}} \text{HOMOGENTISIC ACID}$$

p-HPPA oxidase is apparently present in neonatal liver in adequate concentrations (Kretchmer, 1959) but requires large amounts of ascorbic acid to overcome substrate inhibition with p-HPPA. Inactivation from causes other than ascorbic acid deficiency and substrate inhibition is implied by the studies of Bloxam (Bloxam et al., 1960); this may be related to a postulated quinone cofactor for p-HPPA oxidase activity (Zannoni and La Du, 1961).

Certain developmental characteristics of the p-HPPA oxidase system therefore seem to account for increased neonatal tyrosine excretion (Kretchmer, 1960). The situation is asymptomatic, with rare exception (Menkes and Jervis, 1961) (see section E, Ia, 7) and should not be confused with "tyrosinosis" (La Du, 1960a; Medes, 1942).

ii. Proline and Hydroxyproline. These imino acids have higher excretion rates in early infancy than at any other time of life, although hyperiminoaciduria may not be observed until several days after birth (Woolf and Norman, 1957) (see Fig. 2). Normal excretion rates are only achieved many weeks or months after birth (Berry, 1960c; Jagenburg, 1959; Woolf and Norman, 1957). O'Brien and co-workers (O'Brien, 1961; O'Brien et al., 1961) measured endogenous renal amino acid clearances, noting that prolinuria was of renal tubular origin only, whereas elevated plasma levels of hydroxyproline and high clearance rates suggested that both prerenal and renal tubular mechanisms account for its excretion; these studies confirmed earlier impressions concerning neonatal iminoaciduria (Sereni et al., 1955; Woolf and Norman, 1957) and possibly the increased load of hydroxyproline may compete for proline reabsorption (see section E, Ia, 6), producing prolinuria in this manner. A third imino acid, pipecolic acid, is reported to be present in newborn urine, its excretion rate paralleling that of the other two imino acids (Jagenburg, 1959).

iii. Taurine. Several investigators (Berry, 1960c; Jagenburg, 1959; Norton et al., 1954; Woolf and Norman, 1957) have found the excretion pattern shown in Figure 2. One group noted a temporary rise in excretion rate at the end of the second week (Woolf and Norman, 1957). Recent studies (O'Brien, 1961; O'Brien et al., 1961), performed around forty weeks gestational age, demonstrated that taurine reabsorption rates were comparable to those in the adult, indicating that neonatal hypertaurinuria is probably of prerenal origin.

iv. β-Aminoisobutyric Acid (BAIB). High excretion rates are noted after the first day, particularly when human milk protein feedings are given (Jagenburg, 1959). Renal tubular reabsorption is poor in the infant,

as in the adult (O'Brien, 1961), suggesting that alteration in endogenous BAIB metabolism determines its urinary excretion in the newborn (Jagenburg, 1959).

v. Ethanolamine. Its excretion pattern is similar to that of BAIB except that ethanolamine excretion is high on the first day of life (Jagenburg, 1959; Woolf and Norman, 1957); ethanolamine accumulation in plasma occurs, indicating a prerenal cause for ethanolaminuria. Relatively lower excretion rates in prematures may reflect differences in renal or hepatic function compared to full-term newborns (Jagenburg, 1959; Woolf and Norman, 1957).

vi. Cystine, Lysine, Arginine and Ornithine. Their excretion rates are usually normal in full-term infants, but in premature infants the excretion rates of the four amino acids may often be elevated for a short time after birth. Variations in excretion rates tend to involve this group as a whole, rather than each component separately (Berry, 1960c; Dustin et al., 1955; Woolf and Norman, 1957). It is known that this group has a characteristic and distinctive transport behavior (see section E, II, 1) and it would appear from newborn observations that the pertinent transport system achieves normal activity close to 40 weeks gestational age.

vii. Glycine, Alanine and other Low Molecular Weight Amino Acids. High excretion rates usually exist in the newborn (Berry, 1960c), and endogenous clearance rates of these amino acids are increased compared to clearance rates in adults and older children (O'Brien, 1961; O'Brien et al., 1961).

viii. Other Observations. An unknown compound in the urine of most newborns, described by Fowler and co-workers (1957) as peak no. 9, by Jagenburg (1959) as peak G, and which probably also corresponds to Berry's unknown no. 35 (1960c), has been identified and isolated as homocitrulline (Gerritsen, Lipton, Strong and Waisman, 1961). Its excretion is influenced by diet, is widely variable between individuals and is usually not detected after the first few months of life (Berry, 1960c; Fowler et al., 1957; O'Brien, 1961). The origin of homocitrulline in human urine remains to be discovered.

Whereas the above comments apply to the newborn period and early infancy, aminoaciduria in older children (beyond 6 months) is comparable to the adult excretion pattern, provided comparable excretion coefficients are chosen for adult and childhood age groups (Berry 1960c; Carver and Paska, 1961; Dustin et al., 1955; Fowler et al., 1957; Huisman, 1957; Jagenburg, 1959).

3. *Effect of Sex on Aminoaciduria*

There is variation in aminoaciduria with reference to the sex of the individual; sex-influence is implied, not sex-linkage as written in one review

(Bigwood et al., 1959). Jagenburg has noted (1959) that the choice of excretion coefficient affects interpretation of aminoaciduria data in males and females (see his Tables 23 and 33). When expressed as a 24 hour ratio, males have a greater aminoaciduria; when expressed as a creatinine ratio, the reverse is true; the results are identical when expressed as coefficients of body weight per 24 hours or total nitrogen. Differences in amino acid excretion in men and women observed by other authors, (reviewed by Jagenburg, [1959]), can probably be attributed to the choice of excretion coefficient.

Soupart (1959) investigated normal adults of both sexes, and Carver and associates (1961) performed a similar study with normal children; in both reports, based on ion exchange resin column chromatography data, individual amino acid excretion rates were comparable in male and female subjects.

More specific sex-influence is noted during menstrual and reproductive cycles. Increased histidine excretion during pregnancy was first described by Honda (1923) and by Kappelar-Adler and Bermann (1934), and later Soupart (1954a) noted that hyperhistidinuria began near nidation and continued until the first post-partum week. Soupart (1954b) also detected hyperhistidinuria in relation to ovulation in the menstrual cycle. Pregnancy or ovulatory hyperhistidinuria is relative to the normal range of histidinuria of the particular subject. Absolute values are probably not diagnostic for a woman whose histidine excretion has not been previously studied, unless the excretion rate is greater than 250 mg./24 hours.

Although the major interest has been in histidine excretion, careful endogenous amino acid renal clearance studies (Christensen, Date, Schöheyder and Volqvartz, 1957), performed in three women in the early second trimester, revealed that excretion of other amino acids also differed from that of comparable nonpregnant women. The clearance rates of threonine, serine, the glutamine-asparagine complex, glutamic acid, glycine, α-amino-n-butyric acid, methionine, leucine, tyrosine and lysine, in addition to histidine, were all increased; blood levels of amino acids tended to be slightly lower than in the control women. Glomerular filtration rates interpreted from endogenous creatinine clearance rates, were identical in both groups, a finding at variance with the increased inulin clearance rates observed during pregnancy in a previous study (Page, Glendening, Dingman and Harper, 1954). Whereas this latter study has suggested that the origin of pregnancy aminoaciduria involved an increased filtered load of amino acids in addition to altered renal tubular reabsorption, the more recent study (Christensen et al., 1957) suggests that renal tubular reabsorption, perhaps influenced by increased steroid excretion during pregnancy, is the principal factor altering amino acid excretion rates.

4. Aminoaciduria and Certain Aspects of Renal Function

a. Glomerular function. Certain aspects of early neonatal aminoaciduria may be accounted for by the diminished GFR of this age group (Metcoff, 1959). Patients with renal hyperaminoaciduria, who subsequently develop glomerular failure exhibit a decrease in quantitative hyperaminoaciduria, usually without significant alteration in the excretion pattern (Cusworth and Dent, 1960b). On the other hand, patients without previous disturbance of renal tubular function, may have increased aminoaciduria when failure in whole nephron function affects tubular as well as glomerular function. These relationships are clearly presented in Figure 3.

Certain qualitative features, affecting specific amino acids, are noted in patients with chronic renal disease in which whole nephron failure occurs (Jagenburg, 1959). In this situation, a characteristic increase in urinary excretion of cystine, lysine, arginine and ornithine, as well as proline, citrulline and BAIB is observed (Jagenburg, 1959).

Levinson (1960) has made the interesting observation that despite the effect which drastic changes in glomerular filtration function may have on urinary excretion of amino acids, plasma amino acid concentrations in general remain relatively unaltered. Jagenburg (1959) has reported quantitative analyses of plasma amino acid concentrations in renal failure, confirming the above observations with the exception that significant increases in taurine, citrulline and cystine concentrations exist. β-Aminoisobutyric acid may also accumulate in plasma (Cusworth and Dent, 1960a).

The complexities of this type of problem emphasize the value of determining aminoaciduria as a coefficient of renal function itself (i.e., as endogenous amino acid renal clearance rates or as reabsorbed and filtered amounts), if impairment of renal filtration function is present.

b. Tubular function. Certain aspects of renal tubular function have been studied for their effect on aminoaciduria. Urine flow-rate, that is, axial hydrodynamic flow at right angles to the transport process in cylindrical structures (Bergmann and Dikstein, 1959), may affect transport of a substrate (Weiner, Washington and Mudge, 1960) whose transport is a function of nonionic diffusion processes in lipoid membranes (Milne, Scribner and Crawford, 1958). Amino acid transport, however, does not have these characteristics and therefore should not be affected by urine flow-rate; observations on total amino acid nitrogen excretion (Jagenburg, 1959), and on individual amino acid clearance rates (Scriver, Goldbloom and Roy, unpubl. obs.), suggest that such is the case.

By similar reasoning, changes in hydrogen ion concentration in urine should have no effect on amino acid renal tubular transport. One formal study of this problem revealed no effect when acidosis was induced by

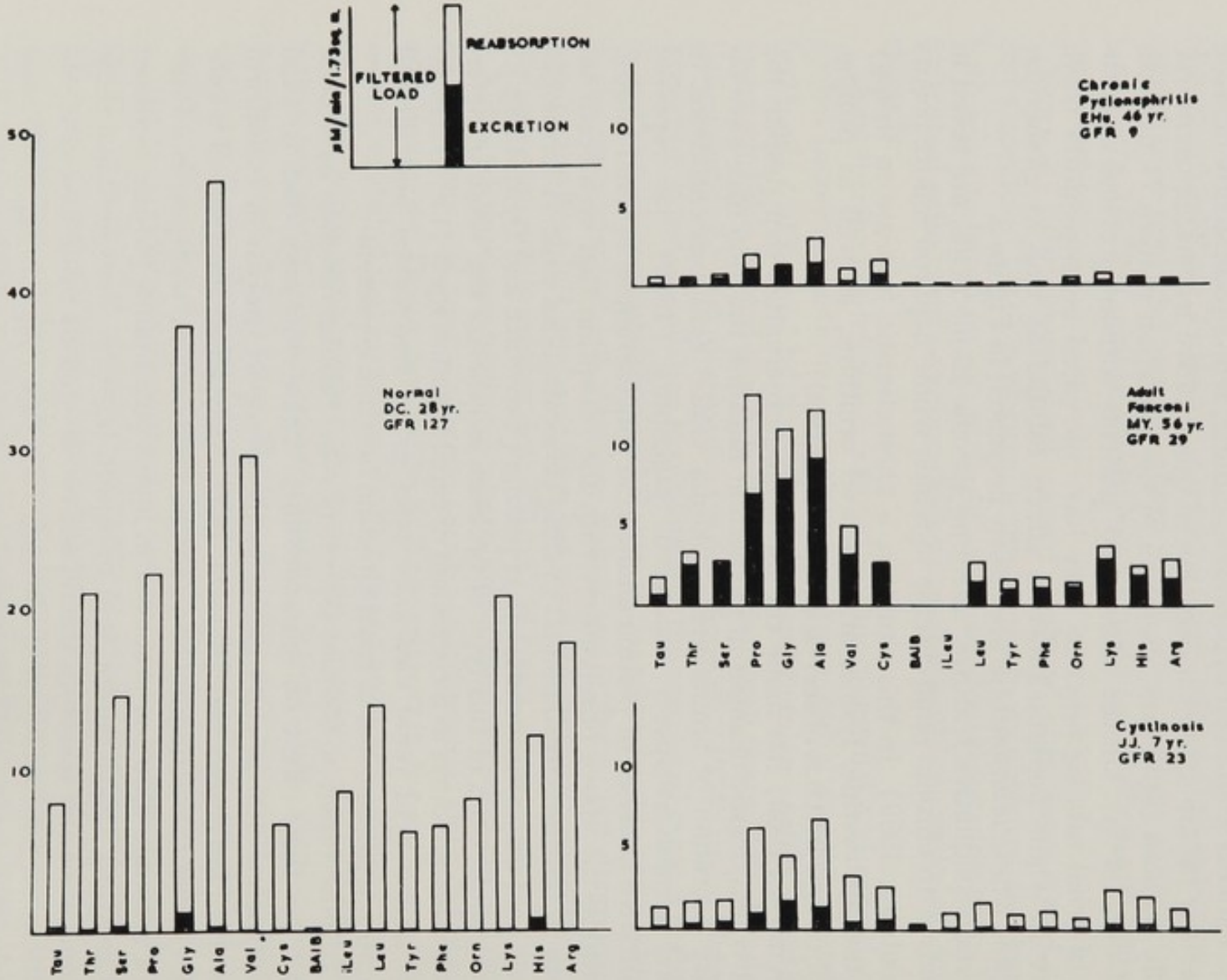


FIG. 3—Illustration of the interrelations between glomerular and tubular dysfunction and their effects on aminoaciduria. (Reproduced from, Cusworth and Dent, in Biology of Pyelonephritis, Henry Ford Hospital Symposium [1960b], by permission of the authors and the publisher.)

ammonium chloride loading (Jagenburg, 1959). The situation appears to be different, however, when acidosis is produced by a congenital disorder of tubular function (see E, IVa, v.).

5. Diurnal Variation in Aminoaciduria

Circadian rhythms in biological activities have attracted great interest in recent years (Halberg, 1960). However, diurnal variations in aminoaciduria have not been studied from the viewpoint of rhythmic activity of amino acid transport or apoenzyme systems. Much of the diurnal variation in aminoaciduria can be satisfactorily accounted for by the intermittent intake of food (McMenamy et al., 1960; Robson and Rose, 1957; Sandler and Pare, 1954). Reasonable constancy of amino nitrogen excretion from day to day (Jagenburg, 1959; McMenamy et al., 1960) or at different times in the same day (Krawczynski et al., 1961), (excluding the effect of diet), is noted in adult patients; it is the impression, however, that variations may be greater in children. Exercise is another diurnal variable which might influence aminoaciduria; no variation in amino acid excretion was observed with moderate exercise (McMenamy et al., 1960).

6. Summary

Interpretation of aminoaciduria is dependent on reliable analytical methods and urinary amino acid excretion coefficients. Age, sex, diet and various physiological phenomena affect aminoaciduria, both qualitatively and quantitatively. Terms of reference must include endogenous compounds which are not true amino acids such as ethanolamine and taurine; account should also be taken of the great variety of compounds (Jagenburg, 1959; Westall, 1955), both free and hydrolyzable, which make up the ninhydrin-reacting ampholytic fraction of urine. Finally, artefacts including nondietary exogenous components, by-products of intestinal bacterial activity or urine contamination, and technical vagaries, should be considered.

Recognition of hereditary aminoaciduria requires comparison with the normal aminoaciduria of a given population for whom environmental and other influences on aminoaciduria are known or controlled. Qualitative two-dimensional chromatograms can provide an easily accessible visual image of this norm. Quantitative data has still to be obtained in many instances. Nevertheless, compilation of available data from 40 adults and older children (Table 2) shows reasonable correlation between different reports when suitable excretion coefficients are chosen. The data, however, are applicable only to Caucasian Northern Europeans and North American subjects beyond infancy. Figure 2 summarizes some current observations on aminoaciduria in infants. An attempt has been made to provide ap-

propriate context for the individual components of aminoaciduria listed in Table 2, by inclusion of available endogenous amino acid renal clearance data.

D. Mechanisms of Aminoaciduria

Current thoughts suggest that one of the products of the genotype, which influences amino acid metabolism (and hence aminoaciduria) is a macromolecular unit whose relation to a smaller amino acid molecule is that of an apoenzyme or a membrane transport complex. Other potential genetic relationships affecting substrate also exist, involving coenzyme factors for apoenzyme activity, and energy systems for transport mechanisms. Most classifications of hereditary aminoaciduria imply distortion of the relationships between amino acid substrate and the apoenzyme or transport systems. "Prerenal" hereditary aminoaciduria places the lesion at an apoenzyme complex, thus affecting the amino acid which serves as substrate; accumulation of unmetabolized substrate follows and this is eventually reflected in its urinary concentration. "Renal" aminoaciduria places the lesion in a defective renal tubular transport system. Other terms, describing aminoacidurias which appear to fit neither "prerenal" or "renal" classifications, are usually reserved for situations when the amino acid or related compound is not normally detected in extracellular biological fluids. These compounds may be transient intracellular metabolites which have accumulated due to impairment in an enzymatic sequence, or they may be unusual substances which have gained access to the body fluids but for which there is limited or no metabolism. In either instance, it is evident that these substances have very low or absent affinity for available reactive sites for mediated transport in renal tubule cells and their appearance in extracellular fluids is followed by rapid loss in the urine. This discussion cannot encompass the difficulties of defining a "gene," nor debate the concepts of "structural" (Bonner, Suyama and De Moss, 1960; Hotchkiss and Evans, 1960) and "regulator" (Jacob and Monod, 1961) genes. However, these concepts do clarify interpretation of many aminoaciduria phenomena; what is not certain at the present time is whether they may also apply to development, structural characteristics, substrate specificity and control of cellular amino acid transport systems. There is every reason to believe that cellular amino acid systems are under direct genetic control, but before going further, some of the present day concepts of amino acid transport should be mentioned.

1. Site of Renal Amino Acid Transport

Correlation of structure and function at all levels in the hierarchy of biology has provoked a variety of publications including such classics as

those by Darwin (1859), Thompson (1942), and Florkin (1949). A single organ such as the kidney has lent itself to a remarkable analysis of the relation of structure to function, in its evolutionary development (Smith, 1959). The structure of the individual nephron has recently been given further functional interpretation (Ullrich, Kramer and Boylan, 1961) and now attention is being given to the meaning of the cytoarchitecture of individual cells (Villee, 1961).

In man, adapted to homeothermic terrestial existence, the kidney receives a large volume of blood under considerable hydraulic pressure. Morphological evolution has resulted in the delivery of renal arterial blood volume under normal circumstances to the complete glomerular vascular bed, with further distribution to peritubular vascular networks only after glomerular vascular transit. The structural characteristics of the glomerulus indicate that it functions as an ultrafilter (Hall, 1957), through which low-molecular weight solutes and fluid may pass into Bowman's space and then into the lumen of the proximal convoluted tubule. At this point, amino acids (along with other solutes and water) are components of the glomerular filtrate which may be lost from the body, unless tubular conservation exists. Approximately 0.4 moles of amino acids would be lost daily by the average human, but since only a few micromoles are actually excreted in the 24 hour urine, conservation must have occurred. Unlike some other organic molecules (Berglund, 1961; Weiner et al., 1960), there is no conclusive evidence that simultaneous secretion and reabsorption of amino acids occurs in the renal tubule (Webber, Brown and Pitts, 1961). Therefore, the main function of the renal tubule in amino acid metabolism is reabsorption of filtered amino acids.

The site of amino acid reabsorption in mammalian kidney has been found by the stop-flow technique (Malvin, 1961) to be in the proximal portion of the proximal convoluted tubule (Brown, Samiy and Pitts, 1961). Darmady (Darmady and Stranack, 1960) and co-workers have made a similar observation using the amino acid I_{131}-diiodotyrosine, the absorption site being detected by microdissection and autoradiography of the rat nephron.

2. The Absorptive Cell

It would be anticipated that the typical epithelial cell of the proximal renal tubule must be highly developed to provide its many functions, in addition to that of reabsorption of amino acids. Pease (1955), Rhodin (1958a; 1958b) and Novikoff (1960) have demonstrated that the proximal renal tubule cell has both highly developed organelles and a remarkable absorptive surface. Contiguous cells are noted to form a monolayer, orientated in an interlocking manner upon a basement membrane, with their

TABLE 2—*Amino Acid Quantitative Data in Humans*

Amino Acid	a) Normal Adults Both Sexes mM. × 10²/24 hrs. Range	a) Average	b) Children (3–11 yrs.) Both Sexes mM. × 10²/24 hrs. Range	b) Average	c) Normal Adults Both Sexes mM. × 10² / 100 g. total nitrogen i) Range	ii) Average	d) Adults Both Sexes Endogenous Amino Acid Renal Clearances ml./min./1.73M.²	Plasma Amino Acids Normal Adults μM./100 ml. e) Range	f) Mean and S.D.	g) Mean
Proline	0	0	0	0	0(-tr)*	0	0	11.5–23.0	22±1.0	20.5
Hydroxy-proline	0	0	0	0	0	0	0	0	0	0
Ornithine	0-8	1.1	—	—	—	—	0	4.5–9.0	9.4±1.0	5.4
Arginine	1–8	2.6	—	—	35–240	35	0.2–0.8	2.0–14.0	10.5±0.75	8.7
α-amino adipic or α-aminobutyric	0–8	3.4	—	—	—	—	0–1.3	0.5–5.0	1.7±0.1	2.9
Methionine	2–8	3.6	2.0–9.5	5.1	50–70	70	(1.1)	0.5–4.5	2.7±0.1	2.5
Aspartic	1–22	4.1	—	—	—	—	(2.4)	0.5–1.0	3.1±0.3	—
Valine	0–26	7.2	1.5–5.0	3.0	55–70	70	0.04–0.3	9.0–29.0	20.2±0.6	24.6
Glutamic					55–95	80	0.3–0.7	2.5–11.5	13.2±2.3	4.8
Lysine	0–12	4.4	7.0–64.0	29.0	40–110	—	0.2–1.9	4.0–16.5	20.5±1.0	18.6
½ Cystine	3–28	8.2	4.0–16.0	10.7	70–115	105	0.7–2.9	△1.5–12.5	7.8±0.7	4.9
Leucine	1–15	7.4	2.7–8.3	4.4	45–90	65	0.2–0.9	7.0–15.5	13.2±0.6	12.9
Isoleucine	4–18	9.4	1.8–5.6	3.4	55–210	75	0.2–1.0	3.5–8.5	6.9±0.6	6.9
Phenylalanine	4–25	8.0	2.4–10.6	4.6	55–170	90	0.7–1.4	3.0–7.0	6.6±0.5	5.1
Tyrosine	4–15	9.4	4.2–16.9	8.4	88–220	105	1.0–1.7	2.5–6.0	6.5±0.3	7.0
Threonine	2–30	16.8	3.5–24.8	15.0	110–290	390	0.8–1.5	5.5–17.0	—	11.7
β-aminoisobutyric	0–51	25.2	4.5–16.6	11.5	80–1500	510	—	0	0	0
Alanine	6–50	27.5	1.2–44.0	23.3	220–510	350	0.3–0.9	§10.0–40.0	41.5±3.5	38.3
3-M-histidine	18–52	32.3	5.5–24.7	11.6	190–340	270	—	0.3–2.0	—	0.6
Serine	21–62	37.4	15.5–54.0	28.2	410–700	550	1.9–3.0	4.0–13.5	—	10.6

Glutamine and Asparagine	29–77	—	14.7–84.7	43.0	¶	¶	0.7–1.8	15.3–89.0†	—	—
1-M-histidine	13–93	43.3	2.3–27.1	11.0	85–2340	230	(17)	(0.3–0.4)		0.6
Histidine	13–137	79.0	30.6–128.0	65.5	170–1400	900	4.7–9.1	3.5–9.5	9.4±0.5	10.8
Taurine	22–185	81.2	6.0–96.9	43.0	300–1590	640	1.7–14	2.5–11.0		4.4
Glycine	71–416	168.7	16.0–142.0	67.0	540–1830	1490	2.7–5.8	§4.5–29.0	28.1±1.8	20.5

Urine data have been converted into millimolar ratios with a factor to shift decimal place (Soupart, 1959). Weights of subjects not available and therefore differences between adult and childhood excretion data are only apparent.

a) Six male and 9 female healthy adults, diet uncontrolled (Soupart, 1959);Dowex 50 × 4 method (Moore and Stein, 1954a).

b) Seven male and 5 female healthy children, aged 3 to 11 years, diet uncontrolled (Carver and Paska, 1961); Amberlite IR-120 method (Moore, Spackman and Stein, 1958). Range includes Fowler's data (1957), obtained by Dowex 50 × 4 method (Moore and Stein, 1954a).

c) i) Healthy adults, including 13 males and 1 female, studied by Stein (1953), Dustin et al. (1955) and Evered (1956); also 3 males and 3 females, studied by Jagenburg (1959).

ii) Average based only on Jagenburg's data (1959).

d) Adult endogenous amino acid renal clearances (Cusworth and Dent, 1960).

e) Range for adults including data from three different reports (Christensen et al., 1957; Cusworth and Dent, 1960a; Knauff and Schabert, 1960) using two column methods (Moore and Stein, 1954a; Moore and Stein, 1954b). Range also includes values for normal children and infants, quoted by Snyderman (1958) and Dancis et al. (1960). Nyhan's data (1961), obtained on Amberlite IR-120 resin, is within or slightly below the lower limit of the stated range.

f) Mean values for adults (Gerok, 1960); Amberlite IR-120 method (Moore et al., 1958).

g) Mean values for adults on Dowex 50 × 4 resin (Stein and Moore, 1954). Age differences for plasma values which may be significant, cannot be determined in any of the sources for these data (columns e, f and g).

° Observed only by Evered (1956).

¶ Data expressed as glutamic acid.

† Higher values for glutamine are generally observed with the Amberlite IR 120-resin technique (Moore et al., 1958).

§ Unusually low values for glycine and alanine were observed by Knauff (1960) and Nyhan (1961) with the new technique (Moore et al., 1958), which did not correspond to the higher range found with the older method (Moore and Stein, 1954); see average values in column g) nor with the data obtained by Gerok (1960) using the Amberlite IR-120 resin.

△ Newer and more accurate data for plasma cystine and cysteine are available (Brigham et al., 1960).

absorptive surfaces upon the apical side of the cell, facing the tubular lumen. The outstanding features of this cell, which has much in common with the intestinal mucosal absorptive cell (Laster and Ingelfinger, 1961), are its rich enzymatic apparatus (Spencer and Knox, 1960), mitochondria and microvilli. The latter are projections of the lipoid membrane on the apical surface of the cell; the simplest interpretation of microvilli is that they increase the surface area of the cell. The large number of mitochondria richly endow the absorptive cell with oxidative phosphorylation and energy production (Green and Goldberger, 1961; Lehinger, 1959).

Investigation of renal amino acid transport is a difficult proposition; hence, several recent publications based on investigation of intestinal transport (Akedo, Sugawa, Yoshikawa and Suda, 1960; anonymous, 1961; Finch and Hird, 1960a; Finch and Hird, 1960b; Gagnon, 1961; Hagihira, Lin, Samiy and Wilson, 1961; Jacobs, Coen and Hillman, 1960; Lin and Wilson, 1960; Metcoff, 1961; Neil, 1959; Quastel, 1960; Wilson and Lin, 1960; Wilson and Wiseman, 1954; Wilson, Lin, Landau and Jorgensen, 1960) are important because their data probably apply to interpretation of renal amino acid transport.

3. Considerations of Cellular Amino Acid Transport

L-amino acid reabsorption in kidney is noted to occur against an electrochemical concentration gradient (Brown et al., 1961; Schachter and Britten, 1961) and is therefore dependent on mediated or active cellular transport in the renal tubule. Transport maxima and competitive inhibition during tubular reabsorption of various L-amino acids has been studied in the dog (Gerok and Gayer, 1961). Variations in rates of urine axial flow in the lumen of the proximal tubule do not alter reabsorption of L-amino acids, which must occur across a "lipoid" membrane; this is further argument that L-amino acid transport characteristics are not in keeping with a simple diffusion process (Bergmann and Dikstein, 1959; Weiner et al., 1960). Effect of urine flow rate on D-amino acid excretion has led some workers to propose that D-amino acids have no active transport (Berry, 1960a; Crampton and Smyth, 1953). However, Finch and Hird (1960b) demonstrated that D-amino acids have low but definite affinity for active transport at L–amino acid reactive sites. Observations in Hartnup disease (see section E, II, 2) also support this conclusion.

a. Terminology. Several recent papers have discussed fundamental concepts of biological transport (Anderson and Ussing, 1960; Berliner, 1959; Christensen, 1959; Lefevre, 1961; Metcoff, 1961; Park, Reinwein, Henderson, Cadenas and Morgan, 1959; Stein, 1961; Wilbrandt, 1959). Hogben has referred to cellular transport of substrate as "the first common pathway" (1960). In general, cellular transport refers to transfer of molecules

across the area of the cell which is morphologically and physicochemically, the "lipoid" cell membrane. It is believed that the typical cell membrane is a lipo-protein structure approximately 100 Å in thickness whose integrity is interrupted by "pores," approximately 4 Å in diameter (Goldstein and Solomon,1960). These pores can allow direct access of small molecules (100 to 200 mol. wt.) into the cell. Although the pores permit a continuous aqueous phase across the membrane, their area is only a small fraction of the total cell surface; in addition, the size of the pores also limits access by diffusion flow of most charged molecules. These two features exclude membrane pores as significant diffusion sites for amino acids.

Passage of molecules across the cell membrane occurs by passive diffusion through lipid or aqueous phases, or by mediated transport. Mediated transport may be either "active," (i.e., "uphill" against an electrochemical gradient) or "facilitated" (where mediated transport is not against a gradient) (Christensen, 1960). Other transport mechanisms which exist in biological systems but whose quantitative importance is either limited or unknown, include bulk transfer of solute in the aqueous phase, as occurs with pinocytosis (Holter, 1960), and transport by membrane flow (Holter, 1960), where the solute is fixed to an external membrane site which then enters the cell interior releasing the solute. Finally, another type of cell access should be included and this is the exchange-diffusion mechanism (Heinz and Walsh, 1958; Widdas, 1952), where a bidirectional flux of two solutes with molecular similarities occurs between separate phases; energy is not required even if accumulation of one solute occurs in one phase against a gradient.

It should be apparent that although diffusion implies no molecular or chemical modification of solute, it might be considered that some form of modification occurs with active or mediated transport. Christensen (1960) has discussed the general term "access system" within this context. Whereas "access systems" may refer to any general chemical system introducing a substance to further metabolism, the term "transport system" has a limited context implying translocation only, of substrate from one phase to another across a physical barrier; the substrate does not undergo structural alteration in this situation and is delivered intact to its new location (Christensen, 1960; Christensen, Parker and Riggs, 1958). (Stein's "dimer" concept of transport (1961) retains the premise that integrity of the main structural features of the molecule is unaltered during transport.) "Transcellular" transport (e.g., transfer across the wall of the proximal convoluted tubule from urine to plasma) implies intracellular accumulation of substrate as a first component of the complete translocation (Oxender and Christensen, 1959.

Of the possible modes of cellular transport available to amino acids,

simple diffusion through lipid or aqueous pore phases seems unlikely to be quantitatively important because of the zwitterion nature of amino acids. Similarly, pinocytosis (Holter, 1960), although an intriguing phenomenon, is not a universal property of all cells to which amino acids have access. Calculations of the capacity for pinocytosis to handle observed rates of amino acid transport have shown that this process does not account for what actually takes place (Christensen, 1960). This means that some form of mediated transport or exchange diffusion must provide access for amino acids into cells, which takes place at recognition or reactive sites on the cell membrane.

b. The reactive site. The nature of the reactive site for biological transport is a most intriguing problem. The independence of this site from other cellular mechanisms which relate to further metabolism of the transferred substrate, is an important consideration. This independence would account for the observations that transport systems have the capacity to transport molecular analogues which have had no evolutionary or metabolic significance for the organism. In other words, the reactive site recognizes molecular or steric features, but not metabolic properties, of the substrate. Reactive sites and transport mechanisms exhibit different affinities and kinetic behavior with different amino acids (Finch and Hird, 1960a; Finch and Hird, 1960b; Scholefield, 1961; Tenenhouse and Quastel, 1960; Wilbrandt, 1956; Wilbrandt, 1959). Other studies suggest limited distribution of reactive sites on the cell surface (Solomon, Gill and Gold, 1956). Specificity, therefore, becomes an important component of reactive site function. Spatial limitation and selective substrate specificity at the reactive transport site, however, do not mean that it must be an enzyme.

The reactive site has been considered from several viewpoints to explain how substrate translocation occurs (Christensen, 1960). In general, transport, whether of active or facilitative diffusion character, is not well accounted for in all cases if a "carrier complex" must undergo translocation as well as its substrate. Several variations have been considered to accommodate this possibility. The mechanism of exchange diffusion provides a model where flow of one solute may stimulate counterflow of another. In a simple exchange diffusion system concentrative accumulation of one solute is accomplished at the expense of the other exchanged solute, so that exchange with the environment by the first does not require energy. This mechanism can still have specificity at the reactive site for its substrate, as shown recently in the study of an exchange diffusion system in ascites cells (Johnstone and Scholefield, 1961). However, for mediated transport to accomplish solute accumulation intracellularly, energy for the carrier reaction must be obtained from a linked exergonic reaction. A shuttling mobile carrier concept to carry amino acid inward or across the cell has

been proposed by Christensen (1959), with the further suggestion that intracellular accumulation of potassium occurs (Riggs, Walker and Christensen, 1958) in association with a net flux of this ion in the opposite direction to solute transport (Hempling, 1961; Hempling and Hare, 1961; Oxender and Christensen, 1959). To overcome the difficulties of a shuttling carrier complex, Burgen suggested a reorientating process of membrane pores (1957) whose walls by alternation of pore size, were able to accept, transmit and discharge a charged molecule through the membrane. Stein (1961) circumvents the problems of "carriers," membrane pores, and resistance to diffusion through lipoid media by polar molecules, by postulating that the reactive site is a "dimerizer" which unites two molecules of a single substrate, thereby reducing charge mass and allowing diffusion; reconstitution into two separate molecules completes the process after cell entry. In any such theory, (of which there are several cited elsewhere; [Christensen, 1960]), energy is presumed to be required for the physical alterations which take place during transport, independent of that which may have been induced in the reactive site by binding of the substrate.

If the accumulation of the substrate intracellularly or transcellularly by mediated transport appears to require energy, the source of this energy is still a matter of speculation (Christensen, 1960). Several observations are of interest. The large mass of mitochondria in absorptive cells has already been suggested to have some teleological importance in relation to transport. ATP'ase has been described in cell membranes with absorptive function (Novikoff, 1960). Transport is affected by temperature, oxygen and glucose concentration under certain conditions, and also by metabolic inhibitors (Tenenhouse and Quastel, 1960; Wilson and Wiseman, 1954). These and other factors to be mentioned later suggest that both glycolysis and respiration may contribute energy to the transport process, and that the energy may be stored as ATP for release in the membrane. The exact source of energy and its manner of availability at the linked reactive site, however, remain unknown.

c. Substrate interaction. So far the discussion of transport systems has implied a certain specificity for their substrates. Christensen has studied some of the steric requirements for amino acid transport, by defining the distribution of radicals around the alpha carbon and the position of the amino group in amino acids (1959; 1960). Different steric, lipophylic and hydrophylic properties of individual amino acids are noted to confer different affinities and kinetics on transport systems; study of their transport behavior has produced a great deal of information concerning competition and interaction between amino acids in cellular transport systems (Scholefield, 1961).

Some of the observations which seem to be of interest in relation to

hereditary aminoaciduria are as follows: (1) D-amino acids are transported at less active rates although these enantiomorphs may still combine with lower affinity for the reactive site (Finch and Hird, 1960b; Lin and Wilson, 1960; Neil, 1959). (2) Certain amino acids do not exhibit the kinetics and affinities of the remainder of the naturally occurring amino acids; this is particularly true of the group comprising lysine, ornithine, arginine and cystine, whose transport, both in intestine (Finch and Hird, 1960b; Hagihira et al., 1961) and in kidney (Webber et al., 1961; see section E), reveals special behavior. (3) The dicarboxylic amino acids (aspartic and glutamic) behave as though they share a separate transport mechanism in comparison with other amino acids (Webber, 1960). (4) A similar situation appears to exist for two imino acids, proline and hydroxyproline; this system in turn appears to share some features in common with a glycine system (Scriver, Schafer and Efron, 1961a; Scriver, Schafer and Efron, 1961b). (5) Glycine has been implicated in many transport systems but a recent study suggests that it can be transported independently (Johnstone and Scholefield, 1961). (6) Taurine and β-aminoisobutyric acid (Gilbert, Ku, Rogers and Williams, 1960) have been observed to act as though sharing common transport mechanisms. (7) Competition for intestinal transport has been demonstrated between valine, leucine and isoleucine (Hagihira, Ogata, Takedatsu and Suda, 1960).

Transport affinities have been studied for many other combinations with less clearly defined results (Christensen, 1960).

d. Factors influencing cellular transport. Several different agents appear to influence transport of amino acids and other substrates. The major difficulty in such studies, that of isolating a presumed specific effect on transport, from a possible effect on intracellular metabolism of substrate subsequent to transport, has been resolved by selecting an amino acid whose structure benefits transport, but prevents further metabolism. Alpha-aminoisobutyric acid (AIB) has these properties, and synthesis of C^{14}-AIB has provided a tool of great use in transport studies.

In the first experiments in which AIB was used (Noall, Riggs, Walker and Christensen, 1957), it was noted that growth, body weight, epinephrine, hydrocortisone, estrogens and pituitary growth hormones, all influenced AIB uptake into different tissues. The effect of certain agents was limited to specific organs in the experimental animal. Riggs and Walker (1958) studied again the effect of growth hormone itself on AIB transport; they observed a positive effect on transport, within minutes after addition of growth hormones to the experimental system. Other work (Kostyo and Engel, 1960; Kostyo, Hotchkiss and Knobil, 1959) suggests that growth hormone effect is independent of any interaction with other hormones.

Insulin appears to have a direct stimulative effect on amino acid transport (Akedo and Christensen, 1961; Manchester, 1961) not mediated through carbohydrate metabolism (Battaglia, Manchester and Randle, 1960). Other hormones with stimulative effects have been found; these are hydrocortisone, which increases liver uptake of AIB (Noall et al., 1957) and estradiol which influences uterine AIB uptake (Akedo et al., 1960).

An important stimulative agent in amino acid transport is vitamin B_6, in either its pyridoxal (PAL) or pyridoxal-5-phosphate (PLP) forms (Jacobs et al., 1960; Oxender and Christensen, 1959). Deficiency of vitamin B_6 in the intact animal inhibits transport of AIB (Riggs and Walker, 1958) and other amino acids (Akedo, Sugawa, Yosikawa and Suda, 1960) and both PAL and PLP will reverse the effect of vitamin B_6 deficiency (Oxender and Christensen, 1959), but with stimulative effect only on L-amino acids (Akedo, Sugawa, Yoshikawa and Suda, 1960). PLP (but not PAL) also can reverse the inhibitive effect of 2, 4-dinitrophenylhydrazine (Jacobs et al., 1960). Carbonyl compounds and other agents which may interfere with cellular metabolism of vitamin B_6 or its coenzyme activity, do not impair the transport activity of PLP and PAL. This anomaly has made it difficult to interpret the mechanism of vitamin B_6 effect on transport. Recently Oxender and Royer (1961) have noted that PAL or PLP, while stimulating mediated transport, both prevent AIB efflux, once intracellular accumulation has occurred. Recent studies with estrogens, whose effect on transport has many features in common with the B_6-effect, suggest that B_6 acts at the reactive site (Christensen and Jones, 1961).

4. Transport and Genetics: Speculations

From all the implications about the nature of mediated transport, it is not unreasonable to believe that there are genetic regulatory mechanisms for transport systems, as there are for apoenzyme systems. Genetic disturbances might affect transport at some enzymatic level (Novikoff, 1960; Spencer and Knox, 1960; Triantaphyllopoulos and Tuba, 1959) in the absorptive cell, (although the meaning and extent of the relationship between activity in the enzyme apparatus and transport mechanisms is as yet unknown); on the other hand, the reactive site on the cell membrane could be the altered gene product. Recent investigations in bacteria have shown that genetic differences in bacterial species are associated with quantitative differences in cell wall components (Ikawa and Snell, 1960), and that mutation may selectively impair membrane transport of an amino acid (Kessel and Lubin, 1961).

The remainder of this paper will present evidence that, in man and animals, mutations in the genotype may affect not only the precise amino acid-

apoenzyme relationships of intracellular metabolism, but also those relationships involving mediated amino acid transport, either at the reactive site on the cell surface or in its energetics.

E. Hereditary Aminoaciduria

The term "hereditary aminoaciduria" is used with two different connotations, which should be clearly distinguished. In one sense, the term refers to genetic control of the normal variations in aminoaciduria, either with age, as in neonatal hydroxyphenyluria and tyrosyluria, or between individuals, as demonstrated by similarities in the excretion patterns of monozygotic twins and differences between certain ethnic groups. More commonly, the term "hereditary aminoaciduria" implies that there is an obvious distortion of normal quantitative and qualitative interrelationships in amino acid excretion in the affected subject, which appears in more than one sib or generation and cannot be attributed to age, diet and other determinants. In certain hereditary aminoacidurias (phenylketonuria, hypophosphatasia, cystinuria, Wilson's Disease and galactosemia), the heterozygote state may be identified (Hsia, 1960). An alteration in genotypic control of an enzyme complex, or transport system, is presumed to be the determining factor for the aminoaciduria.

Hereditary aminoaciduria usually indicates *increased* excretion of amino acids, or excretion of an unusual amino acid or related compound. Holt and Snyderman (1960; Snyderman and Holt, 1960) suggest that "hereditary aminoaciduria" should also include certain conditions with diminished excretion of amino acids. The term "aminoaciduria" may also comprise compounds which are metabolically, but not chemically, related to amino acids, and hence not identified by the usual techniques for detection of amino acids; oxalosis and alcaptonuria would be included in this group.

A classification is given in Table 3, in which aminoaciduria is correlated with the cellular product presumed to be affected by the genetic lesion. The classification is only tentative, since precise studies of the altered product of the genetic lesion is lacking in most hereditary aminoacidurias. There have been several reports in which various hereditary aminoacidurias were discussed in relation to a specific clinical lesion (e.g., mental deficiency [Paine, 1960; Wright, Tarjan, Lippman and Perry, 1958; Zellweger, 1961]), or to the field of biochemical genetics in general (Gerrard, Marko, 1960; Harris, 1959a; Hsia, 1959; Hsia, 1960b; Stanbury, Wyngaarden, Fredrickson, 1960; Sutton and Tashian, 1960; Tashian, 1961b). Reproductions of amino acid chromatograms from several hereditary aminoacidurias have been recently published (Dedmon and Scriver, 1961).

TABLE 3

I. *Probable or proven apoenzyme defect*
  a) *Detectable prerenal accumulation of "substrate"*°
    1) Phenylketonuria
    2) a) Maple syrup urine disease
      b) (Smith and Strang's patient)
    3) Idiopathic hyperglycinemia
    4) Familial hyperhistidinemia
    5) Hypophosphatasia
    6) Familial Hyperprolinemia
    7) Tyrosinosis and related conditions
    8) Argininosuccinicaciduria
  b) *Prerenal accumulation of substrate usually not detectable*
    1) Cystathioninuria
    2) Beta-aminoisobutyricaciduria

II. *Specific disturbance of cellular amino acid transport; closely related to altered gene product*
    1) Cystinuria
    2) Hartnup Disease
    3) Familial Glycinuria

III. *Generalized Hyperaminoaciduria and other disturbances reflecting impaired cellular transport mechanisms; remote from altered gene product*
  a) *Precipitating cause apparent*
    1) Galactosemia
    2) Lactase deficiency?
    3) Vitamin D deficiency
  b) *Undetermined Etiology*
    1) The Fanconi syndrome and related conditions
    2) Oculo-cerebro-renal syndrome of Lowe and coauthors
    3) Wilson's Disease (copper toxicity?)

IV. *Miscellaneous hereditary aminoacidurias, including iminoacidurias*

° Exact relationship between compound which accumulates, and altered gene product, has not been clearly defined in the majority of instances. "Substrate" is therefore used descriptively and not in its strict chemical sense.

I a. Genetically Determined Aminoacidurias for which Affected Amino Acid Presumably Serves as Substrate for an Altered Cellular Enzyme System

1. Phenylketonuria. This disease occurs when the stable fraction II of phenylalanine hydroxylase is functionally abnormal (Mitoma, Auld and Udenfriend, 1957). Homozygous affected, heterozygous carrier and homozygous normal individuals have all been identified biochemically by indirect investigation of phenylalanine hydroxylase activity in-vivo, although

the distinction between heterozygotes and homozygous normals is not absolute. The clinical features of this disease have been documented in early infancy (Partington, 1961) and in older age groups (Paine, 1957) and there is no need to present further details when the superb discussion by Knox is available (Knox, 1960b). The appearance of excessive phenylalaninuria depends on accumulation of this amino acid in extracellular fluids to the point where the normal capacity for phenylalanine reabsorption in the renal tubule is exceeded (Evered, 1956).

Several aspects of the disease continue to be of interest with regard to diagnosis and detection of phenylketonuria. False negative and false positive tests with the ferric chloride reagent remain a hazard. False negative tests have been discussed earlier in section B, 3. False positive urine ferric chloride tests may occur in familial hyperhistidinemia (Ghadimi, Partington and Hunter, 1961), maple syrup urine disease (Mackenzie and Woolf, 1959), "oasthouse urine" syndrome (Smith and Strang, 1958), tyrosyluria and related conditions (Menkes and Jervis, 1961), and various drug excretions (Heffelfinger and LaGro, 1960).

Knox has drawn attention to the changes in plasma concentrations of amino acids other than phenylalanine in phenylketonuria (Knox, 1960b). Many other amino acids have depressed concentrations and further investigation of this problem is already in progress (Efron, 1962). Similar observations in other conditions with amino acid accumulation in extracellular fluids (Dancis, Levitz, Miller and Westall, 1959; Nyhan et al., 1961) bear remembering.

Patients with biochemical changes of phenylketonuria, but with normal average mentality are being detected (Allen and Gibson, 1961; Knox, 1960b; Sutherland, Berry and Shirkey, 1960). The reason for severe mental retardation in the majority of patients still remains the most important question to be answered in phenylketonuria. Low plasma serotonin concentrations (Pare, Sandler and Stacey, 1958) were thought to have significance, but Knox (1960b) has pointed out the difficulty in interpreting these observations. Furthermore, in rats no correlation was found between lowered brain serotonin concentrations induced by phenylalanine feedings, and performance or behavior (Yuwiler and Louttit, 1961). Other disturbances of tryptophan metabolism, affecting excretion of indolic constituents have been studied (cited in Knox, 1960b; Bessman and Tada, 1961; Tada and Bessman, 1960). Investigations with respect to impairment of aromatic amino acid metabolism by phenylpyruvic substrate inhibition have been reviewed (Sutton and Tashian, 1960).

There is potential importance in recent in vitro studies by Tashian (1961a), demonstrating phenylpyruvate substrate inhibition of glutamic acid decarboxylase (GAD) activity; unfortunately, the experiments were

carried out with substrate concentrations far above those encountered in phenylketonuria; it would be important to repeat the experiments with substrate concentrations comparable to those which occur in vivo. GAD is a key enzyme in the γ-aminobutyric acid pathway in brain, which is considered by some workers to have a significant role in cerebral oxidative metabolism (McKhann, Albers, Sokoloff, Mickelson and Tower, 1960). Tashian's observations (1961a), if found to be valid with equivalent in vivo concentrations of phenylpyruvic substrate may explain the apparent decrease in cerebral oxygen consumption noted in phenylketonuric patients (Himwich and Fazekas, 1940).

An excess in phenylalanine concentration has been observed to inhibit amino acid transport selectively, in brain (Neame, 1961). Further study with experimental phenylketonuria in primates (Waisman, Wang, Harlow and Sponholz, 1959; Waisman, Wang, Palmer and Harlow, 1960) and in rats (Goldstein, 1961) may have value in determining the derangement of cerebral metabolism in this disease.

The genetics of phenylketonuria is precisely known and has been discussed by Jervis (1954), Wright and Tarjan (1957) and reviewed by Knox (1960b). A rare single autosomal recessive gene is responsible for the disease. The homozygous state occurs approximately once in 25,000 births in the North American and European populations. Frequency rates of the disease in various matings have been calculated (Wright and Tarjan, 1957). Linkage studies, done mainly with blood groups, have so far been negative (Hsia and Steinberg, 1960; Renwick, Lawler and Cowie, 1960).

The importance of defining the heterozygote carrier state in affected pedigrees has led to the development of several methods for identification of these individuals. Overlapping of discriminant values for normal homozygotes and phenylketonuric heterozygotes obtained with most available tests has resulted in at least 9 per cent uncertainty in the identification of an individual's genotype. A recent publication (Wang, Morton and Waisman, 1961) has suggested a suitable phenylalanine loading test and mathematical handling of the data for reduction of the classification error to less than 4 per cent.

2 a. Maple Syrup Urine Disease (MSUD). This disease was first described by Menkes and co-workers in 1954 (Menkes, Hurst and Craig, 1954). Since then several new patients have been described or reported to Menkes and the author (Dancis, Levitz and Westall, 1960; Dancis et al., 1959; Dent and Westall, 1961; Gairdner, 1961; Lane, 1961; Mackenzie and Woolf, 1959; Menkes, 1960; Menkes et al., 1954; Morris, Lewis, Doolan and Harper, 1961; Patrick, 1961; Westall, pers. comm.). At least 20 patients in 8 families from widely scattered localities are now known and no relationship between the different families is apparent. Four reports are of particular interest (Crome,

Dutton and Ross, 1961; Dent and Westall, 1961; Mackenzie and Woolf, 1959; Patrick, 1961), because they describe the details of continued observations, investigations and necropsy in a single patient.

Menkes (1960) has described the usual clinical features in the MSUD patient. These include onset of symptoms in the first week of life, fluctuating central nervous system symptoms, usually with opisthotonus and decerebrate rigidity, convulsions and mental retardation. A maple syrup odor is noted from the patient and the urine within a few days after birth. Severely affected infants usually die early in infancy. However, less severely affected patients have been observed in whom mental retardation is the principal symptom; delayed onset of the clinical state and intermittent ataxia occurred in one patient (Morris et al., 1961).

A variation of the common clinical picture has been observed in 5 patients (Gairdner, 1961; Lane, 1961) in whom extreme hypotonia, refusal to feed, apparent respiratory distress and seizures occurred following birth, with death occurring within the first two weeks.

Greatly elevated concentrations of the amino acids, valine, leucine and isoleucine, and of "methionine" are found in plasma; several other amino acids, particularly alanine and cystine, are present in reduced amounts (Dancis et al., 1960; Dancis et al., 1959). Accumulation of the branch-chain amino acids, and "methionine," also occurs in urine, saliva and cerebrospinal fluid (Dent and Westall, 1961; Mackenzie and Woolf, 1959). The abnormal "methionine" peak on ion exchange column chromatograms produced by one method (Moore et al., 1958; Spackman et al., 1958) has been shown by another method (Piez and Morris, 1960) probably to be allo-isoleucine (Norton, Roitman, Snyderman and Holt, 1962); methionine concentration is, in fact, normal.

Chromatography of 2, 4-dinitrophenylydrazine derivatives indicates that abnormal amounts of leucine, isoleucine and valine α-keto acids are present in urine and plasma (Dancis et al., 1960; Dent and Westall, 1961; Menkes, 1959a; Menkes, 1960; Patrick, 1961). Confirmatory identification of these compounds has been obtained by other methods (Dancis et al., 1960; Menkes, 1959b), including identification of the equivalent α-hydroxy acids. Cerebrospinal fluid also shows accumulation of keto acids (Dent and Westall, 1961). Detailed investigation of the organic and amino acid patterns in HSUD have been reported by Menkes (1959b).

The abnormal accumulation of α-keto and α-hydroxy acids, in addition to leucine, isoleucine, and valine, suggested to various workers (Dancis et al., 1960; Dancis et al., 1959; Dent and Westall, 1961; Mackenzie and Woolf, 1959) that impairment of appropriate α-keto acid decarboxylase activity might be the biochemical lesion in MSUD. Attempts to investigate branched-chain amino acid transaminase activity in necropsy material

(Dancis et al., 1960; Patrick, 1961) revealed that transaminase activity is present, but that this approach is unsatisfactory for α-keto acid decarboxylase determination because the enzyme is unstable in post mortem tissue. Branched-chain keto acid decarboxylase is a complicated enzyme system requiring several co-factors (Dancis and Levitz, 1960); Dancis and co-workers (Dancis, Hutzler and Levitz, 1960) have studied the system in vivo, with a preparation of white blood cells from an MSUD patient. They believe that a single enzyme, α-keto isocaproic acid decarboxylase, is impaired in this disease and that this enzyme is shared by the three different α-keto acid substrates, thereby accounting for accumulation of three branched-chain keto acids and amino acids. The actual enzymatic impairment is not related to altered cofactor activity (Dent and Westall, 1961).

Other metabolic disturbances in MSUD include hypoglycemia in a few patients (Mackenzie and Woolf, 1959), which may be severe enough to be associated with seizures (Menkes, 1960). These observations appear to be related to the observations made by Cochrane and co-workers (Cochrane, 1960; Cochrane, Payne, Simpkiss and Woolf, 1956) on hypoglycemia induced by leucine and α-keto isocaproic acid. Excessive urinary excretion of indolic compounds has also been observed in MSUD (Mackenzie and Woolf, 1959).

The pathogenesis of the cerebral lesion is unknown. Post mortem studies (Crome et al., 1961; Dancis et al., 1960; Menkes et al., 1954) reveal marked cerebral edema. Defective myelinization, gliosis and status spongiosus are also seen if the patient has survived long enough. Menkes (1959b) has referred to possible impairment in the synthesis of cerebronic acids. Tashian's observation (Tashian, 1961a) on the inhibition of glutamic acid decarboxylase, by high concentrations of α-keto isocaproic acid in vitro, may be significant, particularly if reproducible with substrate concentrations which are likely to occur in vivo. Patrick (1961) described apparent inhibition of oxidative decarboxylative activity in the citric acid cycle in MSUD necropsy tissue. The observation was only partially supported by the in vivo keto-acid data obtained by Westall (Dent and Westall, 1961).

Dietary therapy for MSUD has been attempted using low leucine-isoleucine-valine diets. Correction of the biochemical disorder, including a decrease in "methionine" concentration and increase in cystine levels, was observed in one patient (Dent and Westall, 1961). No change in "methionine" concentration was noted in another patient, although branched-chain amino acid concentrations were lowered (Holt, Snyderman, Dancis and Norton, 1960; Snyderman, Dancis, Norton and Holt, 1960). When started early enough, dietary therapy resulted in normal clinical development in one patient (Snyderman et al., 1960). Dietary depletion of one branched-chain amino acid only (leucine) did not have a biochemical

effect equivalent to depletion of all three (Dent and Westall, 1961), and in fact, the results were an exaggerated replica of the response observed in normal infants (Snyderman, Roitman, Boyer and Holt, 1961). When leucine is omitted from the diet of a normal infant (Snyderman et al., 1961), valine, isoleucine, "methionine," and other amino acids accumulate in plasma and red blood cells. The interpretation of these metabolic interrelationships is not clear, but the need for leucine substrate induction to achieve maximum activity of a common decarboxylase is an attractive possibility. Cysteine feeding and lipoic acid cofactor administration did not improve the biochemical derangement in MSUD (Dent and Westall, 1961).

The genetics of MSUD is not well defined; however, a propositus may have both normal and affected sibs; no sex predominance is noted; affected members in relatives other than sibs have not been positively identified. Reports mentioning parental consanguinity do not describe its occurrence in this disease. Menkes studied organic acid excretion in parents of MSUD patients (Menkes, 1959b) without detection of abnormal excretion patterns. Loading studies with L-leucine have not been carried out in an effort to describe the potential heterozygote state in MSUD. The in vivo investigations by Dancis and co-workers (Dancis et al., 1960) suggest that the disease could be attributed to a single enzymatic defect, which in conjunction with the other observations, is compatible with an autosomal recessive inheritance.

b. Smith and Strang's Patient. Several features in the clinical behavior, odor, biochemistry and pathology of this patient (Smith and Strang, 1958) could be confused with maple syrup urine disease and phenylketonuria. However, biochemical investigation (Jepson, Smith and Strang, 1958) revealed distinctive characteristics of amino acid and organic excretion. Accumulation of methionine, the leucines, phenylalanine and tyrosine was observed in urine; blood levels were not reported. Although phenylpyruvic acid excretion was responsible for the positive ferric chloride reaction, there was also excessive excretion of p-hydroxyphenylpyruvic and lactic acids and indolyl-lactic acid in the urine. Excretion of phenylacetic acid, but not of indolylacetic acid, was increased. Ortho-hydroxyphenylacetic acid excretion was normal. Interpretation of the available data favored selective impairment of decarboxylation of various keto acids (Jepson, Smith and Strang, 1958). Death of the patient prevented further investigation.

3. Idiopathic hyperglycinemia. Childs and co-workers (Childs et al., 1961; Jepson et al., 1958) have described a single child with a newly recognized disorder of amino acid metabolism. It is not proven at present that this is

a genetically determined disorder, or that it can be attributed to a single apoenzyme defect. However, the biochemical features of the disease, the occurrence of persistent symptoms from birth, and the possibility that two previous miscarriages in the mother may have represented lethal expression of the disease in sibs, suggest that the patient's disease may have had a genetic origin.

Symptoms including vomiting, lethargy, ketosis and ketonuria, seizures and failure to thrive. The possibility of mental retardation could not be correlated positively with hyperglycinemia. Biochemical and other findings included neutropenia, intermittently abnormal electroencephalograms, hypoglobulinemia and osteoporosis. Pronounced hyperglycinemia and glycinuria were consistently detected; less marked elevation of other plasma amino acid concentrations was also apparent, but only glycine was in excess in the urine. It was clearly demonstrated that all symptoms correlated with protein intake, and not with plasma glycine concentrations. Lowering of glycine concentrations with benzoate therapy had no beneficial effect, while whole protein hydrolyzate intake remained above 0.5 mg./Kg./day. It was determined that of the protein intake, the leucine component in particular could precipitate symptoms; hypoglycemia did not occur.

The mechanism of hyperglycinemia was investigated but no answer was obtained. Glycine appeared to have access to all its metabolic pathways. Restriction of protein intake was followed by lowering of glycine levels to near-normal range. Glycine tolerance tests, carried out in the patient's parents, were normal. Protein loading studies were not done.

The correlation of clinical and metabolic abnormalities in this patient with protein intake suggested, not a primary disturbance of glycine metabolism, but some other disturbance for which glycine accumulation was a secondary phenomenon.

4. Familial hyperhistidinemia. Ghadimi and co-workers (Ghadimi et al., 1961; Ghadimi, Partington, Hunter and Bailey, 1961) have described a family in which two siblings have an abnormality of histidine metabolism. No significant or consistent clinical findings were associated with the metabolic lesion in their patients. Berlow (discussion, Ghadimi et al., 1961), however, mentions a third unrelated patient in whom mental retardation was present. Detection of this disorder came from a routine ferric chloride test on the urine; the initial green reaction changed within a few minutes to a stable dark brown. Amino acid chromatography of urine, plasma and cerebral spinal fluid demonstrated greatly elevated histidine concentrations and urinary alanine concentrations were also moderately increased. The nature of the metabolic lesion is unknown, but it is postu-

lated that there is a disturbance of histidine α-deaminase or urocanase activity. Whether this is a permanent modification of histidine metabolism or a delayed developmental phenomenon is not apparent.

Familial histidinemia is important to recognize so that it is not mistaken for phenylketonuria, especially if mental retardation is present.

5. *Hypophosphatasia* (*phosphoethanolaminuria*). Subsequent to the first report by Rathburn (1948) of this predominantly childhood disease, many patients have been recognized or recalled. Reviews of the subject were written in 1957 (Currarino, Neuhauser, Reyersbach and Sobel, 1957; Fraser, 1957) and 1960 (Bartter, 1960). A recent paper described two middle-aged sisters with the mild "adult" form of the disease (Bethune and Dent, 1960). In all patients, disturbance of bone formation was the principal clinical manifestation. Neurological manifestations, secondary to involvment of the skull, may occur in severely affected infants.

The identification of a ninhydrin-reacting compound present in excess in the urine of patients with hypophosphatasia was reported simultaneously by McCance and co-workers (McCance, Morrison and Dent, 1955) and Fraser and associates (Fraser, Yendt and Christie, 1955) in 1955; this compound was phosphoethanolamine. Shortly thereafter, elevated plasma phosphoethanolamine was described in the disease by Fraser and Yendt (1955). Later studies by Cusworth and Dent (1960a) confirmed this observation, showing also that urinary excretion of phosphoethanolamine is influenced by its poor renal tubular reabsorption. Urine is therefore a more suitable fluid than plasma for study of changes in phosphoethanolamine metabolism.

The relation of hypophosphatasia to phosphoethanolaminuria is unknown. Serum "alkaline phosphatase" is a heterogeneous mixture (Boyer, 1961; Schlamowitz, 1958) with components derived from many different tissues. Deficient alkaline phosphatase activity is found in bone and other tissues (Fraser, 1957; Nakai, Landing, Pettit and Rathburn, 1948; Rosenthal, Bonting, Hogen and Pirani, 1960) and white cells (Kretchmer, Stone and Bauer, 1958) in most patients with serum hypophosphatasia; however, in a few patients this finding is not always apparent in nonosseous tissues (Scaglioni and Lucey, 1956). Also, although phosphoethanolamine accumulation occurs in plasma and urine in conjunction with hypophosphatasia, this does not occur in all fluids (e.g., saliva [Rose and Kerr, 1958]) in which the compound is a normal constituent. Furthermore, hyperphosphoethanolaminuria is not specific for hypophosphatasia, since it occurs in liver disease and in conditions associated with low alkaline phosphatase activity, other than hereditary hypophosphatasia; phosphoethanolaminuria has even been identified with *hyper*phosphatasia, occurring in a newly described neuromuscular disorder (Roberts, 1960).

The disease is clearly familial and its pattern of distribution is that expected if it were caused by the homozygous state of a rare abnormal gene (Currarino et al., 1957; Fraser, 1957). Serum alkaline phosphatase has not proven to be a reliable parameter for detection of the heterozygous state (Currarino et al., 1957; Fraser, 1957; Kretchmer et al., 1958). Harris and Robson (1959) investigated instead the genetic significance of phosphoethanolamine excretion. Their chromatographic investigations of 381 relatives of sixteen patients with hypophosphatasia indicated that the heterozygote could be identified. The method, however, was only sensitive enough to detect 58 per cent of the potential heterozygote population. There was a positive sib-sib correlation for both heterozygotes (Harris and Robson, 1959) and affected homozygotes (Currarino et al., 1957), whereas parent-child correlation was absent, for expression of the heterozygote state (Harris and Robson, 1959), suggesting that the genetic control of the disease and phosphoethanolamine excretion involves multiple alleles, both mutant and normal at the locus under consideration, or modification by other factors, constitutional or environmental.

6. *Familial hyperprolinemia.* This biochemical disorder was observed in four siblings (Schafer, Scriver and Efron, 1961; Scriver et al., 1961b) who were part of a large pedigree in which 102 members were identified. The pedigree was unusual because it appeared to contain four heritable disorders including dominantly inherited familial nephropathy and deafness of the type recently reviewed by Cohen and co-workers (Cohen, Cassady and Hanna, 1961), photogenic epilepsy showing dominant inheritance, renal hypoplasia (inheritance unproven as yet) and hyperprolinemia apparently recessively inherited. The pedigree data suggested, however, that there was no direct correlation between the hyperprolinemia and the somatic and functional disorders. This was substantiated by the failure to detect hyperprolinemia in patients or relatives in cases with either familial nephropathy and deafness or photogenic epilepsy. The phenotypic manifestation of familial hyperprolinemia is therefore not apparent.

The mechanism of hyperprolinemia was investigated but remains unknown. Elevated concentrations of L-proline were detected in plasma and urine but not in cerebrospinal fluid. Plasma concentration of the related imino acid, hydroxy-L-proline, was not elevated. No accumulation of intermediates in L-proline degradation or alteration in concentration of amino acids (Fig. 4: ornithine and glutamic acid) intimately related with L-proline metabolism was observed. Intravenous L-proline tolerance tests in one affected sib, the mother, and several normal controls produced comparable results in all subjects. Fluctuations in hyperprolinemia in affected sibs appeared to be related to variation in protein intake. Initially, a defect in proline dehydrogenase activity seemed plausible, but present interpreta-

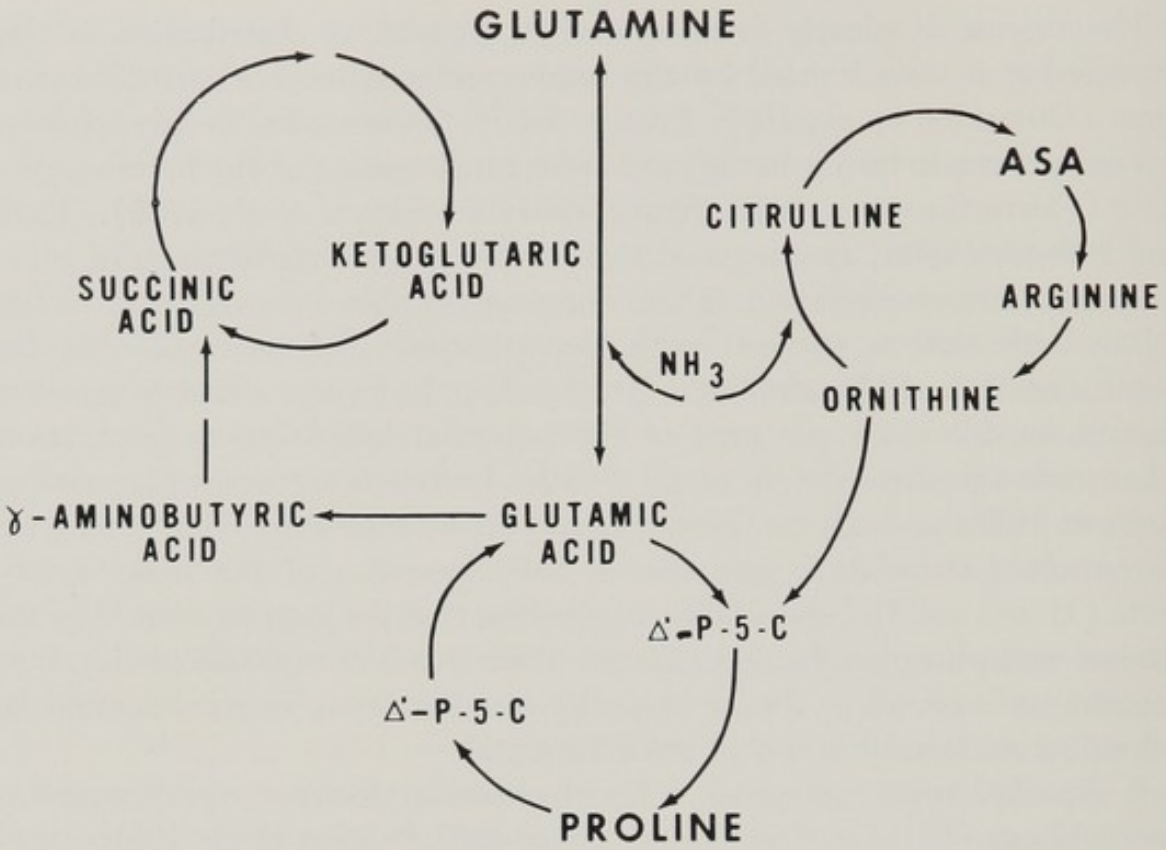


FIG. 4—Hypothetical scheme of interrelations apparently existing between metabolism of proline, arginine, and glutamic acid in brain. Data for proline cycle from Sporn et al. (1959), Strecker (1960), and Tallan et al. 1954; urea cycle from Ratner (1954) and Sporn et al. (1959); glutamic acid and γ-aminobutyric acid from Roberts (1960), and McKhann et al. (1960).

tion of the data does not favor impaired L-proline metabolism at any of the known enzymatic stages of its metabolism. However, human L-proline metabolism is relatively unstudied, and since species differences exist in its metabolism, extrapolation from other mammalian studies may be misleading. The data in familial hyperprolinemia raises a problem analogous to that faced by Childs and co-workers (Childs et al., 1961) in interpreting idiopathic hypoglycemia.

An additional observation was made in the hyperprolinemic subjects. Figure 5 presents a portion of ion exchange column amino acid chromatograms of plasma and urine samples collected simultaneously from a patient. Whereas only L-proline concentration was elevated in plasma, the urinary excretion rates of L-proline, hydroxy-L-proline and glycine were all elevated; other amino acids were not affected. Endogenous amino acid renal clearance data (Scriver et al., 1961b) indicated that prolinuria was related to the high plasma level, but that hydroxyprolinuria and glycinuria were present because of decreased renal tubular reabsorption. There was some correlation between degree of prolinemia and aminoaciduria. These

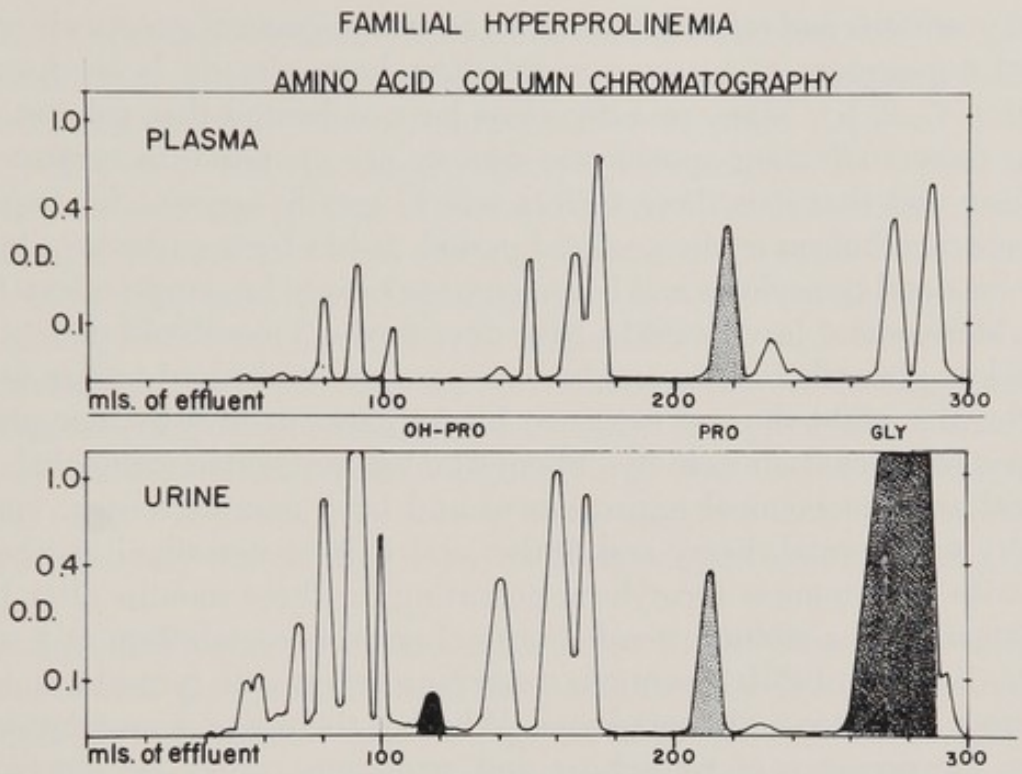


FIG. 5—An example of combined prerenal and renal aminoaciduria (Scriver et al., 1961b). Excessive proline (shaded peak in plasma chromatogram) in glomerular filtrate believed to compete for tubular transport with hydroxyproline and glycine; all three amino acids in excess in urine (three shaded peaks in urine chromatogram). Method: Moore and Stein (1954a).

observations suggest that the two imino acids share a common transport system and either that glycine transport is also shared at some point in this system, or that the imino acids can compete in a separate glycine transport system. Infusion studies with each of the three amino acids given separately to normal subjects, support these interpretations (Scriver et al., 1961a). The independence of imino acid and glycine transport from transport of other amino acids in the renal tubule, is also evident from observations of aminoaciduria in Hartnup disease (see section E, II). A common transport system in the rat intestine, selective for imino acids, sarcosine, betaine and N-dimethylglycine has apparently been demonstrated by Lin and co-workers (Lin, 1961). Final proof for shared transport in this or any system must always rest on calculation of kinetic equations for the individual compounds, under different conditions of competition for transport.

Familial hyperprolinemia has therefore provoked many questions and only a few answers. Its phenotype (other than hyperprolinemia) remains to be identified; its mechanism and identification of the heterozygote requires further investigation; however, it has served to document renal tubular transport behavior of certain amino acids.

7. *Tyrosinosis and related conditions.* Several aspects of genetically determined impairment of tyrosine metabolism have already been discussed (section C, 2, b). Many investigations have indicated that genetic regulating factors affecting apoenzyme activity are operative in tyrosine metabolism and that it is these factors which usually account for impaired tyrosine metabolism in the neonatal period. It has been customary to consider neonatal tyrosyluria and hydroxyphenyluria to be symptomless. However, Menkes and Jervis (1961) have described a 3 month old patient who mimicked phenylketonuria and had seizures, spasticity and failure to gain weight; this child showed evidence for impairment of p-hydroxyphenylpyruvic acid oxidase activity. Phenylalanine restriction coincided with clinical and biochemical improvement and by 8 months of age, enzyme activity was normal. Berry and Sutherland (1960) described another infant with symptomless tyrosyluria persisting for three months after birth; investigation of a sibling revealed normal tyrosine metabolism at 4 weeks of age. Tashian (1961b) mentions a newborn infant with tyrosyluria, hypoglycemia, jaundice and convulsions. Whether there was a correlation between the presence of tyrosyluria and symptoms cannot be determined from the report. Speculation might be raised about the coexistence of hydroxyphenyluria and tyrosyluria with impairment of hepatic function as noted in a patient with galactosemia (Tashian, 1961b). A similar finding was observed in another infant who died with hepatoma (Sakai and Kitagawa, 1957a; Sakai and Kitagawa, 1957b); in this latter patient, despite the authors' claim, the biochemical findings were not identical with those described in the original patient with tyrosinosis (Medes, 1942).

The patient with tyrosinosis, carefully studied by Medes (1942), was believed to have impairment of p-hydroxyphenylpyruvic acid oxidase in adult life. There were no other family members reported. The disease has never been observed again as Medes described it, despite a specific search amongst 20,000 patients with urinary reducing substances (Blatherwick, 1934). LaDu (1960a) has reconsidered Medes' data and postulated that the patient had, in fact, impairment of tyrosine transamination.

It is apparent that these are disorders more often detected by urinary excretion of a metabolite related to amino acid metabolism but not itself detectable by amino acid techniques. Aminoaciduria may, however, also be apparent. Alcaptonuria, another genetically determined disease affecting metabolism of the tyrosine pathway (Knox, 1958b; LaDu, 1960b) is also usually not detected as an aminoaciduria. Holt has suggested that this disease is a "masked aminoaciduria" (Holt and Snyderman, 1960; Snyderman and Holt, 1960). Aminoaciduria per se affecting the aromatic amino acids has, however, been reported in alcaptonuria (Bigwood et al., 1959).

8. *Argininosuccinicaciduria.* The first report of this condition (at that time

unnamed) appeared in 1958 (Allan, Cusworth, Dent and Wilson, 1958). Two siblings in an otherwise normal pedigree were investigated because of mental retardation. Chromatographic investigation detected a large amount of a ninhydrin-reacting compound in urine from both patients; a large amount was also present in cerebral spinal fluid and smaller amounts in plasma. Cusworth and Dent (1960a) determined quantitative relationships of the compound using ion exchange column chromatography; cerebral spinal fluid concentration was approximately three times that in plasma and renal clearance was very high, accounting for the large urinary excess of the compound. In addition, they noted that glycine in plasma was elevated whereas valine, lysine, arginine and ornithine were decreased. Westall (1960) identified the substance as argininosuccinic acid (ASA), and subsequently the disease was called argininosuccinicaciduria (Dent, 1959).

The clinical features are of unusual interest. Unlike other aminoaciduria associated with mental deficiency, the patients' early motor and physical development were normal and nothing was suspected until language function failed to appear normally. When mental assessment was performed in the third and sixth year of life respectively, both siblings were found to be severely mentally retarded (Allan et al., 1958). Unusually friable hair appears to be a hallmark of the disease; however, the hair did not become abnormal until approximately the second year of life and later improved as the children became older. The hair lesion has been histologically identified as "trichorrhexis nodosa" (Dent, 1961b). Electroencephalograms were abnormal, and in one patient seizures had occurred infrequently. Both patients had systolic murmurs audible at the left sternal border, the significance of which remains unclarified.

Levin and associates (Levin, MacKay and Oberholzer, 1961) have just reported the third sib in the original family; argininosuccinicaciduria was present shortly after birth.

Argininosuccinic acid (ASA) is an intermediate in the ornithine-urea cycle (Ratner, 1954) in liver and kidney. It is split by the enzyme argininosuccinase to form arginine and fumaric acid. To the surprise of many, this enzyme has also been identified and its activity studied in mammalian brain (Tomlinson and Westall, 1960); this followed the valuable work by Sporn and co-workers (Sporn, Dingham, Defalco and Davies, 1959) who demonstrated that rat brain can synthesize urea in vivo. It is of interest, then, that in patients with ASA'uria, blood and urine urea concentrations were normal (Allan et al., 1958; Dent, 1959; Westall, 1960). Urea excretion responded to dietary protein manipulation. ASA excretion increased after feeding with ornithine, and with citrulline, but not with arginine.

Baldwin (1959) has discussed the possibility that the ornithine-urea

cycle has significance in arginine synthesis. This is a provocative thought because it has been noted that arginine plus methionine deficiency in mink is associated with friable hair, which impairs the quality of the fur (Leoschke and Elvehjem, 1959). Although arginine is not essential for growth (Snyderman, Boyer and Holt, 1959), relative arginine deficiency secondary to its retention in the ASA complex might account for some of the manifestations in human ASA'uria (1961b). However, preliminary investigation with arginine feeding and intravenous infusion (Dent, 1961a; Dent, 1961b) produced neither clinical nor electroencephalographic improvement in the oldest sib. Arginine supplementation by mouth improved the skin condition in the new patient (Levin et al., 1961) and depletion worsened clinical manifestations.

It is therefore difficult to explain why such a profound disturbance in the ornithine-urea cycle, resulting in the excretion of nearly 3 Gm. of ASA daily, has little effect on systemic urea metabolism, and yet is associated with impaired cerebral function and ASA accumulation in cerebrospinal fluid. Allan and co-workers (1958) originally suggested that the biochemical lesion was limited to brain in this disease. Argininosuccinase has now been identified in brain (Tomlinson and Westall, 1960). If this is the enzyme actually affected in the disease, then brain argininosuccinase must be under different genetic control with different properties from argininosuccinase in other tissues. Observations on chemical, immunological (Henion and Sutherland, 1957) and genetic (Schmid and Hammaker, 1961) differences between muscle and hepatic phosphorylases offer a suitable analogy for such a situation; these considerations are compatible with current thoughts on molecular heterogeneity of enzymes with similar substrate in different tissues (Gregory, 1961).

Certain metabolic "pathways" have been demonstrated or implied to function in mammalian brain (McKhann, Albers, Sokoloff, Mickelson and Tower, 1960; Sporn, Dingman and Defalco, 1959; Sporn et al., 1959; Tallan, Moore and Stein, 1954; Tomlinson and Westall, 1960). The possible interrelations which may exist between the ornithine-urea cycle and other aspects of brain metabolism are presented in Figure 4; this interlocked scheme is only hypothetical and gives no consideration to cellular and subcellular compartmentalization of the reactions in brain tissue.

I b. Presumed Apoenzyme Defect; Plasma Accumulation of Substrate not Detectable

1. Cystathioninuria. This is another disease (Harris, Penrose and Thomas 1959) for which neither genetic origin or apoenzyme disturbance has been rigidly proven. It is very likely that a genetically determined apoenzyme

impairment existed in the original patient, because urinary cystathionine excretion was detected in relatives.

Elevated blood levels of this intermediate in sulfur-containing amino acid metabolism were not detected in the patient; elevated urinary cystathionine excretion was presumed to reflect its rapid renal clearance, secondary to poor renal tubular reabsorption. It seems reasonable that a primary apoenzyme defect can account for cystathioninuria. Coenzyme deficiency (pyridoxal-5-phosphate) may affect cystathionine-cleavage apoenzyme activity; cystathioninuria has been observed both in the rat (Hope, 1957) and in a human infant (Scriver et al., 1961) in the presence of vitamin B_6 deficiency. Coenzyme requirements were not investigated in the cystathioninuria family.

The patient described by Harris and co-workers had mental deficiency. It was uncertain whether this was coincidental with, or the result of, the metabolic lesion. The latter is a possibility; Tallan et al. (1958) have demonstrated significant concentrations of cystathionine in human brain, and it is also detectable in cerebrospinal fluid (Perry and Jones, 1961). Its role in cerebral metabolism is unknown.

2. *-aminoisobutyricaciduria.* L-β-aminoisobutyric acid (α-methyl β-alanine) or BAIB was isolated from normal human urine in 1951 (Crumpler, Dent, Harris and Westall, 1951; Fink, Henderson and Fink, 1951). Subsequent investigations of "high-excretors" and "low-excretors" in human and animal populations served to illustrate many of the problems raised by methodology and nongenetic influences when genetic interpretation is sought to explain urinary excretion of a compound. The thoughtful review by Sutton (1960) is recommended for a clear presentation of the illustrative studies on BAIB excretion.

The principal source of BAIB in human urine remains unknown. Fink and co-workers (1951) were the first to suggest that urinary BAIB was derived from DNA–thymine. Gartler's studies with thymine loading (1959a; 1959b) and more recent investigations with tritium-labelled thymine (Gerber, Gerber and Altman, 1960b) support the conclusion that thymine can be the source of urinary BAIB. Other workers (Kupiecki and Coon, 1957) have shown evidence that valine catabolism in animals can also produce BAIB. The common link between thymine and valine pathways is transamination with α-ketoglutarate, between BAIB and methylmalonate semialdehyde; the latter can also be metabolized to propionic acid. Valine loading in man (Gartler, 1959b), with small doses (500 mg.), did not increase BAIB excretion and Gartler assumed from this, that the transaminase was not active in human tissues. Valine infusion with larger doses (43.5 mMoles [Schafer, Scriver and Efron, 1961]) into a low-excretor adult male, did not produce a significant increase in BAIB excre-

tion. BAIB feeding to low and high excretors (Gartler, 1959a) provided evidence that in the latter the biochemical disturbance followed and did not precede the formation of BAIB in its catabolic sequences. Valine infusion in high excretors, or double tracer studies with valine and thymine differently labelled should identify the main source of urinary BAIB. Only then can one deduce whether a block in BAIB-glutamic acid transamination, or a BAIB "shunt," secondary to impaired propionic acid formation occurs in high excretors.

Because BAIB is not usually detectable in normal body fluids other than urine, there has been interest in the physiological mechanism of its excretion in low and high excretors. BAIB can be formed in the kidney but it is believed that this organ is not the sole source of urinary BAIB. Therefore it is implied that blood flow through the kidney supplies BAIB to the urine; when glomerular filtration rate is greatly diminished, BAIB is detectable in plasma (Cusworth and Dent, 1960a). Renal clearances of BAIB usually cannot be determined accurately in the normal subject, nor in high BAIB excretors. However, the endogenous clearance rate in one high excretor (Evered, 1956) and clearance rates in high excretors whose plasma BAIB level was artificially elevated (Gartler, 1959a) were found to approximate the glomerular filtration rate. The relative absence of renal tubular reabsorption suggests that genetically determined alteration of BAIB transport cannot explain high BAIB excretion rates.

Transport behavior of BAIB raises some questions about its steric and stereospecific properties and their relation to reactive sites. Christensen (1960) has discussed the lower affinity for transport conferred by the β-position of the amino group. Balenovic (1958) has discussed stereospecificity of β-amino acids in general. Human urinary BAIB is present as the L-stereoisomer ((−)α-methyl-β-alanine) (Crumpler et al., 1951), which is in keeping with its being a product of in vivo valine or thymine catabolism. It might be considered that L-BAIB could have anomalous transport behavior and be secreted by the renal tubule, but this is unlikely. Recent experiments in the rat (Gilbert et al., 1960) suggest that mediated transport for BAIB reabsorption may exist in which the reactive site has varying affinity for BAIB and its analogues, β-alanine and β-amino-n-butyric acid, and for taurine. Renal clearance values would indicate that the supposed reactive site has extremely low affinity for BAIB. It is therefore likely that high BAIB excretion is dependent principally on impairment of endogenous BAIB or methyl malonate semialdehyde metabolism. Not all high excretors are genetically determined, however, and a survey of the nongenetic causes of high BAIB excretion (Sutton, 1960) suggests quite strongly that the thymine pathway is the main source of urinary BAIB. Certain malignancies, particularly leukemia, nitrogen mustard therapy,

roentgen radiation, liver disease, tuberculosis and other conditions can all produce prominent BAIB excretion. Recently, reports implicated post-operative catabolism (Berry, 1960b) and thalassemia major with splenectomy (Hillcoat, 1960a), with high BAIB excretion. The effects of prolonged fasting were mentioned earlier (Robson and Rose, 1957; Sandler and Pare, 1954). The common factor to all these agents is probably release of DNA during tissue destruction.

This summary on BAIB excretion has been presented in a manner to suggest that high excretion probably reflects altered cellular metabolism at a single enzyme site. High excretors can be studied to determine whether the altered cell product affecting BAIB metabolism correlates with a single genotypic determinant, even though as yet, the actual enzyme site is not precisely identified.

Nongenetic causes of high BAIB excretion account for only a small fraction of cases. In addition, sex and dietary factors other than fasting have little effect; in general, age is also not a factor (Gartler, 1956), although young children and infants may have high BAIB excretion (Berry, 1960b; Gartler, 1956; Woolf and Norman, 1957). The 4.8 per cent prevalence of high BAIB excretors in the normal British population, estimated by Crumpler and co-workers (1951) would suggest a genetic determinant. Harris investigated this probability (1953) and by his criteria found a 9.8 per cent prevalence; the family distribution of high-excretor behavior was compatible with recessive inheritance. Other studies (cited in Sutton, 1960) indicated similar conclusions. Difficulty in confining BAIB excretion to a bimodal distribution pattern has cast some doubt that a single pair of alleles controls BAIB metabolism and excretion, and has suggested the possibility of more complex genetic interaction or environmental modification. The discrepancy, however, may be a feature of technical difficulties in quantitation, and choosing populations with low frequency of high excretors for study. When a high frequency population of BAIB excretors was investigated, and the data were handled suitably (Gartler, Firschein and Kraus, 1957), quite clear-cut bimodality was demonstrated. High and "very high" excretors have been identified according to recent reports (McEvoy-Bowe and Lugg, 1961) but the genetic or physiological interpretation of this finding is not clear.

Apparent polymorphism for the BAIB excretor genetic system encouraged world-wide investigation of its significance in human evolution and adaptation. Gartler and co-workers (Gartler, Firschein and Gidaspow, 1957) determined that high excretors were probably rare, if present at all, in great apes. On the other hand, the weight of evidence (Blumberg and Gartler, 1959 cited in Sutton, 1960) suggests that genetic polymorphism for BAIB excretion has an early human anthropological origin, and proba-

bly originated in Southeast Asia; recent work supports this hypothesis (McEvoy-Bowe and Lugg, 1961). The advantages conferred by the selective factors acting upon BAIB excretion have not been determined, although some intriguing speculations have been advanced (Gartler, Firschein and Gidaspow, 1957).

In conclusion, it appears that genetic control of BAIB excretion is an old system in human evolution. It is probable that it controls a cell product responsible for further metabolism of BAIB at the cellular level. Once formed, the compound apparently has little significance for the individual and is rapidly lost in the urine. Family studies suggest that BAIB excretion can be influenced by a single gene locus, with the high excretor allele being recessive; however, more complex factors, with non-genetic and perhaps genetic as well, may affect BAIB excretion.

II. Lesion Presumed to be in Renal Tubular Cellular Transport System: Specific Transport Lesion

Harris has suggested (1959b) that genetically determined abnormalities of cellular amino acid transport may occur. These conditions could mimic impaired intermediary metabolism of substrate and might occur as a more general phenomenon than in renal tubule cells alone. The following three diseases illustrate this concept.

1. Cystinuria. This is the oldest aminoaciduria known in medicine (Wallaston, 1810). The history of investigation on the disease, in man or animal, is a remarkable chronicle of observation, deduction, denial and intuition, and the essays by Knox (1958a; 1958b) on the evolution of knowledge about cystinuria make fascinating reading.

Only certain historical references can be quoted here, concerning those publications most influential in defining cystinuria. The paper by Garrod (1908) assigning cystinuria to the category of "inborn errors of metabolism" naturally takes precedence. Much later the report by Yeh and coworkers (Yeh, Frankl, Dunn, Parker, Hughes and György, 1947) describing lysine and arginine, as well as cystine, in the urine of cystinurics, and corroborated and extended by Stein (1951), who also found ornithine in cystinuric urine, brought perspective to former long neglected observations on the "undetermined nitrogen fraction" in urine (Alsberg and Folin, 1905), which was partly accounted for by lysine (Ackermann and Kutscher, 1911). Dent and Rose (1951), with partition chromatography, demonstrated that the quadruple aminoaciduria occurred in the absence of elevated blood concentrations for the affected amino acids; this led them to consider that cystinuria was a specific defect in renal tubular amino acid reabsorption. Robson and Rose (1957) thereafter documented the presence of a renal tubular transport system in normal subjects, shared by

cystine and lysine and ornithine and arginine. Recent studies with arginine infusion have corroborated these findings in man (quoted in Dent, 1961a). Cystine and lysine appear to have lower affinity for transport than the other two amino acids (Harris and Robson, 1957). Further investigations have been concerned with whether genetic factors control the transport system, and whether the latter occurs in cells other than in the renal tubule.

Reliable genetic investigation was not possible provided stone formation was the criteria for case detection, since stones rarely form with cystine excretion rates less than 250 mg./gm. creatinine (Harris, Mittwoch, Robson and Warren, 1955a). Paper chromatography improved case finding, but qualitative methods were still found to be inadequate for accurate definition of inheritance in cystinuria (Dent and Harris, 1951). Quantitative estimation of all four components of the aminoaciduria provided Harris and co-workers (1955b) with the opportunity to describe two modes of inheritance of cystinuria.

The more commonly encountered form exhibited clear-cut modality for abnormal and normal aminoaciduria. Parental consanguinity appeared frequently in the pedigrees, but parental aminoaciduria was completely normal. Positive sib-sib correlation for intensity of aminoaciduria was observed, but widespread variations in intensity of aminoaciduria were found amongst unrelated affected subjects. Segregation ratios suggested that this type of cystinuria was inherited as if affected individuals were homozygous for a rare recessive gene; a single dose of the gene, in the heterozygote, was insufficient to produce any abnormal degree of aminoaciduria. The variance between affected individuals and positive sib-sib correlation suggested that more than one gene might account for this "recessive cystinuria."

A second pedigree type was also found, less frequent but more complex than the first. Three phenotypes existed: one with cystine-lysine-ornithine-argininuria, one with cystine-lysinuria only, and one with normal aminoaciduria. Increased parental consanguinity was again apparent; offspring of all three types were observed from parents who were cystine-lysinurics. When aminoaciduria was expressed as lysine/arginine ratio, poor sib-sib correlation existed. This inheritance pattern was interpreted to represent expression of a gene which, when homozygous, produced gross aminoaciduria, and when heterozygous, produced cystine lysinuria. This form of cystinuria was called "incompletely recessive," and the heterozygote, the "semi-cystinuric" individual. The incidence of the heterozygote with chemically detectable cystinuria is about one in 250. The homozygous form for this disease therefore occurs about one in 250 thousand. The "recessive" homozygote occurs at least twice as often.

It is apparent that nomenclature relying on the terms "recessive" and "dominant" is inadequate when describing cystinuria. "Dominance" and "recessivity" were the subject of reappraisal in a recent article (Allison and Blumberg, 1958). Whether the two forms of cystinuria represent different alleles at a single locus, or alleles at different loci, is unknown, and the answer will depend on the results of matings where an individual known to be carrying the gene for one type of the disease marries a carrier of the other type.

The biochemistry and cellular transport of amino acids is of continuing interest in cystinuria. It is already apparent from the genetic data (Harris and Robson, 1957) and from infusion studies in normals (Dent, 1961a; Robson and Rose, 1957) that transport of cystine and "basic" amino acid occurs at two levels of affinity for the reactive site(s) on the cell surface. Whether it is the membrane reactive site, or some other locus within the cellular transport system which is under genetic control, is not apparent, nor is the genetic basis for two levels of transport affinity at present revealed. Specificity of this transport system seems apparent, not only for its substrates, but also in its chemical properties (Finch and Hird, 1960b). However, the investigations by Webber and co-workers imply that complete physiological independence may not exist (1961). Genetic independence is suggested by the investigations of Dent and Harris (1951) who studied cystine excretion in other aminoacidurias besides cystinuria, and by the selective sparing of the cystine-basic amino acid transport system in Hartnup Disease (see section E, II, 2). Cystinuria can also occur independently in other genetic disturbances (Berry, 1959a; Lehmann, 1960).

Attempts to demonstrate this transport system in other body cells (leucocytes) were at first unsuccessful (Becker and Green, 1958). However, clearcut evidence has been obtained for an active transport system for cystine, lysine, arginine and ornithine in animal intestine (Neil, 1959; Hagihira et al., 1961) and its presence is also implied in man (Milne et al., 1961). The genetic lesion affecting the renal tubule in cystinuria appears also to affect amino acid transport in intestinal mucosal absorptive cells (Milne et al., 1961).

Brigham and co-workers (1960) measured urinary cystine and cysteine quantitatively and demonstrated that cystine, but not cysteine, is affected by the transport lesion. Frimpter (1961) reinvestigated the significance of the unusual leucine peak noted earlier by Stein (1951). Using the newer ion-exchange column technique (Moore et al., 1958) and other appropriate methods, Frimpter isolated a compound which was identified as a disulfide of cysteine and L-homocysteine. The urinary excretion of this compound in cystinuria bore no relation to that of cystine and it was barely detectable

in cystine calculi. The disulfide was not found in other aminoacidurias in which cystine was a component; its origin at present is unknown.

Cystinuria has importance for the individual patient only if calculi are formed. The low solubility coefficient of cystine is responsible for this event and stone formation is maximum at night when urine is concentrated (Dent and Senior, 1955). Many approaches to therapy have been presented (cited in Knox, 1960a). Dent and Senior advocated the simple method of drinking large volumes of water night and day. Recent reports (Dent, 1961b; MacDougall, 1961) suggest that when the patient cooperates and adheres to night water drinking, further calculi formation stops and large calculi slowly dissolve.

2. *Hartnup disease.* In many ways the transport lesion in this disease is complementary to cystinuria and familial hyperprolinemia. The disorder was first described in 1956 (Baron, Dent, Harris, Hart and Jepson, 1956) in a paper with a most descriptive title. Observations over a 5 year period were recorded on the Hartnup family, containing 8 children born to first-cousin parents. Two sibs had histories of intermittent cerebellar ataxia and pellagra-like dermatitis; milder skin changes were also observed in two younger sibs. These 4 patients all had an identical, gross, but specific aminoaciduria. The other children and the parents were clinically normal and had no aminoaciduria. Further biochemical observations were made demonstrating, in particular, abnormal urinary excretion of indolic compounds.

Since the original report, several others have appeared; these are documented in the review by Jepson and Spiro (1960). It is apparent from these reports that whereas mental retardation was thought to be a feature of the syndrome in the index family, this relation is debatable. On the other hand, temporary psychiatric disturbance may occur frequently. In all, 13 patients have been described in 8 families, with parental consanguinity apparent in 3 families. This suggests that the disease may represent the homozygous state of a rare abnormal gene.

The heterogeneity of the clinical state ranging from few if any symptoms to serious neuropsychiatric manifestations makes diagnosis difficult. The differentiation from mental illness and pellagra is one of the primary reasons for recognizing the true diagnosis. Febrile illness, gastrointestinal disorder, sunlight and inadequate diet all have some poorly defined relation to precipitation of symptoms in Hartnup Disease.

Whereas clinical findings are inconstant, the specific aminoaciduria is the only constant lesion in the same or different patients. Other biochemical features are less constant, in particular, the urinary excretion of indolic compounds and related Ehrlich reactors. This latter feature initially fo-

cused attention on the possibility that Hartnup Disease was an abnormality of tryptophan metabolism; the pellagra-like symptoms were correlated with presumed endogenous nicotinic acid deficiency (Baron et al., 1956). Abnormalities of amino acid transport and intestinal bacterial metabolism were also considered in relation to the pathogenesis of Hartnup syndrome.

Jepson investigated the "indoluria" (Jepson, 1956); in addition to identifying many of the indolic constituents he noted that administration of oral chlortetracycline slightly altered the urinary indole pattern. Later Shaw and co-workers (1960) and Scriver and Shaw (in prep.) demonstrated that oral neomycin almost completely suppressed the unusual indole pattern within three or four days; fluctuations in "indoluria" were also observed in the untreated state. Shaw and co-workers (1961; 1960) recognized that many of the Hartnup urinary indolic compounds could have their origin in bacterial metabolism, and intestinal bacterial flora were believed to be the source of the urinary indolic constituents.

Studies were then made on Hartnup and normal urinary excretion of indole-3-acetic acid (IAA) (Milne, Crawford, Girão and Loughridge, 1960a), L-tyrosine (Milne et al., 1960b), indole-3-pyruvic acid (Scriver and Shaw, in prep.; Shaw et al., 1960) and D- and L-tryptophan (Milne et al., 1960b; Scriver and Shaw, in prep.; Shaw et al., 1960), following administration of these compounds by mouth; two-dimensional paper chromatographic techniques were used (with one exception; Milne et al., 1960b), to study the urinary excretion of these and other indolic compounds. Hartnup patients excreted exogenous (Milne et al., 1960a) and endogenous IAA (Scriver and Shaw, in prep.; Shaw et al., 1960) in the urine in a manner typical for excretion and diffusion of a weak organic acid. Indole-3-pyruvic acid was rapidly absorbed from the gut and normally metabolized (Scriver and Shaw, in prep.; Shaw et al., 1960), whereas the corresponding amino acid, L-tryptophan, was poorly absorbed and appeared to be retained in the intestinal luman, later being excreted in the feces in larger amounts than normal (Milne, Crawford, Girão and Loughridge, 1960b; Scriver and Shaw, in prep.; Shaw et al., 1960). Intraluminal tryptophan apparently was converted into indolic derivatives, which were absorbed and eventually excreted in the urine. Neomycin greatly suppressed abnormal indoluria following tryptophan loading (Milne et al., 1960b; Scriver and Shaw, in prep.). L- and D- enantiomorphs both appeared to be affected by the transport defect (Scriver and Shaw, in prep.; Shaw et al., 1960). Milne (1960b) believed that endogenous tryptophan metabolism was also impaired; this observation must be accepted with reservation, in view of the rather unsatisfactory unidimensional chromatographic method employed for kynurenine estimations upon which this opinion was based. It is more likely that observations on diminished excretion of kynurenine (Milne et al., 1960b) and

other products of endogenous intracellular tryptophan metabolism (Hersov and Rodnight, 1960) could be explained by a membrane transport lesion affecting substrate availability. Observations concerning intestinal absorption of L-tyrosine were unsatisfactory (Milne et al., 1960b).

In addition to its constancy, certain characteristic features of the aminoaciduria have also been observed (Baron et al., 1956; Cusworth and Dent, 1960a; Evered, 1956; Hersov and Rodnight, 1960; Milne et al., 1960b; Scriver and Shaw, in prep.). These include relatively low glycine excretion, striking absence of imino acids, minimal ornithine and arginine excretions and only slightly greater cystine and lysine excretions, in contrast to very large excretions of all other filtered plasma amino acids. Endogenous amino acid clearance investigations (Cusworth and Dent, 1960a; Evered, 1956) determined the presence of greatly impaired renal tubular reabsorption of the affected amino acids. Evidence for impairment of intestinal absorption of tryptophan encouraged a search for evidence of impaired transport of other amino acids (Scriver and Shaw, in prep.). Fecal amino acid analysis in a normal subject and in Hartnup Disease revealed remarkable similarity of the abnormal Hartnup fecal amino acid pattern to that in Hartnup urine. The effect of intestinal bacterial brei on amino acid excretion was examined with and without neomycin, with little effect observed. The method of analysis was admittedly crude, and some modification of a newly reported method for obtaining fecal fluid (Wrong, Morrison and Hurst, 1961) would be a valuable asset for future studies.

The most satisfactory interpretation of these data is that Hartnup Disease represents a genetically determined selective disorder of cellular amino acid transport in kidney tubule, intestinal absorptive mucosa and perhaps in other tissues (Anoymous, 1960; Milne, 1960b; Scriver et al., in prep.). Attempts to show that transport inhibition might be caused by acquired factors were unsuccessful (Weyers and Bickel, 1958). The transport lesion is specific and those amino acids which are relatively unaffected (cystine and "basics," imino acids, and probably glycine) are believed to be transported by separate cellular transport mechanisms (see sections E, I, a, 6 and E, II, 1). On the other hand, D- and L- amino acids affected by the Hartnup transport lesion share certain common features in affinities for cellular uptake (Finch and Hird, 1960b). Both D- and L-tryptophan enantiomorphs appeared to be affected by the lesion. The actual site in the cellular transport system, affected by the genetic lesion, has not been determined.

This interpretation provides a tentative explanation for other biochemical and clinical findings in Hartnup Disease. Diminished substrate availability for intracellular metabolism and product deficiency may interact with formation of toxic products derived from gut bacterial action on intra-

luminally accumulated amino acids. Tryptamine (Weissbach, King, Sjoerdsma and Udenfriend, 1959) is one possible product, whose pharmacological activity in conjunction with other potential metabolic lesions (Hersov and Rodnight, 1960; Oates and Sjoerdsma, 1960) might account for the neuropsychiatric symptoms. The pellagra-like lesions may be related to relative deficiency of nicotinic acid.

3. *Familial glycinuria.* Glycinuria associated with urinary calculus formation was observed in four female patients in three generations of a single family; only one report describing this condition is known (Vries, Kochwa, Lazebnik, Frank and Djaldetti, 1957). In the index case, urinary calculi began forming in the patient's fourth year of life. Glycinuria must therefore be considered amongst other metabolic causes of urinary calculi in early life (including oxalosis, xanthinuria and cystinuria).

Metabolic investigation of glycinuria revealed normal plasma glycine levels and defective glycine reabsorption in the renal tubule. Endogenous glycine renal clearance was approximately fifteen times normal in the propositus. Unusual amounts of other amino acids (including proline and hydroxyproline) were not detected in the urine of affected patients by partition chromatography on filter paper.

Calculi, when examined, contained calcium oxalate and approximately 0.5 per cent free glycine. Calcium and oxalate metabolism appeared to be normal in glycinuric patients; endogenous metabolism of glycine also seemed to be normal. Several aspects of glycine metabolism and transport were reviewed in a discussion on familial glycinuria (Wyngaarden, 1960).

It is suggested (Vries et al., 1957; Wyngaarden, 1960) that familial glycinuria is a specific disorder of renal tubular glycine transport, inherited as a dominant trait, possibly sex-limited.

III. Defects of Renal Tubular Amino Acid Transport Producing Generalized Hyperaminoaciduria

Generalized aminoaciduria is observed in many hereditary diseases. The most important and common cause of these aminoacidurias is diminished renal tubular reabsorption of the affected amino acids; in addition, impaired absorption of other metabolites is often detected.* In a few conditions structural as well as functional lesions in the renal tubule are observed.

Many factors demonstrate that aminoaciduria in this group is a secondary manifestation and is not itself directly determined by the genetic trait responsible for the individual disease or syndrome. It would be suspected

* An excellent discussion of tubular diseases in general is available: see Mudge, G. H., (1958), Clinical patterns of tubular dysfunction. Amer. J. Med. *24:* 785–804.

TABLE 4—*Relationship of Genetic Block to Disease Manifestations*

LEVELS			REPRESENTATIVE DISEASES		
GENETIC	GENOTYPE	INHERITED TRAIT	PHENYLKETONURIA	CYSTINURIA	GALACTOSEMIA
MOLECULAR	↓	ALTERED ENZYME ACTIVITY OR ALTERED MEMBRANE REACTIVE SITE(?)	LIVER APOENZYME PHENYLALANINE HYDROXYLASE II	CELLULAR TRANSPORT SYSTEM	CELLULAR APOENZYME GALACTOSE - 1 PHOSPHATE URIDYLTRANSFERASE
PRIMARY OPERATIVE	PHENOTYPE	B A → B ⫽ ····→ C B	ENDOGENOUS ACCUMULATION OF PHENYLALANINE	URINARY LOSS OF FOUR AMINO ACIDS	ACCUMULATION OF GAL. 1-PHOSPHATE
SECONDARY MANIFESTATIONS		X ⫽ ····→ Y	PHENYLALANINE LOSS IN URINE		IMPAIRED RENAL TUBULE FUNCTION
TERTIARY, ETC.	↓				AMINOACIDURIA

from earlier considerations of the complexity of amino acid cellular transport systems, that many different mechanisms are involved in the hyperaminoacidurias discussed below. Extensive study of the causes should eventually contribute to the mapping of transcellular amino acid transport.

Since aminoaciduria is usually of secondary importance, it has no significance in describing the genetics of the individual diseases in this group. The scheme in Table 4 suggests the levels at which genetic defects and their subsequent manifestations occur; the relationships of various aminoacidurias to the altered product of the genetic lesion, is represented in the table by three typical examples.

a. Generalized Aminoaciduria with Apparent Precipitating Cause

1. Galactosemia. Aminoaciduria occurs in untreated patients homozygous for a single autosomal gene which in double-dose is responsible for complete absence, and in single-dose for partial absence of galactose 1-phosphate uridyl transferase activity (Donnell, Bergren, Bretthauer and Hansen, 1960; Isselbacher, 1960); segregation of the three classes on the basis of enzyme measurements is complete (Donnell et al., 1960).

Aminoaciduria is present only when galactose is ingested (Cusworth et al., 1955; Holzel, Komrower and Schwarz, 1957). Simple neutral aliphatic chain and high renal clearance amino acids are primarily affected and the term "central cluster" has been used to describe the appearance of the two-dimensional filter paper chromatogram. Onset of the functional lesion only occurs after several days of galactose ingestion; disappearance of aminoaciduria occurs gradually following galactose withdrawal. Other disturbances of kidney function occur, and proteinuria and impaired acidification have both been observed (Holzel et al., 1957). Aminoaciduria is almost certainly of renal origin (Cusworth et al., 1955; Holzel et al., 1957), although some workers (Vangeffel, Devriendt, Dustin, Vis and Loeb, 1959) claim that prerenal factors are also important; the latter observation may

be accounted for by the degree of liver failure present. Tashian (1961b) noted that liver failure in galactosemia affected tyrosine metabolism.

Delay in onset of aminoaciduria induced by galactose ingestion suggests that aminoaciduria is related to accumulation of a "cytotoxic" product of impaired galactose metabolism (Cusworth et al., 1955; Holzel et al., 1957); galactose 1-phosphate has been implicated. The mechanism of cytotoxicity of this compound is unknown, although inhibition of other enzymes or diversion of ATP to form galactose 1-phosphate, thus depriving the cell of ATP for other requirements, have both been suggested (cited in Isselbacher, 1960).

2. *Lactase deficiency.* Darling and co-workers (1960) have observed two infants in whom failure to thrive, vomiting and frequent bowel movements were associated with the ingestion of lactose. Examination of the urine revealed proteinuria and a generalized aminoaciduria. Unfortunately, the urine volumes placed on chromatograms were not standarized satisfactorily; this feature, and the very young age of the patients (see section C, 2), make interpretation of aminoaciduria in these 2 patients difficult. The authors postulated a deficiency of intestinal lactase activity to account for the syndrome. Another report of a similar or identical condition (Holzel, Schwarz and Sutcliffe, 1959) did not state whether there was abnormal aminoaciduria.

Doell and Kretchmer (1961) have reported a developmental study of intestinal mucosal lactase activity in fetal and newborn rats. Activity was highest in earlier infancy, falling again as the animal matured.

Weijers and co-workers (1961) observed a syndrome with manifestations similar to lactase deficiency, but which they attributed to invertase and/or maltase deficiency. Aminoaciduria was not discussed.

3. *Vitamin D deficiency.* A generalized aminoaciduria is often observed in conjunction with vitamin D deficiency (Chisolm, 1959; Harrison, 1961; Jonxis, 1961; Vries et al., 1957). This aminoaciduria should not be confused with those occurring in several varieties of hypophosphatemic vitamin D "resistant" rickets (e.g., Type I with glycinuria, Type II with iminoaciduria and glycinuria; generalized aminoaciduria in Fanconi syndromes, etc. [after Dent and Harris, 1956]).

Vitamin D deficiency aminoaciduria is of renal tubular origin (Chisolm, 1959; Harrison, 1961; Jonxis, Smith and Huisman, 1952) and is augmented by high-protein diet. A specific transport lesion is implied, which is interesting since vitamin D itself is concentrated by the proximal convoluted portion of the nephron (Darmady and Stranack, 1959).

Jonxis (1961; 1952) has noted that amino acid renal clearances tend to remain elevated longer than usual in patients from certain families in which there appears to be a familial tendency to rickets. He suggested that

these patients represented an hereditary susceptibility to rickets resulting from unusual vitamin D requirements. Further investigation of environmental conditions, intestinal function and other factors seems indicated before acceptance of the genetic hypothesis to explain the rickets in these patients.

b. Generalized Aminoacidurias With Other Tubular Transport Defects, of Undetermined Etiology

1. The Fanconi syndrome(s). "The main features of this syndrome upon which there is general agreement, are rickets or osteomalacia; resistence to vitamin D in usual doses; glucosuria; generalized aminoaciduria and hyperphosphaturia, in spite of normal or reduced plasma concentration of these constituents; and usually, chronic acidosis and hypopotassemia." (Leaf, 1960). Hypouricemia and high uric acid renal clearance probably occurs more frequently than generally suspected (Ben-Ishày, Dreyfuss and Ullmann, 1961).

Disputes over descriptive and interpretive rights for the syndrome, and its own innate complexity, have led to much nosological confusion. Leaf mercifully avoided eponyms in his review and stressed the appellation *syndrome* rather than "disease." It is apparent that several hereditary or acquired forms of the *syndrome*, each presumably with its own pathogenesis, have been described; those which can be clearly recognized include:

i. Childhood and infantile form with cystinosis (cystine accumulation in tissues). This usually fatal form of the *syndrome* is the most common one. A typical case report (Karpinski, 1960) and review (Worthen and Good, 1958) have recently appeared.

ii. Adult form without cystinosis. The *syndrome* often may be apparent biochemically but not symptomatically in this age group. Representative case reports (Ben-Ishày et al., 1961; Dent and Harris, 1951; Wallis and Engle, 1957) and a general discussion (Worthen and Good, 1958) are available.

iii. Many single childhood cases of the *syndrome* without cystinosis have been observed (Allison, 1959; Anonymous, 1957; Anonymous, 1960; Armstrong and Binkley, 1956; Armstrong and Low, 1957) in the review by Worthen and Good (1958); familial cases also occur (Jagenburg, 1959). The functional and clinical manifestations of the childhood *syndrome* without cystinosis are similar to those found in symptomatic adult cases; nevertheless, childhood cases do not occur in families in which adult cases occur. A possible exception to the latter statement may exist in Ben-Ishày's pedigree (1961). Unfortunately, the diagnosis of the *syndrome* in relatives of the propositus in the latter study was made only on the basis of aminoaciduria. In a few members (II, 10; III, 13; IV, 1; IV, 8) who are important

for the conclusions drawn by the authors, hyperaminoaciduria may have been a questionable entity according to Table 4 in the paper (Ben-Ishày et al., 1961). In view of these doubtful features and the unusual high consanguinity rate, the conclusion that the *syndrome* is manifested or inherited differently from other reports of the Fanconi syndrome appears to be made on dangerously tenuous evidence.

iv. Cogan and co-workers (1958; 1957) described a 23 year old male with cystinosis (cystine storage) in ocular and bone marrow tissues. Manifestations of the *syndrome*, however, were not observed in this healthy subject; a similar circumstance noted elsewhere in a 50 year old woman, was referred to in this same report. This condition should not be included in a classification of the *syndrome* but is mentioned for the sake of clarity.

This concludes the nosography of the conditions traditionally called "Fanconi Syndrome" and "cystinosis." Several other acquired or inherited conditions associated with the *syndrome* have been observed whose clinical features distinguish them from the above conditions.

i. Fellers and co-workers (Fellers, Knapp and McKenna, 1960) described three infants without rickets who for no apparent reason had transient but marked manifestations of the *syndrome*. The eponym "Ukari syndrome" was used to describe these children, because of nasal erythema which resembled that present in the Ukari monkey.

ii. Toxic agents, such as lead, have produced the syndrome, even to the appearance of rickets (Chisolm, 1962).

iii. Luder and Sheldon described a family twice (Luder and Sheldon, 1955; Sheldon, Luder and Webb, 1961) at intervals, observing progression of the *syndrome* in twins. Glucosuria and aminoaciduria were detected when the patients were 5 years old; three years later phosphaturia and renal tubular acidosis had developed in association with rickets in one of the twins. Of unusual interest was the father, who was clinically normal but had aminoaciduria and glucosuria. His father in turn had had renal glucosuria at least. Consanguinity was not apparent in this pedigree.

iv. The *syndrome* was noted by Baber (1956) in an infant with hepatic cirrhosis confirmed at autopsy; during life, urinary tyrosine excretion was particularly prominent in conjunction with the generalized aminoaciduria.

v. Multiple myeloma has been associated with the *syndrome* (Engle and Wallis, 1957).

Experimental injection of maleic acid (Harrison and Harrison, 1954) produces renal glycosuria, phosphaturia and aminoaciduria in rats. Worthen corroborated this observation (1960); he also demonstrated concurrent structural changes in the proximal tubular cell wall and inhibition of renal cortical succinic dehydrogenase activity. Fellers and Vawter (1961) produced a similar change in urinary composition with transaconi-

tic acid injections into rats. These observations on citric acid cycle impairment associated with the *syndrome*, suggest that quantitative investigation of plasma and urinary citric acid cycle intermediates in the human *syndrome* might be of interest. Preliminary qualitative studies of urinary organic acid excretion in the human *syndrome* did not reveal any obvious abnormality (Dedmon, Dent, Scriver and Westall, 1961).

Structural as well as functional lesions of the proximal convoluted tubule have been observed in the hereditary infantile and adult forms of the *syndrome* without cystinosis and in the infantile *syndrome* with cystinosis (Clay, Darmady and Hawkins, 1953; Darmady and Stranack, 1957). Marked decrease in luminal volume and surface area of the affected portion in the proximal convoluted tubule results from shortening and narrowing by the "swan-neck" lesion. The majority of the nephrons are affected. It is not clear whether the lesion follows or precedes onset of metabolic tubular dysfunction. Worthen and Good (1958) found no "swan-neck" lesion in their patient, tending to refute the suggestion that the structural lesion might be congenital and hence *a-priori* to the *syndrome*.

Symptoms in the infantile *syndrome* with cystinosis typically appear only after the first few months of life. Bickel (1955) followed from birth a patient who later developed cystinosis; tubular dysfunction occurred only after five months of age. Delay in appearance of functional and clinical abnormalities in an hereditary *syndrome* suggests at least two possibilities: (a) that the observed lesions are at best secondary to a primary unidentified "toxic" defect, which is itself the genetically determined phenomenon (the sequence of events presumably resembles, in extended fashion, the time scale of events following galactose ingestion in galactosemia); (b) that the function for which the "normal" allele is responsible is not required by the organism until a certain stage of differentiation; possibly a regulator gene complex could be involved here.

It is probable that in most forms of the *syndrome* there is a single biochemical lesion responsible for the several biochemical abnormalities. It is possible that a cellular system, supplying and distributing energy for maintaining cytoarchitectural relationships necessary for transcellular transfer and membrane transport of various substrates is affected by a variety of hereditary or acquired lesions which may produce the *syndrome*. There may be several points within this cellular system which may be affected. Finer analysis of the functional and cytological abnormalities in various forms of the *syndrome* may reveal even more accurately than at present that each form of the *syndrome* has distinctive functional and cytological features. Varying severity in the manifestations of the functional lesion, among different individuals with the same form of the *syndrome*, has been noted. This could be accounted for by environmental and other

factors, influencing the susceptibility of cellular mechanisms at the secondary level, to the effect of the disturbance at the primary level.

Worthen and Good (1958) carefully studied the different abnormalities of urinary composition in the *syndrome* and demonstrated that each was dependent on impaired proximal convoluted tubular function.

Aminoaciduria in particular has been the subject of investigation in the *syndrome*. The pattern of the typical aminoaciduria resembles that of the plasma amino acids (Fig. 3). A prerenal mechanism has been postulated for the aminoaciduria (Bickel, 1955), but endogenous amino acid renal clearances, analyzed by ion exchange chromatography (Cusworth and Dent, 1960a) unequivocably demonstrated the renal tubular origin of the aminoaciduria. The quantitative differences in aminoaciduria observed between patients, can be partly accounted for by varying degrees of nephron failure and glomerular-tubular imbalance. This has been discussed by Worthen and Good (1958) and by Cusworth and Dent (1960b). The apparent therapeutic efficacy of vitamin D on aminoaciduria (Bickel, 1955) and other functional defects appears to result from depression of glomerular filtration rate by this type of therapy (Worthen and Good, 1958); no beneficial effect on tubular reabsorption has been observed with vitamin D.

There appears to be little support for one suggestion (Fellers, Ko and Nicolaidou, 1960) that aminoaciduria in the *syndrome* is dependent on tubular secretion of amino acids.

The quantitative variation and pathogenesis of aminoaciduria has also been discussed in terms of tubular reabsorptive activity alone. Lathem and co-workers (1955) considered the transport lesion to be minimal in the patients at their disposal and to be restricted to a small number of nephrons, a finding which is at variance with morphological observations. Other workers (Piel and Harper, 1961), on the other hand, have determined that the tubular transport lesion may be either minimal or severe in different patients, and that all nephrons are probably affected by the lesion.

Butler and Flynn (1958) have stressed the occurrence of an unusual proteinuria in the *syndrome*. Sulphosalicylic acid added to urine from patients with the *syndrome* produces turbidity which clears on boiling and reappears on cooling. Electrophoretic analysis of urinary protein reveals an unusual pattern with relatively high concentration of a_2, β, and γ globulins. A special mechanism for "tubular" proteinuria was implied and discussed in the report.

Observations in the experimental *syndrome*, in lead poisoning and in cystinosis, have suggested that unavailability of free sulfhydryl groups may be responsible for the syndrome. Hereditary inhibition of cysteine reductase activity was postulated for the pathogenesis of cystinosis (Worthen

and Good, 1958). Recently, Brigham and co-workers (1960) reported accumulation of cystine but not cysteine in plasma from patients with cystinosis. Clayton and Patrick (1961) observed elevated plasma pyruvate and α-ketogluterate levels in vivo, and low succinic dehydrogenase activity in necropsy tissue, in cystinosis. These findings, they believed, were in accordance with the speculations presented above. Penicillamine and dimercaprol were given to 3 patients for a prolonged period, during which marked biochemical and clinical improvement was observed. Unfortunately, the 3 patients had somewhat atypical *syndromes* and further details are awaited before a full assessment of this report is possible. Dimson (1952) briefly reported a different approach. He gave the biological antioxidant, α-tocopherol, 100 mg. daily for 6 months, in conjunction with other therapy. Solubilization of cystine deposits in the eye, and improvement of renal function was claimed. Repetition of these studies with quantitation of changes in cystine content in available tissue, such as circulating white blood cells (Korn, 1960), should be tried for corroboration of this report.

2. *Oculo-cerebro-renal syndrome* (Lowe-Terrey-MacLachlan Syndrome). This is another syndrome (Lowe et al., 1952) whose primary cause remains unknown. Multiple renal tubular dysfunctions, partially resembling those of the Fanconi syndrome, are associated with a striking clinical picture. The typical phenotype is a male infant or child who first comes for medical attention because of ocular abnormalities (cataracts or buphthalamos), or mental retardation. Hypotonia, diminished or absent reflexes, and frequently hypophosphatemic rickets, are also detected. Lowe has referred to the characteristic appearances of all patients (discussion in Richards, Donnell, Wilson and Stowens, 1960); this feature is also visible in photographs of published cases (many of which are cited in McKusick's reviews, items 629 and 931 [1961]).

Manifestations of renal tubular dysfunction include generalized aminoaciduria and defective urinary acidification. Formation of both titratable acid and ammonia is defective (Richards et al., 1960; Schoen and Young, 1959). Glucosuria and hyperphosphaturia, the latter being of renal tubular and not hyperparathyroid origin (Richards et al., 1960), are less constant findings. Proteinuria is also frequently observed and is of the "tubular" variety (Butler and Flynn; Terslev, 1960; Richards et al., 1960). Organicaciduria, stressed in the original report (Lowe, Terrey and MacLachlan, 1952) is probably accounted for by the aminoaciduria. Specific paper chromatography of urinary organic acids has not revealed any significant abnormality in one patient with the O.C.R. syndrome (Dedmon et al., 1961). Guthrie (1961; 1960) reported the presence of an unidentified compound in urine from these patients; it was also observed in normal newborn infants.

Aminoaciduria is the most consistent sign of renal tubular dysfunction. The amino acid reabsorption defect apparently exists in all nephrons (Piel and Harper, 1961). Vitamin D is claimed to have reduced hyperaminoaciduria (Terslev, 1960) but since uremia, during vitamin D therapy, was also described in this report, the claim may not be valid.

"Swan-neck" structural lesions of the nephron have been reported in a patient believed to have this syndrome (Scholten, 1960). Mitochondrial changes in tubular cells, observed by electron microscopy of renal biopsy tissue (Schoen and Young, 1959), may have been fixation artefacts. No unusual pathology was mentioned in a study in which necropsy material was available (Richards et al., 1960).

The quite consistent over-all similarity of clinical and functional abnormalities probably indicate a single common cause for the syndrome. The variations in degree of tubular dysfunction observed between individual patients suggest that this manifestation is of secondary importance and not itself a direct manifestation of altered gene product. Familial occurrence of the syndrome is frequent. All reported cases, with one exception (Scholten, 1960) are phenotypic males; no reduction in the number of females in the pedigrees has been noted. Father-to-son transmission has not been encountered and pedigrees indicate female-carrier transmission. Overtly unaffected potential carriers have normal urinary composition and mentality, but detailed investigation of tubular function capacity in these subjects has not been reported. Donnell (discussion, Dent and Smellie, 1961) has reported that crystalline lenticular abnormalities are unusually frequent in potential carrier females. It has been suggested that the syndrome has X-linked "recessive" inheritance, and partial expression may be apparent in the female. A recent report (Scholten, 1960) described what was believed to be fatal Lowe's syndrome in a 2 month old phenotypic female. A female sib died with a similar disease. To account for this occurrence, sex chromosome anomalies (e.g., an X0 state) should be ruled out; alternatively, misdiagnosis or a different form of inheritance of the true syndrome could be the explanation.

4. Wilson's disease (hepato-lenticular degeneration). Wilson's disease is well enough known, that detailed description is unnecessary. Several excellent discussions by Bearn (1957; 1960b; 1961) have reported successive developments in its investigation.

Aminoaciduria occurs as one component of extensive renal tubular dysfunction, which in turn is one of the generalized systemic manifestations of the disease. Many genetic problems are raised by Wilson's disease. It most certainly is familial in occurrence, and investigation of 30 families by Bearn (1960a) revealed a very high consanguinity rate among parents of affected individuals. The ratio of normal to affected sibs suggested the lat-

ter were homozygous for a rare abnormal autosomal gene. However, certain interesting clinical features were apparent: (1) The same clinical pattern of the disease, either neurological or hepatic, was usually observed in sibs when more than one was affected in a family. (2) Time of onset of symptoms was usually the same in each sib. (3) Within certain ethnic groups there was a tendency for one form of the disease to occur more frequently than another.

These observations have suggested that genetic heterogeneity may account for the different forms of the disease; variation and delay in times of onset may also represent, amongst other possibilities, the presence of moderating or "regulator" genetic activity.

The pleiomorphic manifestations in Wilson's disease suggest that they are only secondary expressions of an altered gene product. Genetic analysis is likely to be unreliable if based on these functional disorders. A search for the altered gene product has focused interest on ceruloplasmin (Sternlieb and Scheinberg, 1961) as the possible altered gene product, which is in turn responsible for the various clinical and biochemical manifestations. Ceruloplasmin is a copper protein which has oxidase activity; copper dissociation results in loss of enzymatic activity. Ceruloplasmin exhibits molecular heterogeneity with at least two forms present in human plasma (Hirschmann, Morell and Scheinberg, 1961). Recent evidence (Poulik and Bearn) indicates that much of the heterogeneity of purified ceruloplasmin is artefactual.

In Wilson's disease, with few exceptions, ceruloplasmin concentration is decreased. However, some individuals with the disease have been observed to have normal levels of ceruloplasmin oxidase activity (Bearn, 1957; Sass-Kortsak, Cherniak, Geiger and Slater, 1959). Ceruloplasmin in these subjects does not bind copper normally and in this respect the copper protein is similar to that detected in the usual Wilson's disease patient. The molecular characteristics of the detectable ceruloplasmin in typical Wilson's disease are believed to be similar to those of ceruloplasmin in normal individuals (Hirschmann et al., 1961). Whether there is molecular heterogeneity has not been reported in Wilson's disease patients with normal ceruloplasmin levels. If ceruloplasmin happens to be the gene product affected in Wilson's disease, several alterations may affect it, including impaired production of one or more protein fractions, or molecular rearrangement producing functional impairment of one or more fractions.

Although interest in ceruloplasmin has dominated investigation for the altered gene product in Wilson's disease, Uzman has suggested (1956) that other possibilities may exist for the pathogenesis of this disease. Whatever the gene product may prove to be in Wilson's disease, the possibility of genetic heterogeneity remains to be resolved.

Identification of the heterozygote has been attempted in Wilson's disease (Bearn, 1960a; Blattner, 1961). The most promising method to date measures the endogenous ceruloplasmin turnover of Cu^{64} to define normals, carriers and affected subjects (Sternlieb, Morell, Bauer, Combes, Bobes-Sternberg and Scheinberg, 1961). However, there is overlap between heterozygotes and affected homozygotes, and only 50 per cent of known heterozygotes can be so classified. Findings in the study by Sternlieb and co-workers (1961) were consistent with the "recessive" gene hypothesis, thereby attributing the multiple manifestations of Wilson's disease to a single biochemical lesion; the type of Wilson's disease patient studied was not stated in the report. As implied above, it is possible, though unproven, that multiple alleles at the same or different loci may be responsible for different gene products and different manifestations of Wilson's disease.

Despite the inconclusive identification of the altered gene product, it is known eventually to alter copper metabolism (Scheinberg and Sternlieb, 1960). Tissue accumulation of copper follows, in turn impairing cellular function in different organs. As one reflection of this, renal tubular transports of phosphate, uric acid, glucose, bicarbonate, organic acid (PAH) and amino acids may all be impaired. The extent of the functional abnormality varies between different patients (Stein et al., 1954) and with the progression of the disease in the same patient. The aminoaciduria has been studied in some detail (Stein et al., 1954; Dent and Harris, 1951) and is clearly renal in origin, except when liver failure contributes pre-renal components.

Therapy in the disease is based on the assumption that copper in excess inhibits free sulfhydryl groups in enzymes; Walshe (1961) believes that results of plasma pyruvate determinations in patients with Wilson's disease support this hypothesis. Penicillamine and BAL have been used (Blattner, 1958; Walshe, 1960), and functional improvement frequently follows treatment with these agents.

IV. a. Miscellaneous Causes of Hyperaminoaciduria

There are a number of other apparently hereditary conditions associated with abnormal aminoaciduria. These disorders have not been included in the previous sections because the pathogenesis or significance of their aminoaciduria is relatively unknown at present.

i. A syndrome including familial growth retardation, hypoplastic muscle development, renal aminoaciduria and cor pulmonale was described by Rowley and co-workers (1961). The aminoaciduria was investigated by Rosenberg and associates (1961) who demonstrated its renal origin. Smaller neutral aliphatic-chain amino acids, lysine, histidine and tyrosine

were primarily affected; none was associated with an elevated plasma concentration. Other aspects of glomerular and tubular function were normal.

Several asymptomatic relatives, but not the parents, of the propositus also appeared to have the aminoaciduria and two sibs had died with the syndrome before aminoaciduria was investigated. Occurrence of aminoaciduria and growth failure coincided only in the propositus, suggesting that these features were either independently inherited, or were both secondary, variable manifestations of a primary genetic condition.

ii. Paine described apparently sex-linked microcephaly with mental deficiency and other neurological abnormalities (1960). "Central-cluster" aminoaciduria was detected in the chromatograms. Normal plasma amino nitrogen concentration and elevated α-amino nitrogen level in cerebrospinal fluid in the propositus were reported, but not investigated further. This may be a relatively nonspecific finding since other workers (McKay and Thom, 1961), examining mentally retarded children, have noticed some correlation between intensity and distribution of amino acid patterns in urine and cerebrospinal fluid when studied by paper chromatography. Quantitative investigation (Perry and Jones, 1961) detected no specific abnormality of cerebrospinal fluid in several forms of mental retardation. Unfortunately, for technical reasons, cerebrospinal fluid was pooled in this latter report and the individual variations could not be determined; urine was not investigated. Wright (1960) investigated aminoaciduria in sibs with mental retardation without specific cause, and also observed mild "central-cluster" aminoaciduria in about 10 per cent of 110 sibs in 61 families.

iii. The syndrome of ichthyosis, tetraplegia and severe mental retardation (Anonymous, 1957) was first described in Sweden by Sjögren and Larsson (1957) and has also been found elsewhere in the world (Link and Roldan, 1958; Wallis and Kalushiner, 1960; Williams and Lingtang, 1960). This interesting familial condition appears to show recessive inheritance. The constant association of the principal manifestations, often associated with macular changes, indicate a single genetic component in its inheritance. Recent investigators (Williams and Lingtang, 1960) have suggested that mild generalized aminoaciduria also occurs; amino acids which normally have relatively high renal clearance rates were those mainly affected. A very definite generalized aminoaciduria has been found in the propositus of an unreported pedigree (Scriver; unpublished data).

iv. Hereditary hyperparathyroidism (Jackson, Talbert and Caylor, 1960) has been observed in families which previously would have been classified as pedigrees of hereditary pancreatitis (Gross, Comfort and Ulrich, 1957). Prerenal aminoaciduria has been detected in some of these patients (Gross et al., 1957). The feature of particular interest is the high frequency and

predominance of lysinuria. The significance of this does not appear to have been investigated, but the recorded interrelation of calcium and lysine transport in the rat intestine (Raven, Langemann and Wasserman, 1960) may prove to have some bearing on lysinuria in hereditary hyperparathyroidism.

v. Hyperaminoaciduria is occasionally observed as part of the spectrum of tubular dysfunction occurring in congenital renal tubular acidosis with nephrocalcinosis. The aminoaciduria is generalized, resembling that in the Fanconi syndrome and related conditions, and is of renal origin. Treatment with sodium and potassium citrate alone can correct the defect in amino acid transport in the renal tubule (Chisolm, 1962). The aminoaciduria is not the result of acidosis itself (Jagenburg, 1959) and its mechanism is probably similar to that occurring in the Fanconi syndrome, and other generalized disturbances of renal tubular function.

vi. "Masked aminoaciduria" (Snyderman and Holt, 1960) may occur with certain congenital or hereditary disorders of tryptophan metabolism. Knapp (1960) described a family with urticaria and other allergic manifestations in which affected members excreted abnormal amounts of xanthurenic acid following tryptophan loading by mouth. This biochemical disturbance was corrected only when vitamin B_6 supplements were given continuously. The correlation of the biochemical and clinical manifestations remains obscure.

Marver (1961) recorded distortion in the excretion pattern of certain tryptophan metabolites in association with congenital hypoplastic anemia. These observations were obtained with quantitative analysis of tryptophan metabolites on ion exchange columns, in two siblings with the disease; the mother also had a similar excretion pattern of tryptophan metabolites. The controversy over previous investigations on this problem was discussed.

Tryptophan metabolism has been studied in mongolism with particular interest, in view of the chromosomal abnormality. Jerôme and co-workers (1960) reported impaired 5-HIAA synthesis in mongolism. O'Brien and Groshek (1962) performed tryptophan loading by mouth in mongols and normal controls. Significant impairment of xanthurenic acid formation was detected, suggesting decreased hydroxykynurenine transaminase activity. Significant differences in 5-HIAA excretion were not detected, upon statistical analysis, in contrast to other studies (Jerôme et al., 1960). McCoy (1961) observed no abnormality of plasma amino acid concentrations in mongols.

vii. Muscular dystrophies have been investigated for aminoaciduria. The results vary with different neuromuscular diseases. Jackson and Carey (1961) noted no aminoaciduria in the autosomal recessive form of progressive muscular dystrophy, in contrast to other forms of the disease. Car-

ver and associates (1961) found a variety of changes in aminoaciduria, particularly hypoaminoaciduria in different muscular disorders; they stressed the effect of clinical status, diet and other factors on the results.

viii. Menano and colleagues (1960) claimed detection of "hereditary aminoaciduria" in a propositus, one sib and the mother, in whom dermatological changes were noted. The only reliable statement concerning this aminoaciduria seems to be that glycine excretion was moderately elevated. Bacterial contamination artefacts could account for the other changes in urinary amino acids, described by the authors.

b. Iminoaciduria (L-Proline and Hydroxy-L-Proline) and Glycinuria in Disease

Hyperiminoaciduria does not normally occur after the first few months of life (section C, 2). Several generalized aminoacidurias of renal tubular origin, as well as specific aminoacidurias, such as familial glycinuria, familial hyperprolinemia and idiopathic hyperglycinemia, have abnormal urinary excretion of imino acids or glycine; these diseases have already been considered. Other disorders are known which specifically affect imino acid and glycine concentrations in urine, and these will now be discussed.

Detection of imino acids in biological fluids is an important technical problem. In general, the ninhydrin reaction combined with filter paper partition chromatography is an insensitive method; however, special modifications of the ninhydrin reaction have improved detection of proline on filter paper chromatograms (Pasieka and Morgan, 1956). The isatin reaction (Block et al., 1958; Smith, 1960) is often used for identification of imino acids in these circumstances. Microbiological inhibition assay systems (Guthrie, 1961; Guthrie and Tieckelmann, 1960) may be adaptable to proline detection when a screening method is required.

Quantitative analysis of both proline and hydroxyproline on ion-exchange resin columns has become more reliable with recent modifications (Moore et al., 1958). Specific chemical techniques in the past usually possessed inadequate specificity; newer methods (Messer, 1961; Mitoma, Smith, Davidson, Udenfriend, DaCosta and Sjoerdsma, 1959; Prockop and Udenfriend, 1960) have improved quantitative analysis for both proline and hydroxyproline.

Iminoaciduria may occur when the imino acid transport system, which seems to be present in the normal renal tubule (Scriver et al., 1961a; Scriver et al., 1961b), is impaired. The relation of iminoaciduria to endogenous metabolism of the affected amino acids is less clearly understood and is a field of current interest. Degradation and synthesis of L-proline have been reinvestigated in certain animal tissues (Strecker, 1960), but much work remains to be done. Hydroxy-L-proline may share the same catabolic path-

ways as proline. The two imino acids, as well as glycine, together constitute over 30 per cent of the amino acid residues in collagen (Eastoe, 1955) and KMEF proteins (keratin, myosin, epidermin and fibrinogen (Szent-Györgyi and Cohen, 1957). The structural configuration of these latter molecules is in part determined by their L-proline content (Szent-Györgyi and Cohen, 1957). Hydroxy-L-proline is uniquely present in large amounts in the collagen protein molecule; changes in hydroxyproline content of collagen occur in diseases associated with structural and functional changes of connective tissue (Bonting, deBruin and Pollak, 1961). Whereas L-proline is incorporated intact into the growing collagen molecule, hydroxy-L-proline is apparently synthesized from proline in situ within the polypeptide strand at some early stage in collagen synthesis (Hausmann and Neuman, 1961). Investigation of L-proline and hydroxy-L-proline concentration in biological fluids could possibly provide insight into altered callagen metabolism (Gerber, Gerber and Altman, 1960a); as an example, experimental scurvy (Martin, Mergenhagan and Prockop, 1961) is associated with decreased hydroxyprolinuria.

Other areas of metabolism are open to L-proline, as indicated in Figure 4. Small amounts of free L-proline are detectable in mammalian brain and other tissues (Tallan et al., 1954), and L-proline is actively metabolized in rat brain (Sporn, Dingman and Defalco, 1959).

Quantitative estimates of free imino acid concentrations in several human biological fluids have been obtained by ion-exchange chromatography. Urine from normal adults and children beyond early infancy contains no detectable amount of either imino acid. In plasma, only proline is present in significant amounts. In cerebral spinal fluid, only trace amounts of proline are present (Perry and Jones, 1961). Chemical methods indicate that small amounts of hydroxy-L-proline are detectable in a hydrolyzable form in normal urine (Mitoma et al., 1959).

Therefore, when hereditary iminoaciduria occurs, it should reflect some alteration either of cellular metabolism, or of transport.

1. Possibly altered cellular metabolism. i. The Marfan syndrome has recently been discussed exhaustively from the clinical and genetic viewpoints (McKusick, 1960), and McKusick both reviews and interprets yearly (1961) the considerable volume of literature on this topic. The Marfan syndrome is inherited in an autosomal dominant manner. Evaluation of inherited manifestations suggest that the Marfan syndrome is determined by a single gene with irregularly expressed multiple effects; the possibility may also exist that multiple alleles or modifiers are present.

The nature of the disease suggests an inherited disturbance of connective tissue metabolism. A recent comment states, "From analogy with the hemoglobinopathies, it is likely that one or more of the heritable disorders

of connective tissue . . . will be found to have their basis in a highly pinpointed amino acid substitution (in collagen)" (item No. 831, [McKusick, 1961]). The discovery of hydroxyprolinuria in the Marfan syndrome (Mitoma et al., 1959; Sjoerdsma, Davidson, Udenfriend and Mitoma, 1958) is therefore of much interest. This was determined by a chemical method (Mitoma et al., 1959), which revealed that the urinary hydroxyproline occurred mainly in an hydrolyzable form and not as the free imino acid. The observation has been confirmed by Summer (1961a) who noted that a quantitative correlation existed between degree of iminoaciduria and severity of clinical manifestations. Others (Prockop, Mitoma and Sjoerdsma, 1959) have suggested that increased turnover of collagen accounts for the biochemical lesion in the Marfan syndrome. Reports on bound hydroxyproline concentration in serum in the Marfan syndrome should be informative, but none has been presented so far.

Other workers (Jagenburg, 1959; Lehmann, 1960) have not confirmed the iminoaciduria, but it seems most likely that this can be attributed to differences in analytical methods. Little of the Marfan iminoaciduria is accounted for by the free imino acid (Mitoma et al., 1959), and hence chromatographic ninhydrin methods (used by Jagenburg [1959]) may detect no abnormality.

Experimental lathyrism in animals, which bears similarities to the human Marfan syndrome, is associated with increased urinary hydroxyproline excretion (Martin et al., 1961).

ii. Osteogenesis imperfecta and odontogenesis imperfecta: This hereditary disease has been discussed at length from clinical, genetic and other viewpoints (Caniggia, Stuart and Guideri, 1958; Heys, Blattner and Robinson, 1961; McKusick, 1960). The consensus is that the abnormality responsible for bone fractures, blue sclera, impaired conductive hearing and defective dentin is inherited as a single factor in an autosomal dominant manner. The possibility of one or more abnormal alleles, and the role of environmental and other factors affecting the degree of manifestation of the syndrome, has not been established.

It has been implied that collagen formation in affected tissues is abnormal in the disease (Caniggia, Ravenni and del Giovane, 1961). It has not been determined whether an unusual structural form of collagen exists, or whether the rate of collagen synthesis itself is altered. Certain findings point to either of these possibilities. Urinary hydroxy-L-proline excretion is below average in osteogenesis imperfecta (Mitoma et al., 1959); other urinary amino acids appear to be unaffected (Caniggia et al., 1958). Uptake of L-proline into plasma, following loading by mouth, is abnormally decreased in the disease, when compared to normal subjects (Summer, 1961b), and there is smaller than normal subsequent rise in hydroxypro-

line concentration. A very ambiguous paper by Caniggia and co-workers (1961) claims that collagen in tissues affected by osteogenesis imperfecta is different from other tissue collagens in its proline content. The paper does not make clear whether this observation was found in affected or normal subjects. Furthermore, their finding, assuming it applies to normal tissues, does not correspond to quantitative data published elsewhere (Eastoe, 1955; Piez, 1961). Careful studies in the future should provide interesting data on this disease.

iii. The "super-glycine" form of hypophosphatemic rickets (Dent and Harris, 1956), although not proven to be genetic, shows evidence of alteration in cellular imino acid metabolism and renal tubular transport of glycine and imino acids. Evered (1956) observed that two patients with this disease had increased urinary excretion of proline, hydroxyproline and glycine. The same patients were reinvestigated at a later date (Cusworth and Dent, 1960a) at which time high endogenous glycine renal clearance was demonstrated; iminoaciduria was absent, but plasma proline concentration was elevated in both patients. The causes of amino acid and mineral metabolic abnormalities remain unknown. However, the mechanism of the aminoaciduria appears to resemble that which occurs in familial hyperprolinemia (Scriver et al., 1961b). In retrospect, the author believes that Evered, who only investigated urinary amino acids, probably would have found higher plasma proline levels than were later noted by Cusworth and Dent, thus accounting for the iminoaciduria which he observed.

2. *Altered tubular reabsorption.* i. Another form of hypophosphatemic rickets associated with glycosuria, glycinuria and phosphaturia has been observed (Dent and Harris, 1956). Glycinuria was associated with elevated endogenous renal clearance rate (Cusworth and Dent, 1960a) in 2 patients who were investigated; no alterations in urine or plasma imino acid concentrations were detected. Intravenous calcium infusions in another patient with this disease established that phosphaturia was determined by a primary renal tubular defect and not by hyperparathyroidism (Scriver et al., unpubl. obs.). The interrelation of the transport defect of phosphate to transport defects of glycine and glucose is an interesting problem at present under study.

ii. Joseph and co-workers (1958) have described a familial convulsive disorder affecting three sibs with onset in the neonatal period. Aminoaciduria involving proline, hydroxyproline and glycine in particular was observed. Blood levels of the affected amino acids were considered to be normal (by paper chromatographic methods) and it was suggested that defective renal tubular reabsorption accounted for the aminoaciduria. A lesion common to brain and renal tubule was postulated; unfortunately cerebrospinal fluid amino acids were not investigated.

Jonxis (1962) has studied a male infant who had convulsions in the first

week of life. The patient was somnolent with slow mental development until the end of the first year of life. Ion exchange column chromatography revealed normal plasma amino acids but striking iminoaciduria (proline and hydroxyproline) and glycinuria. The epileptic mother had normal aminoaciduria; the father was clinically normal but excreted abnormal amounts of proline in his urine. The relationship of this disease to that described by Joseph (1958) and to familial hyperprolinemia is being investigated.

F. Recently Identified Unusual Ninhydrin Reacting Compounds in Human Urine

New ninhydrin reacting compounds are still being identified in human urine. Westall (1955) detected a large number of free and hydrolyzable substances which reacted with ninhydrin following separation by column and partition chromatography. Jagenburg (1959) subsequently investigated several compounds which appeared as peaks on the elution chromatogram, following chromatography on ion exchange resin columns. One of these peaks has been recently identified as homocitrulline by Gerritson and co-workers (1961) (see section C, 2, b).

β-L-aspartyl-L-histidine has recently been identified as a normal constituent of human urine (Kakimoto and Armstrong, 1961).

Two mentally defective sisters, age 8 and 6 years, have been found to excrete moderate amounts of an amino acid not present in normal urine and which has been tentatively identified as homocystine (Carson, Cusworth, Dent, Field, Neill and Westall, 1961). The identification of a sulfur-containing sulphide composed of L-homocysteine and L-cysteine from cystinuric urine (Frimpter, 1961) was discussed earlier. A recent report tentatively mentions identification of S-isopropylcarboxymethylcysteine in a hypothyroid patient (Mizuhara, 1961); the relation of this to the drug therapy of the patient was not discussed.

Coles and co-workers (1960) observed the appearance of an unusual compound during routine amino acid chromatography in a phenol system. The original patient was a mentally retarded child; small amounts were subsequently identified in other members of the family and in unrelated patients. The compound was identified tentatively as an alloxan-like substance.

G. Stable Genetic Factors Influencing Aminoaciduria

The examples of unusual human aminoaciduria which have been quoted in this discussion were in most cases the result of rare mutations which, when fully expressed, were decidedly disadvantageous. However, amino-

aciduria in normal individuals is also under genetic control to a degree which may be detected under the proper conditions. Studies on monozygotic and dizygotic twins (Berry, Dobzhansky, Gartler and Osborne, 1955; Gartler, Dobzhansky and Berry, 1955) have revealed that genetic factors participate in control of excretion rates of β-aminoisobutyric acid, threonine, tyrosine and lysine. Glycine, proline, taurine and valine may also have heritable control of their execretion rates. These effects are not of such strength, however, that they will be obvious in random populations (Berry, 1960c), except in the case of BAIB excretion.

Urinary BAIB excretion is an example of probable polymorphism. Urinary excretion of amino acids other than BAIB has also been studied in segregated racial groups (McEvoy-Bowe and Lugg, 1961), but marked ethnic differences were not so apparent. The significance of the racial distribution of polymorphic genes affecting amino acid excretion, the disadvantages or advantages associated with their presence or absence, and their contribution to human evolution and adaptation (Allison, 1959; Dobzhansky, 1961) have not been determined.

Insight into evolutionary and adaptive aspects of urinary amino acid excretion has been sought in studies on interspecific and interphyllic variations. Variability of aminoaciduria was detected in several mammalian species which could not be attributed solely to dietary differences (Datta and Harris, 1953); variability was absent in plasma amino acid patterns, implying that renal mechanisms may have been significant in determining the different urinary patterns. More detailed analysis of three strains of mice (Harris and Searle, 1953) revealed striking difference in taurine excretion in the C57BL strain; this appeared to be related to a difference in renal tubular transport of taurine in this strain. Aminoaciduria in apes has been studied (Gartler, Firschein and Dobzhansky, 1956) with differences quoted between subspecies of apes and from human aminoaciduria. Interphyllic variability of individual amino acid excretion patterns apparently has not been investigated in any detail.

The prominent influence of renal tubular amino acid transport mechanisms on interspecific variability of aminoaciduria in mammals is probably expected in view of the central position of the kidney in their evolution and adaption (Smith, 1959). Of possible relevance in this regard is the comparative study of mitochondria in intestinal absorptive cells of fish, toad, lizard, chicken, rat and guinea pig (Stanbury, 1961). Structural similarity but certain enzymatic differences were detected (Stanbury, 1961); further study of transport behavior in these organisms would be of interest.

The occurrence of amino acid transport systems in different species has not been defined, with the exception of identification of the cystine- "basic"

amino acid transport system in dog kidney, and the spectrum of systems in the rat intestine (see section D). Unusual amino acid patterns, such as the exaggerated cystinuria, without lysinuria, observed in the Kenya genet (Datta and Harris, 1953) may represent a successful, or at least unharmful, experiment in amino acid transport. In the domestic cat, cysteine probably has an additional route of excretion in the form of cysteine-S-*iso*-pentanol (felinine) (Westall, 1953). This compound appears to be synthesized endogenously, perhaps in the kidney itself. Less successful mutations have produced cystinuric calculi in several breeds of dog and mink, the mutation in some cases having striking differences of inheritance from the human disease (cited in Knox, 1960b). These are but a few examples of what awaits the enterprising investigator.

Finally, investigation remains to be done on the functional significance of specific amino acid patterns detectable in saliva (Rose and Kerr, 1958), sweat (Clarke, Elian and Shwackman, 1961) and other body fluids. "Markers" of acquired or genetic significance in health and disease may be found by such investigation.

Conclusion

This paper has not attempted a complete review of hereditary aminoaciduria, for this would be a phenomenal task. Instead, the discussion centered on four main topics. The problem of methods is of great importance in the proper investigation of aminoaciduria in general, and particularly when genetic interpretation is the objective of the investigation. Many methods and techniques are available which can be tailored to the needs of the investigator.

Normal aminoaciduria must be defined before either normal genetic variability or inherited and acquired abnormalities of urinary amino acid excretion can be identified. Several nonheritable factors influence amino acid excretion in man and these were reviewed. A summary of data available in the literature, covering amino acid levels in urine and plasma was presented. Unfortunately, only a range of values (and where possible, mean values) could be presented. It is obvious that where genetic and environmental factors can influence aminoaciduria, data such as those found in Table 2 are only a limited guide for assessment of the individual patient.

Hereditary aminoaciduria occurs when either intermediary metabolism or cellular transport is affected by a heritable abnormality. When an apoenzyme appears to be the altered gene product, the amino acid(s) serving as substrate for the altered enzyme accumulates endogenously and eventually appears in the urine. Specific transport systems may also be affected in a manner suggesting that some part of the transport mechanism with spe-

cific affinity for certain amino acids may be altered by a heritable abnormality. Less specific heritable disorders of cellular transport also occur, affecting many substrates including most amino acids. In these latter diseases, the altered gene product is less directly related to the membrane transport mechanism and may instead be related to maintenance of cellular energetics and cytoarchitecture. In general, all of these hereditary disorders observe established patterns of inheritance provided the known or presumed altered gene product and its primary manifestations are identified. The various heritable aminoacidurias were discussed within this context.

Addendum

Several recent publications warrant notation.

General

Proceedings of 2 symposia have been published, containing information which bears directly on topics presented in this review. Academic Press (1961) published "A Symposium on Membrane Transport and Metabolism (Prague, 1960)," edited by Kleinzeller and Kotyk. Elsevier (1961) published "Amino Acid pools: distribution, formation and function (City of Hope Medical Center, California, 1961)," edited by Holden.

Christensen's group presented data on transport behavior of a new inert amino acid exhibiting active transport which they named 1-aminocyclopentane-1-carboxylic acid (J. Biol. Chem. 237: 113–117, 1962). Its renal reabsorption in living rats was reported in J. Biol. Chem. 237: 1203–1206, 1962, the data indicating concentrative transport.

Lin and co-workers (Am. J. Physiol. 202: 919–925, 1962) studied the specificity of the transport system for neutral amino acids in the hampster intestine. A detailed analysis of the affects of optical specificity, modifications of the α amino group, carboxyl group, side chain, and α hydrogen and the influence of vitamin B_6, on active transport were evaluated.

Specific

Histidine. Histidinemia (Ghadimi et al., 1961, see section Ia4) has been identified in two new families (Auerbach et al., J. Pediat. 60: 487–497, 1962, and LaDu et al., Biochem. and Biophys. Res. Comm. 7: 398–402, 1962); in the latter report two sibs were affected. Speech retardation, positive urine ferric chloride test, elevated plasma histidine concentration and increased urinary histidine, imidazolepyruvic acid, imidazolelactic acid, and imidazoleacetic acid excretions are now recognized as characteristic identifying features. All three groups of workers identified the enzymatic abnormality as a disturbance of histidase activity with subsequent impairment of urocanic acid formation. This enzyme defect is readily investigated in skin (LaDu et al., 1962) and correlated with absence of urocanic acid in sweat in this disease. The report by LaDu (1962) discusses, in addition, the potential heterozygote state in a clinically normal sib and the father.

Imidazoleaminoaciduria was described by Bessman and Baldwin (Science 135: 789–791, 1962). They found high urinary excretion of histidine, 1-methyl histidine and of the related peptides, carnosine and anserine in 3 families with Tay-Sachs dis-

ease. Plasma levels of these compounds were normal. Imidazoleaminoaciduria exhibited dominant inheritance and was not necessarily associated with cerebromacular degeneration; the neurological disease was recessively inherited, always in conjunction with the biochemical abnormality in these pedigrees. The authors favored a common genetic defect for clinical and biochemical abnormalities.

Citrullinuria. This new amino aciduria was detected in a mentally retarded infant born to first-cousin parents. Citrulline levels in cerebrospinal fluids, plasma and urine were elevated. Blood and urinary urea concentrations, however, were normal. Loading studies have been done (Rathbun, personal comm.) but data are not available. In the meantime, the analogy between this new disease and argininosuccinic-aciduria (see section Ia8) is of considerable interest.

Homocitrullinuria. Further data has been published (Gerritsen et al. Arch. Biochem. and Biophys. 97: 34–36, 1962). This amino acid is normally present in amounts of 1–65 mg/24 hours in urine of children under 6 years of age. Dietary components or intravesical lysine-urea interaction, do not account for the formation of this compound, which is metabolically inert and not detected in plasma.

Dr. Stanford Moore has drawn the present author's attention to past literature (Scriver, Pediatrics 26: 62, 1960), suggesting that "unknown peak #2" in urine from a patient with vitamin B_6 dependency probably was a large homocitrulline peak.

Cystinuria. Renal tubular transport of amino acids in four homozygous cystinuric patients was evaluated by Frimpter and colleagues (J. Clin. Invest. 41: 281–288, 1962). Of particular interest was one female patient who showed evidence for net tubular secretion of cystine; in addition, glycine reabsorption was depressed in this patient (viz. also Webber et al., 1961). Frimpter's earlier work (1961) concerning a disulfide in cystinuric urine and this recent data provoke new thinking concerning renal tubular cell metabolic activity in cystinuria.

Cystinosis. Patrick (Biochem. J. 83: 249–256, 1962) investigated cystine reductase and glutathone-cysteine transhydrogenase activities in autopsy specimens from livers of 3 cystinosis patients. No abnormality could be detected.

References

Ackermann, D., and F. Kutscher. 1911. Ueber das Vorhommen von Lysin im Harn bei Cystinurie. Ztschr. Biol. 57: 355–359.

Akedo, H., T. Sugawa, S. Yoshikawa, and M. Suda. 1960. Intestinal absorption of amino acids: I: Effect of vitamin B6 on L-amino acids through the intestine. J. Biochem. (Tokyo) 47: 124–130.

——, and H. M. Christensen. 1961. Effect of insulin on the transfer of α-amino iso butyric acid (abstr.) Fed. Proc. 20: 140(d).

Allan, J., D. C. Cusworth, C. E. Dent, and V. K. Wilson. 1958. A disease, probably hereditary, characterized by severe mental deficiency and a constant gross abnormality of amino acid metabolism. Lancet 1: 182–187.

Allen, R. J., and R. M. Gibson. 1961. Phenylketonuria with normal intelligence. Am. J. Dis. Child. 102: 115–122.

Allison, A. C., and B. S. Blumberg. 1958. Dominance and recessivity in medical genetics. Am. J. Med. 25: 933–941.

——. 1959. Metabolic polymorphisms in mammals and their bearing on problems of biochemical genetics. Am. Naturalist. 93: 5–14.

Alsberg, C., and O. Folin. 1905. Protein metabolism in cystinuria. Am. J. Physiol. 14: 54–72.

Andersen, B., and H. H. Ussing. 1960. Active transport. In Florkin, M., and Mason, H. S., Eds.: Comparative Biochemistry. New York: Academic Press, pp. 371–404, vol. 2.

Anonymous. 1957. Mental defect, tetraplegia and ichthyosis. Lancet 2: 337.

——. 1960. Hartnup disease. Lancet 2: 1384–1385.

——. 1961. Absorption of amino acids. Nutrition Rev. 19: 16–17.

——. 1961. Amino acid excretion in infants and children. Nutrition Rev. 19: 68–70.

Armstrong, M. D., and E. L. Binkley. 1956. Studies on phenylketonuria V: Observations on a new born infant with phenylketonuria. Proc. Soc. Exper. Biol. & Med. 93: 418–420.

——, and N. L. Low. 1957. Phenylketonuria VIII: Relation between age serum phenylalanine level and phenylpyruvic acid excretion. Proc. Soc. Exper. Biol. & Med. 94: 142–146.

Baber, M. D. 1956. A case of congenital cirrhosis of the liver with renal tubular defects akin to the Fanconi syndrome. Arch. Dis. Child. 31: 335–339.

Baldwin, E. 1959. Pages 315 and 333. Baldwin, E. Dynamic Aspects of Biochemistry, ed. 3. Camb. Univ. Press, pp. 315 and 333.

Balenović, K. 1958. The stereospecificity of naturally occuring β-amino acids. In Wolstenholme, G. E. W., and O'Connor, C. M., Eds.: Amino Acids and Peptides with Antimetabolic Activity. Ciba Foundation Symposium. Boston: Little, Brown & Co., pp. 5–18.

Baron, D. M., C. E. Dent, H. Harris, E. W. Hart, and J. B. Jepson. 1956. Hereditary pellagra-like skin rash with temporary cerebellar ataxia, constant renal amino aciduria and other bizarre biochemical features. Lancet 2: 421–428.

Bartter, F. C. 1960. Hypophosphatasia. In Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S., Eds.: The Metabolic Basis of Inherited Disease. New York: McGraw-Hill, pp. 1367–1378.

Battaglia, F. C., K. L. Manchester, P. J. Randle. 1960. Effects of insulin on monosaccharide transport and incorporation of amino acids into protein in diaphragm, differentiated with phlorizin. Biochim. Biophys. Acta. 43: 50–54.

Bearn, A. G. 1957. Wilson's disease: an inborn error of metabolism with multiple manifestations. Am. J. Med. 22: 747–757.

——. 1960a. A genetical analysis of thirty families with Wilson's disease (hepato-lenticular degeneration). Ann. Human Genet. 24: 33–43.

——. 1960b. Wilson's disease. In Stanbury, J. B., Wyngaarden, J. B., and Frederickson, D. S., Eds.: The Metabolic Basis of Inherited Disease. New York: McGraw Hill, pp. 809–838.

——. 1961. Wilson's disease. Am. J. Clin. Nutr. 9: 695–699.

Becker, F. F., and H. Green. 1958. Incorporation of cystine and lysine by normal and cystinuric leukocytes. Proc. Soc. Exper. Biol. & Med. 99: 694–696.

Beecher, H. K. 1960. Ed.: Disease and Advancement of Basic Science. Cambridge, Mass.: Harvard Univ. Press.

Beerman, W. 1956. Nuclear differentiation and functional morphology. Cold Springs Harbor Symposium on Quantitative Biology 21: 217–230.

Berglund, F. 1961. Urinary excretion patterns for substances with simultaneous secretion and reabsorption by active transport. Acta physiol. scandinav. 52: 276–290.

Bergmann, F., and S. Dikstein. 1959. The theory of transfer through walls of biological tubes of solute in a moving fluid. J. Physiol. 145: 14–21.

Bergstedt, J., D. O'Brien, and L. O. Lubchenco. 1960. Interrelationships in the urinary excretion of creatinine, creatine, free alpha-amino acid nitrogen and total nitrogen, in premature infants. J. Pediat. 56: 635–639.

Berliner, R. W. 1959. Membrane transport. Rev. of Modern Physics 31: 342–348. (Also In Oncley, J. L. et al., Ed.: Biophysical Science—a Study Program. New York: J. Wiley & Son, pp. 342–348.)

Ben-Ishày, D., F. Dreyfuss, and T. D. Ullmann. 1961. Fanconi syndrome with hypouricemia in an adult: family study. Am. J. Med. 31: 793–800.

Berry, H. K. 1959a. Cystinuria in mentally retarded siblings with a-typical osteogenesis imperfecta. A.M.A. J. Dis. Child. 97: 196–197.

——. 1959b. Procedures for testing urine dried on filter paper. Clin. Chem. 5: 603–608.

——. 1960a. Individual metabolic patterns. I: amino acid excretion in 700 children. Metabolism 9: 363–372.

——. 1960b. Individual metabolic patterns. II: Excretion of beta-aminoisobutyricacid. Metabolism 9: 373–376.

——. 1960c. Amino acid excretion in urine of normal infants and children. Paper chromatographic methods for amino acid analyses. Pediatrics 25: 983–996.

——, Th. Dodzhansky, S. M. Gartler, and R. H. Osborne. 1955. Chromatographic studies on urinary excretion patterns in monozygotic and dizygotic twins. I: methods and analysis. Am. J. Human Genetics 7: 93–107.

——, and B. Sutherland. 1960. "Tyrosinosis" in an infant (abstr.) Am. J. Dis. Child. 100: 571.

——, ——, and G. M. Guest. 1960. Detection of metabolic disorders among young infants: a case finding program. (abstr.) Am. J. Dis. Child. 100: 570.

——, ——, ——, and J. Warkany. 1958. Simple method for detection of phenylketonuria. J.A.M.A. 167: 2189–2190.

——, H. E. Sutton, L. Cain, and J. S. Berry. 1951. II: Development of paper chromatography for use in the study of metabolic patterns. Biochem. Institute studies IV. University of Texas Pub. No. 5190, pp. 22–55.

Bessman, S. P., and K. Tada. 1961. An enzyme basis for the indole lesion in phenylketonuria (abstr.). Am. J. Dis. Child. 102: 606.

Bethune, J. E., and C. E. Dent. 1960. Hypophosphatasia. Am. J. Med. 28: 615–622.

Bickel, H. 1955. Die Entwicklung der Biochemischen Lasion bei der Lignac-Fanconischen Krankheit. Helvet. paedeat. acta 10: 259–267.

——. 1961. Melleturia, a paper chromatographic study. J. Pediat. 59: 641–656.

Bigwood, E. J., R. Crokaert, E. Schram, P. Soupart, and H. Vis. 1959. Amino aciduria. Advances Clin. Chem. 2: 201–265.

Blatherwick, N. R. 1934. Tyrosinosis: a search for additional cases. J.A.M.A. 103: 1933.

Blattner, R. J. 1958. Chelating agents in the treatment of hepatolenticular degeneration (Wilson's disease). J. Pediat. 52: 758–761.

——. 1961. Heterozygous carrier in Wilson's disease (comment on current literature). J. Pediat. 59: 460–463.

Block, R. J., E. L. Durrum, and G. Zweig. 1958. A Manual of Paper Chromatography and Paper Electrophoresis, ed. 2. (rev.). New York: Academic Press.

Bloxam, H. R., M. G. Day, N. K. Gibbs, and L. I. Woolf. 1960. An inborn defect in the metabolism of tyrosine in infants on a normal diet. Biochem. J. 77: 320–325.

Blumberg, B., and S. M. Gartler. 1959. High prevalence of high-level β-aminoisobutyric acid excretors in Micronesians. Nature 184: 1990.

Bonetti, E., and C. E. Dent. 1954. The determination of optical configuration of naturally occuring amino acids using specific enzymes and paper chromatography. Biochem. J. 57: 77–81.

Bonner, D. M., Y. Suyama, and J. A. DeMoss. 1960. Genetic fine structure & enzyme formation. Fed. Proc. 19: 926-930.

Bonting, S. L., H. deBruin, and V. E. Pollak. 1961. Quantitative histo-chemistry of the nephron. J. Clin. Invest. 40: 177–180.

Bowden, C. H. 1959. An improved solvent combination for amino acid chromatography. Clin. Chim. Acta. 4: 539–544.

Boyer, S. H. 1961. Alkaline phosphatase in human sera and placentae. Science 134: 1002–1004.

Brigham, M. P., W. H. Stein, and S. Moore. 1960. The concentrations of cysteine and cystine in human blood plasma. J. Clin. Invest. 39: 1633–1638.

Brown, J. L., A. H. Samiy, and R. F. Pitts. 1961. Localization of amino-nitrogen reabsorption in the nephron of the dog. Am. J. Physiol. 200: 370–372.

Burgen, A. S. V. 1957. The physiological ultrastructure of cell membranes. Can. J. Biochem. & Physiol. 35: 569–576.

Butler, A. M., and R. H. Richie. 1960. Simplification and improvement in estimating drug dosage and fluid and dietary allowances for patients of varying size. New England J. Med. 262: 903–908.

Butler, B. A., and F. V. Flynn. 1958. The proteinuria of renal tubular disorders. Lancet 2: 978–980.

Caniggia, A., G. Ravenni, and L. del Giovane. 1961. On the pathogenesis of fragilitas ossium hereditaria. Panminerva Med. 3: 67–72.

——, C. Stuart, and R. Guideri. 1958. Fragilitas ossium hereditaria tarda, Ekman-Lobstein disease. Acta. Med. Scandinav. 162 (suppl. 340): 1–172.

Carson, N. A., D. C. Cusworth, C. E. Dent, C. M. B. Field, D. W. Neill, and R. G. Westall. 1961. Personal communication.

Carver, M. J., S. J. Dutch, and C. L. Wittson. 1961. Aminoaciduria in representative neuromuscular disorders. Metabolism 10: 582–584.

——, and R. Paska. 1961. Ion exchange chromatography of urinary amino acids. I: Normal children. Clin. Chim. Acta. 6: 721–723.

Chadwick, J. M., J. S. Fawcett, and F. G. Warburton. 1959. Amino-aciduria in a case of scurvy. Am. J. Clin. Nutr. 7: 721–725.

Chapelle, E. W., and J. M. Luck. 1957. The decarboxylation of amino acids, proteins and peptides by N-bromo succinimide. J. Biol. Chem. 229: 171–179.

Childs, B. 1952. Urinary excretion of free alpha-amino nitrogen by normal infants and children. Proc. Soc. Exper. Biol. & Med. 81: 225–226.

——, W. L. Nyhan, M. Borden, L. Bard, and R. E. Cooke. 1961. Idiopathic hyperglycinemia and hyperglycinuria: a new disorder of aminoacid metabolism. J. Pediat. 27: 522–538.

Chisolm, J. J., Jr. 1959. The clinical significance of aminoaciduria. J. Pediat. 55: 303–314.

——. 1962. Aminoaciduria as a manifestation of renal tubular injury in lead intoxication and a comparison with patterns of aminoaciduria seen in other diseases. J. Pediat. 60: 1–17.

——, and H. E. Harrison. 1960. Amino-aciduria. Pediat. Clin. N. America 7: 333–351.

Christensen, H. M. 1959. Active transport, with special reference to amino acids. Perspectives in Biol. and Med. 2: 228–242.

——. 1960. Reactive sites and biological transport. Adv. in Protein Chem. 15: 239–314.

——, and J. C. Jones. 1961. Action of estrogens on mediated transports into red blood cells. J. Biol. Chem. 236: 76–80.

——, H. M. Parker, and T. R. Riggs. 1958. Non-exchange of carboxyl oxygen in mammalian amino acid transport. J. Biol. Chem. 233: 1485–1487.

Christensen, P. J., J. W. Date, F. Schöheyder, and K. Volqvartz. 1957. Amino acids in

blood plasma and urine during pregnancy. Scandinav. J. Clin. & Lab. Invest. 9: 54–61.

Clarke, J. T. 1961. Urinary creatinine and creatine. Clin. Chem. 7: 271–283.

——, E. Elian, and H. Shwackman. 1961. Components of sweat. Am. J. Dis. Child. 101: 490–500.

Clay, R. D., E. M. Darmady, and M. Hawkins. 1953. The nature of the renal lesion in the Fanconi syndrome. J. Path. Bact. 65: 551–558.

Clayton, B. E., and A. D. Patrick. 1961. Use of dimercaprol and penicillamine in the treatment of cystinosis. Lancet 2: 909–910.

Cochrane, W. 1960. Idiopathic infantile hypoglycemia and leucine sensitivity. Metabolism 9: 386–399.

Cochrane, W. A., W. W. Payne, M. J. Simpkiss, and L. I. Woolf. 1956. Familial hypoglycemia precipitated by amino acids. J. Clin. Invest. 35: 411–422.

Cogan, D. G., T. Kuwabara, C. S. Hurlbut, and V. McMurray. 1958. Further observations on cystinosis in the adult. J.A.M.A. 166: 1725–1726.

——, ——, J. Kinoshita, L. Sheehan, and L. Merola. 1957. Cystinosis in an adult. J.A.M.A. 164: 394–396.

Cohen, M. M., G. Cassady, and B. L. Hanna. 1961. A genetic study of hereditary renal dysfunction with associated nerve deafness. Am. J. Human Genet. 13: 379–389.

Cohn, E. J., and J. T. Edsall. 1943. Proteins, Amino Acids and Peptides as Ions and Dipolar Ions. New York: Reinhold.

Coles, H. M. T., A. Priestman, and J. H. Wilkinson. 1960. "T-substance" anomaly. An inborn error of purine metabolism. Lancet 2: 1220–1223.

Connell, G. E., G. H. Dixon, and C. S. Hanes. 1955. Quantitative chromatographic methods for the study of enzymic transpeptidation reactions (part 1). Can. J. Biochem. Physiol. 33: 416–427.

Consden, R., A. H. Gorden, and A. J. P. Martin. 1944. Qualitative analysis of proteins: a partition chromatographic method using paper. Biochem. J. 38: 224–232.

Cope, F. W. 1961. A logarithmic integrator for the Spackman-Stein-Moore amino acid analyzer. Report No. 14 from the Aviation Medical Accel. Lab. NADC-MA-6103.

Crampton, R. F., and D. H. Smyth. 1953. The excretion of the enantiomorphs of amino acids. J. Physiol. 122: 1–10.

Crawhall, J. C., E. F. Scowen, and R. W. E. Watts. 1959. Conversion of glycine to oxalate in primary hyperoxaluria. Lancet 2: 806–809.

Crome, L., G. Dutton, and C. F. Ross. 1961. Maple syrup urine disease. J. Path. Bact. 81: 379–384.

Crumpler, H. R., C. E. Dent, H. Harris, and R. G. Westall. 1951. β-amino isobutyric acid (α-methyl-β-alanine): a new amino-acid, obtained from human urine. Nature 167: 307–308.

Currarino, G., E. B. D. Neuhauser, G. C. Reyersbach, and E. Sobel. 1957. Hypophosphatasia. Am. J. Roentgenol. 78: 392–419.

Cusworth, D. C., and C. E. Dent. 1960a. Renal clearances of amino acids in normal adults and in patients with aminoaciduria. Biochem. J. 74: 550–561.

——, and ——. 1960b. Inborn errors of tubular function page 535–549 in Quinn E., and Kass E., (eds.) "Biology of Pyelonephritis" Henry Ford Hosp. Sympos., Little Brown & Co., Boston.

——, ——, and F. V. Flynn. 1955. The aminoaciduria in galactosaemia. Arch. Dis. Child. 30: 150–154.

Dancis, J., and M. Levitz. 1960. Maple syrup urine disease. In Stanbury, J. B., Wyngaarden, and Fredrickson, D. S., Eds.: The Metabolic Basis of Inherited Disease. New York: McGraw-Hill, pp. 473–485.

——, J. Hutzler, and M. Levitz. 1960. Metabolism of the white blood cells in maple syrup urine disease. Biochem. et biophys. acta. 43: 342–343.

——, M. Levitz, and R. G. Westall. 1960. Maple syrup urine disease: branched-chain ketoaciduria. Pediatrics 25: 72–79.

——, ——, S. Miller, and R. G. Westall. 1959. "Maple-syrup urine disease." Brit. J. 1: 91–93.

Darling, S., O. Mortensen, and G. Søndergaard. 1960. Lactosuria and aminoaciduria in infancy. A new inborn error of metabolism? Acta. paediat. 49: 281–290.

Darmady, E. M., and F. Stranack. 1957. Microdissection of the nephron in disease. Brit. M. Bull. 13: 21–25.

——, and ——. 1959. Autoradiography of the isolated nephron. Proc. Soc. Exptl. Biol. and Med. 100: 658–660. (Also published as Kodicek et al. 1961. The localization of 14 C-labeled vitamin D_2 in the nephron. Clin. Sc. 20: 185–196).

——, and ——. 1960. Morphological expression of functional changes in the nephron. In Quinn, E., and Kass, E., Eds.: Biology of Pyelonephritis. Henry Ford Hosp. Sympos. Boston: Little Brown & Co., pp. 173–181.

Darwin, C. 1859. On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life. London.

Datta, S. P., and H. Harris. 1951. Dietary origin of urinary methylhistidine. Nature 168: 296–297.

——, and ——. 1953. Urinary amino acid patterns of some mammals. Ann. Eugen. 18: 106–115.

——, C. E. Dent, and H. Harris. 1950. An apparatus for the simultaneous production of many two-dimensional paper chromatograms. Science 112: 621.

Dedmon, R. E., and C. R. Scriver. 1961. Chromatography and related techniques in human biochemical genetics. New Physician 10: 13–27.

——, C. E. Dent, C. R. Scriver, and R. G. Westall. 1961. The urinary excretion of organic acids in man: a survey of a variety of metabolic disturbances by two-dimensional paper partition chromatography. Clin. Chim. Acta. 6: 291–295.

Dent, C. E. 1946. Detection of amino-acids in urine and other fluids. Lancet 2: 637–639.

——. 1947. The aminoaciduria in Fanconi syndrome. A study making extensive use of techniques based on paper partition chromatography. Biochem. J. 41: 240–253.

——. 1948. A study of the behavior of some sixty amino-acids and other ninhydrin reacting substances on phenol- "collidine" filter-paper chromatograms, with notes as to the occurrence of some of them in biological fluids. Biochem. J. 43: 169–180.

——. 1957a. Foreword: Symposium on inborn errors of metabolism. Am. J. Med. 22: 671–675.

——. 1957b. Clinical applications of amino acid chromatography. Scand. J. Clin. and Lab. Invest. (supp. 31), 10: 122–127.

——. 1959. Argininosuccinicaciduria. A new form of mental deficiency due to metabolic causes. Proc. Roy. Soc. Med. 52: 885.

——. 1961a. Argininosuccinicaciduria and maple syrup urine disease. Bull. Schweiz. Akad. Med. Wiss. 17: fasc. 5.

——. 1961b. Personal communication.

——, and H. Harris. 1951. The genetics of cystinuria. Ann. Eugen. 16: 60–87.

——, and ——. 1956. Hereditary forms of rickets and osteomalacia. J. Bone & Joint Surg. 38B: 204–226.

——, and G. A. Rose. 1951. Amino acid metabolism in cystinuria. Quart. J. Med. 20: 205–219.

——, and B. Senior. 1955. Studies on the treatment of cystinuria. Brit. J. Urol. 27: 317–332.

——, and J. M. Smellie. 1961. Two children with oculo-cerebro-renal syndrome of Lowe-Terrey and MacLachlan. (With comment by G. N. Donnell). Proc. Roy. Soc. Med. 54: 335–357.

——, and R. G. Westall. 1961. Studies in maple syrup urine disease. Arch. Dis. Child. 36: 259–268.

Dimson, S. B. 1952. The Fanconi syndrome in two siblings. Proc. Roy. Soc. Med. 45: 454.

Dobzhansky, Th. 1961. Man and natural selection. Am. Scientist 49: 285–300.

Doell, R. G., and N. Kretchmer. 1961. A developmental study of lactase in intestine (abstr.) Am. J. Dis. Child. 102: 224–225.

Donnell, G. N., W. R. Bergren, R. K. Bretthauer, and R. G. Hansen. 1960. The enzymatic expression of heterozygosity in families of children with galactosemia. Pediatrics 25: 572–581.

Doolan, P. D., H. A. Harper, M. E. Hutchin, and W. W. Shreeve. 1955. Renal clearances of eighteen individual amino acids in human subjects. J. Clin. Invest. 34: 1247–1255.

Drèze, A., S. Moore, and E. J. Bigwood. 1954. On the desalting of solutions of amino acids by ion exchange. Analyt. Chim. Acta. 11: 554–558.

Driscoll, S. G., and D. Y-Y. Hsia. 1958. The development of enzyme systems during early infancy. Pediatrics 22: 785–845.

Dubois, R., H. Vis, H. Loeb, and M. Vincent. 1959. Alterations de l'amino-acidurie observée dans des cas de kwashiorkor. Étude par chromatographie sur colonnes d'echangeurs d'ions. Helvet paediat. acta. 14: 13–43.

Durrum, E. L. 1951. Two-dimensional electrophoresis and ionophoresis. J. Colloid. Sc. 6: 274–290.

Dustin, J. P., S. Moore, and E. J. Bigwood. 1955. Chromatographic studies on the excretion of amino acids in early infancy. Metabolism 4: 75–79.

Eastoe, J. E. 1955. The amino acid composition of mammalian collagen and gelatin. Biochem. J. 61: 589–603.

Eastoe, J. E. et al. 1961. Coloquium on: "The quantitative estimation of amino acids. Contributors: Eastoe, J. E., A. L. J. Buckle, S. M. Partridge, D. F. Elsden, J. I. Harris, J. K. Whitehead, and B. S. Hartley. Biochem. J. 80: 33–37P.

Eckhardt, R. D., and C. S. Davidson. 1949. Urinary excretion of amino acids by a normal adult receiving diets of varied protein content. J. Biol. Chem. 177: 687–695.

Edozien, J. C., E. J. Phillips, and W. R. F. Collis. 1960. The free amino acids of plasma and urine in kwashiorkor. Lancet 1: 615–618.

Efron, M. L. 1959. Two way separation of amino acids and other ninhydrin-reacting substances by high-voltage electrophoresis followed by paper chromatography. Biochem. J. 72: 691–694.

——, E. S. Kang, and J. K. Visakorpi. 1962. Plasma amino acids in phenylketonuria (abstr.). Soc. Ped. Rev. p. 112.

Engle, R. L. Jr., and L. A. Wallis. 1957. Multiple myeloma and the adult Fanconi syndrome. Am. J. Med. 22: 5–12.

Evered, D. F. 1956. Excretion of aminoacids by the human. A quantitative study with ion-exchange chromatography. Biochem. J. 62: 416–427.

——. 1959. Ionophoresis of acidic and basic aminoacids on filter paper using low voltages. Biochim. Biophys. Acta. 36: 14–19.

Fellers, F. X., and G. F. Vawter. 1961. Hypophosphatemia and aminoaciduria in rats fed transaconitic acid. (abstr.) Fed. Proc. 20: 417.

——, J. Knapp, and J. McKenna. 1960. The Ukari syndrome: a transient tubular nephropathy (abstr.) Am. J. Dis. Child. 100: 763–764.

——, K. W. Ko, and M. Nicolaidou. 1960. A defect in tubular secretory function in the de Toni-Fanconi syndrome. (abstr.) Am. J. Dis. Child. 100: 588–589.

Filteau, G., and F. Martel. 1956. Analyse quantitative des acides aminés librés du sérum sanguin par chromatographie bi-dimensionelle sur papier-filtre. Rev. Canad. Biol. 15: 212–230.

Finch, L. R., and F. J. R. Hird. 1960a. The uptake of amino acids by isolated segments of rat intestine: i) a survey of factors affecting the measurement of uptake. Biochim et biophys. acta. 43: 268–277.

——, and ——. 1960. The uptake of amino acids by isolated segments of rat intestine: ii) a survey of affinity for uptake from rates of uptake and competition for uptake. Biochim. biophys. acta. 43: 278–287.

Fink, K., R. B. Henderson, and R. M. Fink. 1951. Beta-aminoisobutyric acid, a possible factor in pyrimidine metabolism. Proc. Soc. Exper. Biol. & Med. 78: 135–141.

Florkin, M. 1949. Biochemical evolution (edited and translated by S. Morgulis). New York: Academic Press.

Forbes, G. B. 1959. Body surface as a basis for dosage. Pediatrics 23: 3–5.

Fowler, D. I., P. M. Norton, M. W. Cheung, and E. L. Pratt. 1957. Observations on the urinary amino acid excretion in man: influence of age and diet. Arch. Biochem. 68: 452–466.

Frame, E. G., J. A. Russell, and A. E. Wilhelmi. 1943. The colorimetric estimation of amino nitrogen in blood. J. Biol. Chem. 149: 255–270.

Fraser, D. 1957. Hypophosphatasia. Am. J. Med. 22: 730–746.

——, and E. R. Yendt. 1955. Metabolic abnormalities in hypophosphatasia (abstr. and discussion). A.M.A. J. Dis. Child. 90: 552–554.

——, ——, and F. H. E. Christie. 1955. Metabolic abnormalities in hypophosphatasia. Lancet 1: 286.

Frimpter, G. W. 1961. The disulfide of L-cysteine and L-homocysteine in urine of patients with cystinuria. J. Biol. Chem. 236: 51–53PC.

Gagnon, A. 1961. The absorption of amino acids by the isolated intestine of the green frog, Rana Clamitans. Rev. Can. Biol. 20: 7–14.

Gairdner, D. 1961. Unpublished data.

Garrod, A. E. 1908. The Croonian lectures on "inborn errors of metabolism," lecture III. Lancet 2: 142–148.

Gartler, S. M. 1956. A family study of urinary β-aminoisobutyric acid excretion. Am. J. Human Genet. 8: 120–126.

——. 1959a. An investigation into the Biochemical genetics of β-aminoisobutyric aciduria. Am. J. Human Genet. 11: 257–262.

——. 1959b. A metabolic investigation of urinary β-aminoisobutyric acid excretion in man. Arch. Biochem. 80: 400–409.

——, and Th. Dobzhansky. 1954. Excretion in human urine of an unknown amino acid derived from dates. Nature 174: 533.

——, I. L. Firschein, and Th. Dobzhansky. 1956. A chromatographic investigation of urinary aminoacids in the great apes. Am. J. Phys. Anthropol. 14: (N.S.) 41–57.

——, Th. Dobzhansky, and H. K. Berry. 1955. Chromatographic studies on urinary excretion patterns in monozygotic and dizygotic twins. II: Heritability of the excretion rates of certain substances. Am. J. Human Genetics 7: 108–121.

——, I. L. Firschein, and T. Gidaspow. 1957. Some genetical and anthropological considerations of urinary β-aminoisobutyric acid excretion. Acta. Genet. 6: 435–446.

——, ——, and B. S. Kraus. 1957. An investigation into the genetics and racial variation of BAIB excretion. Am. J. Human Genet. 9: 200–207.

Gerok, W. 1960. Quantitative Bestimmung der Freien und Gebundenen Amionsäuren im Blutserum dur Elutionschromatographie an Ionenaustauschern. Klin. Wschr. 38: 1212–1215.

——, and J. Gayer. 1961. Die tubulare Rückresorption der L-Aminosäuren in der Niere des Hundes, Klin. Wchnschr. 39: 540–546.

Gerber, G. B., G. Gerber, and K. I. Altman. 1960a. Some interrelated aspects of proline and hydroxy proline metabolism. Nature 185: 767.

——, ——, and ——. 1960b. Urinary excretion of β-aminoisobutyric acid after injection of thymidine labelled with tritium. Nature 187: 956–957.

Gerrard, J. W., and A. M. Marko. 1960. Congenital enzyme defects. Ann. Rev. Med. 11: 353–376.

Gerritsen, T., S. H. Lipton, F. M. Strong, and H. A. Waisman. 1961. On the isolation and identification of homocitrulline from urine. Biochem. Biophys. Res. Comm. 4: 379–383.

Ghadimi, H., and H. Shwachman. 1959. A screening test for aminoaciduria. New England J. Med. 261: 998–1001.

——, and ——. 1960. Evaluation of aminoaciduria in infancy and childhood. A.M.A. J. Dis. Child. 99: 457–475.

——, J. T. Clarke, and H. Shwachman. 1961. Simultaneous screening for mellituria and aminoaciduria. C.M.A.J. 84: 1308–1312.

——, M. W. Partington, and A. Hunter. 1961. A familial disturbance of histidine metabolism. New England J. Med. 265: 221–224.

——, ——, ——, and J. D. Bailey. 1961. An inborn error of histidine metabolism mimicking phenylketonuria (abstr. and discussion). Am. J. Dis. Child. 102: 454–457.

Gilbert, J. B., Y. Ku, L. L. Rogers, and R. L. Williams. 1960. Increase in taurine after intraperitoneal administration of amino acids to mice. J. Biol. Chem. 235: 1055–1060.

Glendening, M.B., A. J. Margolis, and E. W. Page. 1961. Aminoacid concentrations in fetal and maternal plasma. Am. J. Obstet. & Gynec. 81: 591–593.

Goldstein, D. A., and A. K. Solomon. 1960. Determination of equivalent pore radius for human red cells by osmotic pressure measurement. J. Gen. Physiol. 44: 1–18.

Goldstein, F. B. 1961. Biochemical studies in phenylketonuria. I: Experimental hyperphenylalaninemia in the rat. J. Biol. Chem. 236: 2656–2662.

Green, D. E., and R. F. Goldberger. 1961. Pathways of metabolism in the heart. Am. J. Med. 30: 666–678.

Greenstein, J. P., and M. Winitz. 1961. Chemistry of the Aminoacids. 3 vols. New York: Wiley J. & Son.

Gregory, K. F. 1961. Introductory remarks to the conference on "Multiple molecular forms of enzymes," pp. 657–658. Conf. ed. by Wróblewski F., Ann. New York Acad. Sc. 94: 655–1030.

Gross, D. 1959. Two-dimensional high-voltage paper electrophoresis of amino and other organic acids. Nature 184: 1298–1301.

Gross, J. B., M. W. Comfort, and J. A. Ulrich. 1957. Abnormalities of serum and urinary amino acids in hereditary and non hereditary pancreatitis. Tr. A. Am. Physicians 70: 127–139.

Guthrie, R. 1961. The inhibition assay: a method for detecting chemical differences in clinical specimens (abstr.) Am. J. Dis. Child. 102: 583–584.

——, and H. Tieckelmann. 1960. The inhibition assay: its use in screening urinary speci-

mens for metabolic differences associated with mental retardation. In Proc. of the London Conf. on Scientific Study of Mental Deficiency (in press).

Hagihira, H., E. C. Lin, A. H. Samiy, and T. H. Wilson. 1961. Active transport of lysine ornithine arginine and cystine by the intestine. Biochem. Biophys. Res. Comm. 4: 478–481.

——, M. Ogata, N. Takedatsu, and M. Suda. 1960. Intestinal absorption of amino acids. III: Interference between amino acids during intestinal absorption. J. Biochem. (Tokyo) 47: 139–143.

Hais, I. M., and K. Maçek. Eds. 1958. Handbuch der Papier Chromatographie. Band I. Grundlagen und Technik. Jena: Gustav Fischer.

Halberg, F. 1960. The 24-hour time scale; a time dimension of adaptive functional organization. Perspect. in Biol. and Med. 3: 491–527.

Hall, V. 1957. The protoplasmic basis of glomerular ultrafiltration. Am. Heart J. 54: 1–9.

Hanes, C. S. et al. 1961. Quantitative chromatographic methods (parts 2–7). Can J. Biochem. and Physiol. 39: 119–194, 417–451.

Hare, R. S. 1950. Endogenous creatinine in serum and urine. Proc. Soc. Exper. Biol. & Med. 74: 148–151.

Harris, H. 1953. Family studies on the urinary excretion of β-aminoisobutyric acid. Ann. Eugen. 18: 43–49.

——. 1959a. Human Biochemical genetics. Cambridge Univ. Press.

——. 1959b. Some recent developments in human biochemical genetics. Proc. X Int. Cong. Genetics 1: 100–107.

——, and E. B. Robson. 1957. Cystinuria. Am. J. Med. 22: 774–783.

——, and ——. 1959. A genetical study of ethanolamine phosphate excretion in hypophosphatasia. Ann. Human Genet. 23: 421–441.

——, and A. G. Searle. 1953. Urinary amino acids in mice of different genotypes. Ann. Eugenics 17: 165–167.

——, L. S. Penrose, and D. H. H. Thomas. 1959. Cystathioninuria. Ann. Human Gent. 23: 442–453.

——, U. Mittwoch, E. B. Robson, and F. L. Warren. 1955a. Pattern of amino acid excretion in cystinuria. Ann. Human Genet. 19: 195–208.

——, ——, ——, and F. L. Warren. 1955b. Phenotypes and genotypes in cystinuria. Ann. Human Genet. 20: 57–91.

Harris, M., and G. A. Morrison. 1961. Multiple inhibitions of cell metabolism by a single substance. Nature 191: 1276–1277.

Harrison, H. 1961. Vitamin D and calcium and phosphate transport. Pediatrics 28: 531–544.

Harrison, H. E., and H. C. Harrison. 1954. Experimental production of renal glycosuria, phosphaturia and aminoaciduria by injection of maleic acid. Science 120: 606–608.

Hausmann, E., and W. F. Neuman. 1961. Conversion of proline and hydroxyproline and its incorporation into collagen. J. Biol. Chem. 236: 149–154.

Heffelfinger, J. C., and R. J. LaGro. 1960. False negative urine tests in surveys for phenylketonuria. Pediatrics 25: 1086–1087.

Heinz, E., and P. O. Walch. 1958. Exchange diffusion, transport and intracellular level of amino acids in Ehrlich carcinoma cells. J. Biol. Chem. 233: 1488–1493.

Hempling, H. G. 1961. Potassium fluxes and glycine transport in the Ehrlich mouse ascites tumor cell: the effect of pyridoxal. (abstr.) Fed. Proc. 20: 139.

——, and D. Hare. 1961. The effect of glycine transport on potassium fluxes in the Ehrlich ascites tumor cell. J. Biol. Chem. 236: 2498–2503.

Henion, W. F., and E. W. Sutherland. 1957. Immunological differences of phosphorylases. J. Biol. Chem. 224: 477–488.

Hersov, L. A., and R. Rodnight. 1960. Hartnup disease in psychiatric practice: clinical and biochemical features of three cases. J. Neurol. & Neurosurg. & Psychiat. 23: 40–45.

Heys, F. M., R. J. Blattner, and H. B. G. Robinson. 1961. Osteogenesis imperfecta and odontogenesis imperfecta: clinical and genetic aspects in eighteen families. J. Pediat. 59: 234–245.

Hillcoat, B. L. 1960a. The occurrence of β-aminoisobutyric acid in the urine of patients with thalassemia Major. Aust. J. Exp. Biol. and Med. Sc. 38: 441–445.

——. 1960b. Urinary amino acid excretion in health and disease. Aust. Ann. Med. 9: 276–282.

Himwich, H. E., and J. F. Fazekas. 1940. Cerebral metabolism in mongolian idiocy and phenylpyruvic oligophrenia. Arch. Neurol. & Psychiat. 44: 1213–1218.

Hirschmann, S. Z., A. G. Morell, and I. H. Scheinberg. 1961. The heterogeneity of the copper-containing protein of human plasma, ceruloplasmin. Ann. New York Acad. Sc. 94: 960–969.

Hogben, C. A. M. 1960. The first common pathway. Fed. Proc. 19: 864–869.

Holzel, A., G. M. Komrower, and V. Schwarz. 1957. Galactosemia. Am. J. Med. 22: 702–711.

——, V. Schwarz, and K. W. Sutcliffe. 1959. Defective lactose absorption causing malnutrition in infancy. Lancet 1: 1126–1128.

Holt, L. E. Jr., and S. Snyderman. 1960. Disturbances of amino acid metabolism. Bull. New York Acad. Med. 36: 431–450.

——, ——, J. Dancis, and P. M. Norton. 1960. The treatment of a case of maple syrup urine disease (abstr.) Fed. Proc. 19: 10.

Holter, H. 1960. Pinocytosis. Int. Rev. of Cytol. 8: 481–504.

Honda, M. 1923. Untersuchung des Harns Gravider Frauen. J. Biochem. (Japan) 2: 351–359.

Hope, D. B. 1957. L-cystathionine in urine of pyridoxine deficient rats. Biochem. J. 66: 486–489.

Hotchkiss, R. D., and A. H. Evans. 1960. Fine structure of a genetically modified enzyme as revealed by relative affinities for modified substrate. Fed. Proc. 19: 912–925.

Hsia, D. Y-Y. 1959. Inborn errors of metabolism. Chicago: Year Book Publishers.

——. 1960a. Recent advances in biochemical detection of heterozygous carriers in hereditary disease. Metabolism 9: 301–315.

——. 1960b. Medical genetics. New England J. Med. 262: 1172–1178, 1222–1227, 1273–1278, 1318–1323.

——, and A. G. Steinberg. 1960. Studies on linkage between phenylketonuria and the blood groups. Am. J. Human Genet. 12: 277–286.

Huggins, A. K., and V. Moses. 1961. Breakdown of (2-14C)–glycine during paper chromatography. Nature 191: 668–670.

Huisman, T. H. J. 1957. L'elimination des acides amines chez des enfants normaux d'âges differents. Arch. franç. pédiat. 14: 166–180.

Ikawa, M., and E. E. Snell. 1960. Cell wall composition of lactic acid bacteria. J. Biol. Chem. 235: 1376–1381.

Isselbacher, K.J. 1960. Galactosemia, page 208–225 in reference 446.

Jagenburg, O. R. 1959. The urinary excretion of free amino acids and other amino compounds by the human. Scandinav J. Clin. & Lab. Invest. 11: Supp. 43.

Jackson, C. E., and J. H. Carey. 1961. Progressive muscular dystrophy: autosomal recessive type. Pediatrics 28: 77–84.

——, P. C. Talbert, and H. D. Caylor. 1960. Hereditary hyperparathyroidism. J. Indiana M. A. 53: 1313–1316.

Jacob, F., and J. Monod. 1961. Genetic regulatory mechanisms in the synthesis of proteins. J. Molec. Biol. 3: 318–356.

Jacobs, F. A., L. J. Coen, and R. S. L. Hillman. 1960. Influence of pyridoxine, pyridoxal phosphate, deoxypyridoxine and 2, 4,-dinitrophenol on methionine absorption. J. Biol. Chem. 235: 1372–1375.

Jepson, J. B. 1956. Indolylacetyl-glutamine and other indole metabolites in Hartnup disease. Biochem. J. 64: 14P.

——, and M. J. Spiro. 1960. Hartnup disease. In Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S., Eds.: The Metabolic Basis of Inherited Disease. New York: McGraw Hill, pp. 1338–1364.

——, A. J. Smith, and L. B. Strang. 1958. An inborn error of metabolism with urinary excretion of hydroxyacids, ketoacids and amino acids. Lancet 2: 1334–1335.

Jerôme, H., J. Lejeune, and R. Turpin. 1960. Étude de l'excretion urinaire de certains metabolites du tryptophane chez les enfants mongoliens. C. R. Acad. Sc. (Paris). 251: 474–476.

Jervis, G. A. 1954. Phenylpyruvic oligophrenia. A. Res. Nerv. & Ment. Dis. Proc. 33: 259–282.

Johnson, D. E., S. J. Scott, and A. Meister. 1961. Gas-liquid chromatography of amino acid derivatives. Anal. Chem. 33: 669–673.

Johnstone, R. M., and P. G. Scholefield. 1961. Factors controlling the uptake and retention of methionine and ethionine by Ehrlich ascites carcinoma cells. J. Biol. Chem. 236: 1419; 1424.

Jonxis, J. H. P. 1951. The influences of differences in food on the amino acid excretion (B.P.A. Proc. 22nd Gen. Meeting). Arch. Dis. Child. 26: 272.

——. 1961. Some investigations on rickets. J. Pediatrics 59: 607–615.

——. 1962. Personal communication.

——, P. A. Smith, and T. H. J. Huisman. 1952. Rickets and amino-aciduria. Lancet 2: 1015–1017.

Joseph, R., M. Ribierre, J-C. Job, and M. Girault. 1958. Maladie familiale assosciant des convulsions a début très precoce, une hyperalbuminorachie et une hyperamunoacidurie. Arch. franç pediat. 15: 374–387.

Kakimoto, Y., and M. D. Armstrong. 1961. β-L-aspartyl-L-histidine, a normal constituent of human urine. J. Biol. Chem. 236: 3280–3282.

Kapellar-Adler, R., and H. Bermann. 1934. Zur Frage der Histidinurie bei der Gravidität. Klin. Wchnschr. 13: 1220.

Kaplansky, S. Ya. 1959. Disorders of amino acid metabolism in protein deficiency and their correction. Clin. Chem. 5: 186–202.

Karpinski, F. E. 1960. Cystinosis a clinical report of three cases in one family. Pediatrics 25: 1018–1024.

Keller, R. A., G. H. Stewart, and J. C. Giddings. 1960. Chromatography. Ann. Rev. Phys. Chem. 11: 347–368.

Kessel, D., and M. Lubin. 1962. Transport of proline in escherichia coli, Biochim. Biophys. Acta 57: 32–43.

Khachadurian, A., W. E. Knox, and A. M. Cullen. 1960. Colorimetric ninhydrin method for total alpha amino acids. J. Lab. & Clin. Med. 56: 321–332.

Knapp, A. 1960. Über eine neue, Hereditäre, von Vitamin B_6 Abhängige Störung in Tryptophan-stoffwechsel. Clin. Chim. Acta. 5: 6–13.

Knauff, H. G., and P. Schabert. 1960. Zur Quantitative Aminosäurebestimmung in Körperflüssigkeiten und Organ Extrakten nach Elutionschromatographie an Ioenaustercher säulen nut Automatischer Registrierung der Ergebnisse. Klin. Wchrschr. 38: 1206–1212.

Knight, C. S. 1959. A new two-dimensional paper chromatographic method applied to the separation of amino acids. Nature 184: 1486–1487.

——. 1960. Separation of amino acid mixtures on sulphonated polystyrene resin-loaded papers. Nature 188: 739–740.

Knox, W. E. 1958a. Sir Archibald Garrod's "Inborn errors of Metabolism." I: Cystinuria. Am. J. Human Genet. 10: 3–32.

——. 1958b. Sir Archibald Garrod's "Inborn errors of metabolism." II: Alkaptonuria. Am. J. Human Genet. 10: 95–124.

——. 1960a. Cystinuria. In Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S., Eds.: The Metabolic Basis of Inherited Disease. New York: McGraw Hill, pp. 1302–1337.

——. 1960b. Phenylketonuria. In Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S., Eds.: The Metabolic Basis of Inherited Disease. New York: McGraw Hill, pp. 321–382.

Korn, D. 1960. Demonstration of cystine crystals in peripheral white blood cells in a patient with cystinosis. New England J. Med. 262: 545–548.

Kostyo, J. L., and F. L. Engel. 1960. In-vitro effects of growth hormone and corticotropin preparations on amino acid transport by isolated rat diaphragms. Endocrinology 67: 708–716.

——, J. Hotchkiss, and E. Knobil. 1959. Stimulation of amino acid transport in isolated diaphragm by growth hormone added in-vitro. Science 130: 1653–1654.

Krawczyński, J., J. Kujawa, W. Tyburczyk, and M. Zelazowska. 1961. Diurnal variations in renal excretion of electrolytes, uric acid, and total amino nitrogen in various renal diseases and in essential hypertension. Clin. Chim. Acta. 6: 637–640.

Kretchmer, M. 1959. Enzymatic patterns during development. Pediatrics 23: 606–617.

——. 1960. An enzymatic mechanism for tyrosinuria. (abstr.) Am. J. Dis. Child. 100: 605.

——, S. Z. Levine, and H. McNamara. 1957. The *in-vitro* metabolism of tyrosine and its intermediates in the lever of the premature infant (abstr.) A.M.A. J. Dis. Child. 93: 19–20.

——, M. Stone, and C. Bauer. 1958. Hereditary enzymatic effects as illustrated by hypophosphatasia. Ann. New York Acad. Sc. 75: 279–285.

——, S. Z. Levine, H. McNamara, and H. L. Barnett. 1956. Certain aspects of tyrosine metabolism in the young. I: The development of the tyrosine oxidizing system in human liver. J. Clin. Invest. 35: 236–244.

Kupiecki, F. P., and M. J. Coon. 1957. The enzymatic synthesis of β-amino-isobutyrate, a product of valine metabolism, and of β-alanine, a product of β-hydroxypropionate metabolism. J. Biol. Chem. 229: 743–754.

LaDu, B. N. 1960a. Tyrosinosis. In Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S., Eds.: The Metabolic Basis of Inherited Disease. New York: McGraw Hill, pp. 383–393.

——. 1960b. Alcaptonuria. In Stanbury, J. B., Wyngaarden, J. B., and Fredrickson D. S., Eds.: The Metabolic Basis of Inherited Disease. New York: McGraw Hill, pp. 394–427.

Lahey, M. E., and P. F. Bray. 1960. Unique neuromuscular disorder (? familial) characterized by hyperphosphatasemia and phosphoethanolaminuria (abstr.) Am. J. Dis. Child. 100: 606.

Lane, M.R. 1961. Maple syrup urine disease. J. Pediatrics 58: 80–86.
Laster, L., and F. J. Ingelfinger. 1961. Intestinal absorption: aspects of structure, function and disease of the small-intestine mucosa. New England J. Med. 264: 1138–1147 (part 1).
Lathem, W., K. Baker, and S. Bradley. 1955. Urinary amino acid excretion in renal disease, with observations on the Fanconi syndrome. Am. J. Med. 18: 249–258.
Leaf, A. 1960. The syndrome of osteomalacia, renal glycosurie aminoaciduria and hyperphosphaturia (the Fanconi syndrome), pages 1222–1245 in Reference 442.
Lefevre, P. G. 1961. Sugar transport in the red blood cell: structure-activity relationships in substrates and antagonists. Pharmacol. Rev. 13: 39–70.
Lehmann, O. 1960. A family with Marfan's syndrome traced through an affected newborn. Acta. Pediatrica. 49: 540–550.
Lehninger, A. L. 1959. Respiratory-energy transformation. Rev. Modern Physics 30: 136–146 (also In Biophysical Science, a Study Program. New York: J. Wiley & Sons, pp. 136–146).
Leoschke, W. L., and C. A. Elvehjem. 1959. The importance of arginine and methionine for growth and fur development of mink fed purified diets. J. Nutrition 69: 147–150.
Levenson, S. M. 1960. Page 78–79 Ross. Pediatric Research Conference No. 25, Fomon S. (ed.), Ross Laboratories Columbus.
Levin, B., H. M. M. Mackay, and V. G. Oberholzer. 1961. Argininosuccinicaciduria. Arch. Dis. Childhood 36: 622–632.
Levine, S. Z. 1947. Tyrosine and phenylalanine metabolism in infants and the role of vitamin C. Harvey Lectures Series 42: 303–331.
——. 1961. Personal communication.
——, and T. H. Wilson. 1960. Transport of L-tyrosine by the small intestine in-vitro. A. J. Physiol. 199: 127–130.
Link, J. K., and E. C. Roldan. 1958. Mental deficiency, spasticity and congenital ichthyosis. J. Pediat. 52: 712–714.
Lowe, C. U., M. Terrey, and E. A. MacLachlan. 1952. Organic-aciduria, decreased renal ammonia production, hydrophthalmos and mental retardation; a clinical entity. Am. J. Dis. Child. 83: 164–184.
Luder, J., and W. Sheldon. 1955. A familial tubular absorption defect of glucose and amino acids. Arch. Dis. Child. 30: 160–164.
Lundquist, F., and H. Galatius-Jensen. 1960. Quantitative determination of amino acids by combined displacement and paper chromatography. Scandinav. J. Clin. & Lab. Invest. 12: 342–352.
Ma, T. S., and G. Zuazago. 1942. Micro-kjeldahl determination of nitrogen, a new indicator, and an improved rapid method. Indus. and Engin. Chem. (Anal. Ed.) 14: 280–282.
MacDougall, J. A. 1961. Dissolution of cystine calculi. B. M. J. 2: 809–810.
Mackenzie, D. Y., and L. I. Woolf. 1959. "Maple syrup urine disease." An inborn error of metabolism of valine, leucine and isoleucine associated with gross mental deficiency. Brit. M. J. 1: 90–91.
Malvin, A. L. 1961. The stop-flow method and its use in studying renal function. Prog. Cardiovasc. Dis. 3: 432–448.
Manchester, K. L. 1961. Insulin and incorporation of amino acids into protein of muscle. Biochem. J. 81: 135–147.
Martin, G. R., S. E. Mergenhagen, and D. J. Prockop. 1961. Influence of scurvy and lathyrism (odoratism) on hydroxyproline excretion. Nature 191: 1008–1010.

Marver, H. S. 1961. Studies on tryptophan metabolism. I: Urinary tryptophan metabolites in hypoplastic anemias and other hematological disorders. J. Lab. & Clin. Med. 58: 425–436.

McCance, R. A., A. B. Morrison, and C. E. Dent. 1955. The excretion of phosphoethanolamine and hypophosphatasia: preliminary communication. Lancet 1: 131.

McCoy, E. E. 1961. Free amino acids, γ-globulin and fatty acid levels in mongols (abstr.) Am. J. Dis. Child. 100: 611.

McDonald, H. J., M. C. Urbin, and M. B. Williamson. 1951. Ionography: some aspects of ion migration on paper in an electrical field. J. Colloid Sc. 6: 236–244.

McEvoy-Bowe, E., and J. W. H. Lugg. 1961. A direct quantitative paper chromatography of amino acids and its application to the urinary excretion of some human ethnic groups. Biochem. J. 80: 616–623.

McKay, E., and H. Thom. 1961. A chromatographic study of the amino acids in urine and C.S.F. of mentally deficient children. J. Ment. Sc. 107: 261–267.

McKhann, G., R. W. Albers, L. Sokoloff, O. Mickelson, and D. B. Tower. 1960. The quantitative significance of the γ-aminobutyricacid pathway in cerebral oxidative metabolism. In Roberts, E., Ed.: Inhibition in the Central Nervous System and γ-Aminobutyricacid. New York: Pergamon Press, pp. 169–181.

McKusick, V. A. 1961. Medical Genetics, 1958–1960. St. Louis: C. V. Mosby Co.

——. 1960. Heritable disorders of connective tissue, ed. 2. St. Louis: C. V. Mosby.

McMenamy, R. H., C. C. Lund, G. J. Neville, and D. F. H. Wallach. 1960. Studies of unbound amino acid distributions in plasma, erythrocytes leukocytes and urine of normal human subjects. J. Clin. Invest. 39: 1675–1687.

Medes, G. 1942. A new error of tyrosine metabolism: tyrosinosis. The intermediary metabolism of tyrosine and phenylalanine. Biochem. J. 26: 917–940.

Meister, A. 1957. Biochemistry of the Amino Acids. New York: Academic Press.

Menano, H., S. F. G. da Costa, M. E. Relvas, F. Ferraz, M. Halpern, and F. Teixeira. 1960. Cheilosis-like mucous and cutaneous lesions, seborrheic dermatitis, and hereditary aminoaciduria. Helvet. pediat. acta. 15: 487–494.

Menkes, J. H. 1959a. Maple syrup urine disease: isolation and identification of organic acids in the urine. Pediatrics 23: 348–353.

——. 1959b. Maple syrup urine disease: investigations into the metabolic disease. Neurology 9: 826–835.

——. 1960. Maple syrup disease. Maandschr. kindergeneesk. 28: 54–59.

——, and G. A. Jervis. 1961. Developmental retardation associated with an abnormality in tyrosine metabolism. Pediatrics 28: 399–409.

——, P. L. Hurst, and J. M. Craig. 1954. A new syndrome: progressive familial infantile cerebral dysfunction associated with an unusual urinary substance. Pediatrics 14: 462–466.

Messer, M. 1961. Interference by amino acids and peptides with the photometric estimation of proline. Anal. Biochem. 2: 353–359.

Metcoff, J. 1959. Some aspects of renal structure and function concerned with regulation of the body fluids. Pediat. Clin. N. Am. 6: 43–80.

——. 1961. (Editor) Intermediary metabolism and transport in the intestine page 1–68 and kidney page 183–285. Proc. 12th Ann. Conf. Nephrotic Syndrome, Nat. Kidney Dis. Found. New York.

Meyer, H. 1957. The ninhydrin reaction and its analytical applications. Biochem. J. 67: 333–340.

Milne, M. D., A. M. Asatoor, K. D. G. Edwards, and L. W. Loughridge. 1961. The intestinal absorption defect in cystinuria. Gut. 2: 323–337.

——, B. H. Scribner, and M. A. Crawford. 1958. Non-ionic diffusion and the excretion of weak bases and acids. Am. J. Med. 24: 709–729.

——, M. A. Crawford, C. B. Girão, and L. W. Loughridge. 1960a. The excretion of indolyl-acetic acid and related indolic acids in man and the rat. Clin. Sc. 19: 165–179.

——, M. A. Crawford, C. B. Girão, and L. W. Loughridge. 1960b. The metabolic disorder in Hartnup disease. Quart. J. Med. 29: 407–421.

Mitoma, C., R. M. Auld, and S. Udenfriend. 1957. On the nature of the enzymatic defect in phenylpyruvic oligophrenia. Proc. Soc. Exper. Biol. & Med. 94: 634–635.

——, T. E. Smith, J. D. Davidson, S. Udenfriend, F. M. DaCosta, and A. Sjoerdsma. 1959. Improvements in methods for measuring hydroxyproline. J. Lab. & Clin. Med. 53: 970–976.

Mizuhara, S. 1961. New sulphur-containing amino acids in urine. Seikagaku (J. Jap. Biochem. Soc.) 33: 353–361.

Moore, S., and W. H. Stein. 1951. Chromatography of aminoacids on sulfonated polystyrene resins. J. Biol. Chem. 192: 663–681.

——, and ——. 1954a. Procedures for the chromatographic determination of aminoacids on four per cent cross-linked sulfonated polystyrene resins. J. Biol. Chem. 211: 893–906.

——, and ——. 1954b. A modified ninhydrin reagent for the photometric determination of aminoacids and related compounds. J. Biol. Chem. 211: 907–913.

——, D. H. Spackman, and W. H. Stein. 1958. Chromatography of aminoacids on sulfonated polystyrene resins. Anal. Chem. 30: 1185–1190.

Morris, M. D., B. D. Lewis, P. D. Doolan, and H. A. Harper. 1961. Clinical and biochemical observations on an apparently non-fatal variant of branched-chain ketoaciduria (maple syrup urine disease). Pediatrics 28: 918–923.

Nakai, H., B. J. Landing, and M. D. Pettit. 1960. Distinguishing hypophosphatasia from cretinism by means of alkaline phosphatase stain of skin biopsy. Am. J. Clin. Path. 33: 115–117.

Neame, K. D. 1961. Phenylalanine as an inhibitor of transport of amino acids in brain. Nature 192: 173–174.

Neil, M. W. 1959. The absorption of cystine and cysteine from rat small intestine. Biochem. J. 71: 118–124.

Noall, M. W., T. R. Riggs, L. M. Walker, and H. N. Christensen. 1957. Endocrine control of amino acid transfer. Distribution of an unmetabolized amino acid. Science 126: 1002–1005.

Northrop, J. H. 1926. Convenient method for formol titration. J. Gen. Physiol. 9: 767–769.

Norton, P. M., E. L. Pratt, and E. Hasselmeyer. 1954. Urinary amino acid excretion by premature infants (abstr. and discussion). A.M.A. J. Dis. Child. 88: 659–660.

——, E. Roitman, S. E. Snyderman, and L. E. Holt Jr. 1962. A new finding in Maple Syrup Urine Disease. Lancet 1: 26–27.

Novikoff, A. B. 1960. The rat kidney: cytochemical and electron microscopic studies. In Quinn, E., and Kass, E., Eds.: The Biology of Pyelonephritis. Henry Ford Hosp. Sympos. Boston: Little Brown & Co. pp. 113–114.

Nyhan, W. L., M. Borden, and B. Childs. 1961. Idiopathic hyperglycinemia: a new disorder of amino acid metabolism. II: The concentrations of other amino acids in the plasma and their modification by the administration of leucine. Pediatrics 27: 539–550.

Oates, J. A., and A. Sjoerdsma. 1960. Neurologic effects of tryptophan in patients receiving a monoamine oxidase inhibitor. Neurology 10: 1076–1078.

O'Brien, D. 1961. Personal communication.
——, A. P. Groshek. 1962. The abnormality of tryptophane metabolism in children with *mongolism* (abstr.) Arch. Dis. Childhood 37: 17–20.
——, J. Bergstedt, J. Butterfield, F. Ibbott, and L. O. Lubchenco. 1960. Observations on the urinary excretion of amino acids by the premature infant. Acta. paediat. 49: 258–264.
——, J. Butterfield, D. Walker, F. A. Ibbott, and L. O. Lubchenco. 1961. The renal clearances of free amino acids in the premature infant (abstr.) Am. J. Dis. Child. 102: 627–628.
Oxender, D. L., and H. N. Christensen. 1959. Transcellular concentration as a consequence of intracellular accumulation. J. Biol. Chem. 234: 2321–2324.
——, and M. Royer. 1961. Action of pyridoxal phosphate and related agents on uptake of amino acids by Ehrlich ascites cells (abstr.) Fed. Proc. 20: 140(e).
Page, E. W., M. B. Glendening, W. Dingman, and H. A. Harper. 1954. The cause of histidinuria in normal pregnancy. Am. J. Obst. & Gynec. 68: 110–118.
Paigen, W., and W. K. Noell. 1961. Two linked genes showing a similar timing of expression in mice. Nature 190: 148–150.
Paine, R. S. 1957. The variability in manifestations of untreated patients with phenylketonuria (phenylpyruvic aciduria). Pediatrics 20: 290–301.
——. 1960. Evaluation of familial biochemically determined mental retardation in children with special reference to aminoaciduria. New England J. Med. 262: 658–665.
Pare, C. M. B., M. Sandler, and R. S. Stacey. 1958. Decreased 5-hydroxytryptophan decarboxylase activity in phenylketonuria. Lancet 2: 1099–1101.
Park, C. R., D. Reinwein, M. J. Henderson, E. Cadenas, and H. E. Morgan. 1959. The action of insulin on the transport of glucose through the cell membrane. Am. J. Med. 26: 674–684.
Parry, T. E. 1957. Paper chromatography of 56 amino compounds using phenol and butanol acetic acid as solvents. Clin. Chim. Acta. 2: 115–125.
Partington, M. W. 1961. The early symptoms of phenylketonuria. Pediatrics 27: 465–473.
Pasieka, A. E., and J. F. Morgan. 1956. Specific determination of hydroxy-L-proline in biological materials. Proc. Soc. Exper. Biol. & Med. 92: 96–98.
Patrick, A. D. 1961. Maple syrup urine disease. Arch. Dis. Child. 36: 269–272.
Pease, D. C. 1955. Electron microscopy of the tubular cells of the kidney cortex. Anat. Rec. 121: 723–743.
Perry, T. L., and R. T. Jones. 1961. The amino acid content of human cerebral spinal fluid in normal individuals and in mental defectives. J. Clin. Invest. 40: 1363–1372.
Piel, C., and H. A. Harper. 1961. Excretion of D- and L-methionine in children with generalized aminoaciduria. J. Pediat. 59: 861–871.
Piez, K. A. 1961. Amino acid composition of some calcified proteins. Science 134: 841.
——, and L. Morris. 1960. A modified procedure for the automatic analysis of amino acids. Anal. Biochem. 1: 187–201.
Povlik, M. D., and A. G. Bearn. 1962. Heterogeneity of ceruloplasmin. Clin. Chim. Acta 7: 374–382.
Prockop, D. J., and S. Udenfriend. 1960. A specific method for the analysis of hydroxy proline in tissues and urine. Anal. Biochem. 1: 228–239.
——, C. Mitoma, and A. Sjoerdsma. 1959. Observations on hydroxyproline metabolism in man (abstr.) Fed. Proc. 18: 434(c).
Quastel, J. H. 1960. Intestinal absorption of sugars and amino acids. Am. J. Clin. Nutr. 8: 137–146.

Ratner, S. 1954. Urea synthesis and metabolism of arginine and citrulline. Advances Enzymol. 15: 319–387.

Rathburn, J. C. 1948. Hypophosphatasia. A new developmental anomaly. Am. J. Dis. Child. 75: 822–831.

Raven, A. M., F. W. Langemann, and R. H. Wasserman. 1960. Studies of the effect of lysine on the absorption of radiocalcium and radiostrontium by the rat. J. Nutrition 72: 29–36.

Reed, G. 1942. The polarographic estimation of cystine in urine. J. Biol. Chem. 142: 61–64.

Renwick, J. H., S. D. Lawler, and V. A. Cowie. 1960. Phenylketonuria, a genetical linkage study using phenylalanine tolerance tests. Am. J. Human Genet. 12: 287–322.

Rhodin, J. 1958a. Electron microscopy of the kidney. Am. J. Med. 24: 661–675.

——. 1958b. Anatomy of kidney tubules. Inter. Rev. Cytol. 7: 485–534.

Richards, W., G. M. Donnell, W. A. Wilson, and D. S. Stowens. 1960. The oculo-cerebro-renal syndrome (abstr. and discussion). Am. J. Dis. Child. 100: 707–708.

Riggs, T. R., and L. M. Walker. 1958. Diminished uptake of C^{14} α-aminoisobutyric acid by tissues of Vitamin B_6 deficient rats. J. Biol. Chem. 233: 132–135.

——, and ——. 1960. Growth hormone stimulation of amino acid transport in rat tissues in-vivo. J. Biol. Chem. 235: 3603–3607.

——, ——, and H. N. Christensen. 1958. Potassium migration and amino acid transport. J. Biol. Chem. 233: 1479–1484.

Roberts, E. 1960. Free amino acids of nervous tissue: some aspects of metabolism of gamma-aminobutyricacid. In Roberts, E., Ed.: Inhibition in the Nervous System and γ-Aminobutyric Acid. New York: Pergamon Press, pp. 144–152.

Robson, E. B., and G. A. Rose. 1957. The effect of intravenous lysine on the renal clearances of cystine, arginine and ornithine in normal subjects, in patients with cystinuria and Faconi syndrome, and in their relatives. Clin. Sc. 16: 75–93.

Rose, G. A., and A. C. Kerr. 1958. The amino acids and phosphoethanolamine in salivary gland secretions of normal men and of patients with abnormal calcium, phosphorus, and amino acid metabolism. Quart. J. Exper. Physiol. 43: 160–168.

Rosenberg, L. E., P. S. Mueller, and D. M. Watkin. 1961. A new syndrome: familial growth retardation renal aminoaciduria and cor pulmonale. II: Investigation of renal function, amino acid metabolism and genetic transmission. Am. J. Med. 31: 205–215.

Rosenthal, I. M., S. L. Bonting, W. Hogen, and C. L. Pirani. 1960. Tissue alkaline phosphatase in hypophosphatasia. A.M.A. J. Dis. Child. 99: 185–192.

Rowley, P. T., P. S. Mueller, D. M. Watkin, and L. E. Rosenberg. 1961. Familial growth retardation, renal aminoaciduria and cor pulmonale. I: Description of a new syndrome with case reports. Am. J. Med. 31: 187–204.

Rutstein, D. D. 1961. Maintaining contact with medical knowledge. New England J. Med. 265: 321–324.

Sakai, K., and I. Kitagawa. 1957a. An atypical case of tyrosinosis (1-para-hydroxyphenyllacticaciduria) I: clinical and laboratory findings. Jikeikai M. J. 4: 1–10.

Ibid. 1957b. Ibid. II: A research on the metabolic block. Jikeikai M. J. 4: 11–15.

Sandler, M., and C. M. B. Pare. 1954. Starvation aminoaciduria. Lancet. 1: 494–495.

Sass-Kortsak, A., M. Cherniak, D. W. Geiger, and R. J. Slater. 1959. Observations on ceruloplasmin in Wilsons disease. J. Clin. Invest. 38: 1672–1682.

Scaglioni, P. R. and J. F. Lucey. 1956. Further observations on hypophosphatasia (abstr. plus discussion). A.M.A. J. Dis. Child. 92: 493–495.

Schachter, D., and J. S. Britten. 1961. Active transport of non electrolytes and the potential gradients across intestinal segments in-vitro. Fed. Proc. 20: 137.

Schafer, I. A., C. R. Scriver, and M. E. Efron. 1961. Multiple congenital anomalies in a family with an error in proline metabolism. (Abstr.) Am. J. Dis. Child. 102: 632.

Scheinberg, I. H., and I. Sternleib. 1960. Copper metabolism. Pharmacol. Rev. 12: 355–381.

Schendel, H. E., A Antonis, and J. D. L. Hansen. 1959. Increased aminoaciduria in infants with kwashiorkor, Fed Natural and Synthetic Diets. Pediatrics 23: 662–675.

Schlamowitz, M. 1958. Immunochemical studies on alkaline phosphatase. Ann. New York Acad. Sc. 75: 373–379.

Schmid, R. and L. Hammaker. 1961. Hereditary absence of muscle phosphorylase (McArdle's syndrome). New England J. Med. 264: 223–225.

Schoen, E. J., and G. Young. 1959. Lowe's syndrome: abnormalities in renal tubular function in combination with other congenital defects. Am. J. Med. 27: 781–792.

Scholefield, P. 1961. Competition by amino acids for transport into Ehrlich ascites tumour cells. Can. J. Biochem. and Physiol. 39: 1717–1736.

Scholten, H. G. 1960. Ein meisje met het syndroom van Lowe. Maandschrift V. Kindergn. 28: 251–255.

Schram, E., and E. J. Bigwood. 1953. Fraction collector for chromatography. Anal. Chem. 25: 1424.

——, J. P. Dustin, S. Moore, and E. J. Bigwood. 1953. Application de la chromatographie sur échangeur d'ions à l'étude de la composition des aliments en acides aminés. Anal. Chim. Acta. 9: 149–162.

Scriver, C. R., and K. N. F. Shaw. Hartnup disease: a genetically determined abnormality affecting transcellular transport of certain amino acids (to be submitted to the New England J. Med.).

——, R. Goldbloom, and C. Roy. Unpublished observations.

——, J. H. Hutchison, and D. B. Coursin. 1961. Vitamin B_6 deficiency in a human infant: biochemical observations of aminoacid and coenzyme metabolism (abstr.) Am. J. Dis. Child. 102: 632–633.

——, I. A. Schafer, and M. L. Efron. 1961a. Evidence for a renal tubular amino acid transport system common to glycine, L-proline and hydroxy-L-proline. J. Clin. Invest. 40: 1080.

——, ——, and ——. 1961b. A new renal tubular amino acid transport system and a new hereditary disorder of amino acid metabolism. Nature 192: 672–673.

Sereni, P., H. McNamara, M. Shibuya, N. Kretchmer, and H. L. Barnett. 1955. Concentrations in plasma and rate of urinary excretion of amino acids in premature infants. Pediatrics 15: 575–585.

Shaw, K. N. F. 1961. Personal communication.

——, D. Redlich, S. W. Wright, and J. B. Jepson. 1960. Dependence of urinary indole excretion in Hartnup disease upon gut flora (abstr.) Fed. Proc. 19: 194.

——, T. L. Perry, E. Jacobs, M. Gutenstein, D. Walker, and R. Koch. 1961. Screening of urine specimens for metabolic abnormalities. Chem. and Eng. Report Calif. Instit. Technol. page 97.

Sheldon, W., J. Luder, and B. Webb. 1961. A familial tubular absorption defect of glucose and amino acids. Arch. Dis. Child. 36: 90–95.

Simon, S. 1911. Zur stichstoffverteilung in Urin des Neugeborenen. Ztschr. Kinderh. 2: 1–17.

Sjoerdsma, A., J. D. Davidson, S. Udenfriend, and C. Mitoma. 1958. Increased excretion of hydroxyproline in Marfan's syndrome. Lancet. 2: 994.

Sjögren, T., and T. Larsson. 1957. Oligophrenia in combination with congenital ichthyosis and spastic disorder: clinical and genetic study. Acta. Psychiat. 32: Supplement 113.

Smith, A. J., and L. B. Strang. 1958. An inborn error of metabolism with the urinary excretion of α-hydroxy-butyric acid and phenylphruvic acid. Arch. Dis. Child. 33: 109–113.

Smith, H. 1959. From fish to philosopher. Boston: Little, Brown & Co.

Smith, I. 1960. Editor; Chromatographic and electrophoretic techniques. New York: Interscience Publisher. 2 vols.

Snyderman, S. E. 1958. Metabolism of amino acids. Pediatrics 21: 117–142.

——, and L. E. Holt, Jr. 1960. Aminoaciduria and certain related disorders of amino acid metabolism. Adv. Pediatrics 11: 209–241.

——, A. Boyer, and L. E. Holt, Jr. 1959. The arginine requirement of the infant. A.M.A. J. Dis. Child. 97: 192–195.

——, ——, S. V. Phansalkar, and L. E. Holt, Jr. 1961. Essential amino acid requirements of infants: tryophan. Am. J. Dis. Child. 102: 163–167.

——, J. Dancis, P. M. Norton, and L. E. Holt, Jr. 1960. The treatment of a case of maple syrup urine disease (abstr.) Am. J. Dis. Child. 100: 633.

——, E. L. Roitman, A. Boyer, and L. E. Holt, Jr. 1961. Essential amino acid requirements of infants: leucine. Am. J. Dis. Child. 102: 157–162.

Sobel, C., R. J. Henry, N. Chiamori, and M. Segalove. 1957. Determination of α-amino acid nitrogen in urine. Proc. Soc. Exper. Biol. & Med. 95: 808–813.

Solomon, A. K., T. J. Gill 3rd, and G. L. Gold. 1956. The kinetics of cardiac glucoside inhibition of potassium transport in human erythrocytes. J. Gen. Physiol. 40: 327–350.

Sörbo, B. 1961. A method for determination of taurine in urine. Clin. Chim. Acta. 6: 87–91.

Soupart, P. 1954a. Contributions à l'étude de l'histidinurie au cours de la grossesse. Acta. Clin. Belg. 9: 297–318.

——. 1954b. Le débit urinaire de l'histidine au cours du cycle menstruel normal. Acta. Clin. Belg. 9: 319–324.

——. 1959. Urinary excretion of free amino acids in normal adult men and women. Clin. Chim. Acta. 4: 265–271.

Spackman, D. H., W. H. Stein, and S. Moore. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30: 1190–1206.

Spencer, R. P., and W. E. Knox. 1960. Comparative enzyme apparatus of the gut mucosa. Fed. Proc. 19: 886–897.

Sporn, M. B., W. Dingman, and A. Defalco. 1959. A method for studying metabolic pathways in the brain of the intact animal. J. Neurochem. 4: 141–147.

——, W. Dingman, A. Defalco, and R. K. Davies. 1959. The synthesis of urea in the living rat brain. J. Neurochem. 5: 62–67.

Stanbury, J. B., J. B. Wyngaarden, and D. S. Fredrickson. 1960. The metabolic basis of inherited disease. New York: McGraw Hill.

Stanbury, P. J. 1961. Comparison of the mitochondria of the small intestine of vertebrates. Nature 192: 67.

Stanbury, S. W. 1958. Some aspects of disordered renal tubular function. Adv. Int. Med. 9: 231–282.

Stearns, G. 1956. Creatine and creatinine metabolism in infancy: pages 157–161 in, Scheinberg, I. H. Infant Metabolism. Proc. W.H.O. Seminars. 1950. MacMillan New York.

Stein, W. D. 1961. Dimer formation and glucose transfer across the membrane of the red blood cell. Nature 191: 1277–1280.

Stein, W. H. 1951. Excretion of amino acids in cystinuria. Proc. Soc. Exper. Biol. and Med. 78: 705–708.

——. 1953. A chromatographic investigation of the amino acid constituents of normal urine. J. Biol. Chem. 201: 45–58.

——, and S. Moore. 1954. Free amino acids of human blood plasma. J. Biol. Chem. 211: 915–926.

——, A. G. Bearn, and S. Moore. 1954. The amino acid content of the blood and urine in Wilson's disease. J. Clin. Invest. 33: 410–419.

Sternlieb, I., and I. H. Scheinberg. 1961. Ceruloplasmin in health and disease. Ann. New York Acad. Sc. 94: 71–76.

——, A. G. Morell, C. D. Bauer, B. Combes, S. de Bobes-Sternberg, and I. H. Schienberg. 1961. Detection of the heterozygous carrier of the Wilson's disease gene. J. Clin. Invest. 40: 707–715.

Strecker, H. J. 1960. The interconversion of glutamic acid and proline. III: δ-pyrroline-5-carboxylic acid dehydrogenase. J. Biol. Chem. 235: 3218–3223.

Summer, G. K. 1961a. Iminoaciduria in the Marfan syndrome (abstr.) Am. J. Dis. Child. 102: 587–588.

——. 1961b. Oral proline tolerance in osteogenesis imperfecta. Science 134: 1527–1528.

Sutherland, B. S., H. K. Berry, and H. C. Shirkey. 1960. A syndrome of phenylketonuria with normal intelligence and behavior disturbance. J. Pediatrics 57: 521–525.

Sutton, H. E. 1960. Beta-aminoisobutyricaciduria. In Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S., The Metabolic Basis of Inherited Disease. New York: McGraw Hill, pp. 792–806.

——, and R. E. Tashian. 1960. Inherited variations in aromatic metabolism. Metabolism 9: 284–292.

Szent-Györgyi, A., and C. Cohen. 1957. Role of proline in polypeptide chain configuration of proteins. Science 126: 697.

Tada, K., and S. P. Bessman. 1960. Indicanuria in phenylketonuria. Metabolism 9: 377–385.

Tallan, H. H., S. Moore, and W. H. Stein. 1954. Studies on the free amino acids and related compounds in the tissues of the cat. J. Biol. Chem. 211: 927–939.

——, ——, and W. H. Stein. 1958. L-cystathionine in human brain. J. Biol. Chem. 230: 707–716.

Tashian, R. E. 1961a. Inhibition of brain glutamic acid decarboxylase by phenylalanine, valine and leucine derivatives. A suggestion concerning the etiology of the neurological defect in phenylketonuria and branched-chain ketonuria. Metabolism 10: 393–402.

——. 1961b. Problems in the chemical diagnosis of some hereditary metabolic diseases. Clin. Chem. 7: 441–456.

Tenenhouse, A., and J. H. Quastel. 1960. Amino acid accumulation in Ehrlich ascites carcinoma cells. Can. J. Biochem. 38: 1311–1326.

Terslev, E. 1960. Two cases of aminoaciduria, ocular changes and retarded mental development (Lowe's syndrome). Acta paediat. 49: 635–644.

Thompson, D. W. 1942. On Growth and Form, ed. 2. Cambridge University Press, 2 vols.

Tomlinson, S., and R. G. Westall. 1960. Argininosuccinase activity in brain tissue. Nature 188: 235–236.

Triantaphyllopoulos, E., and J. Tuba. 1959. Changes in intestinal and serum alkaline

phosphatase levels during absorption of certain amino acids. Can. J. Biochem. & Physiol. 37: 711–719.

Ullrich, K. J., K. Kramer, and J. W. Boylan. 1961. Present knowledge of the countercurrent system in the mammalian kidney. Prog. Cardiovasc. Dis. 3: 395–431.

Uzman, L. L., F. I. Iber, and T. C. Chalmers. 1956. The mechanism of copper deposition in the liver in hepatolenticular degeneration (Wilson's disease). Am. J. M. Sc. 231: 511–518.

Van Geffel, R., A. Devriendt, J-P, Dustin, H. Vis, and H. Loeb. 1959. La maladie du galactose. Considerations genetiques: étude de l'amino-acidémie et de l'aminoacidurie. Arch. franç. pediat. 16: 158–184.

Van Slyke, D. D., R. T. Dillon, D. A. MacFayden, and P. B. Hamilton. 1941. Gasometric determination of carboxyl groups in free amino acids. J. Biol. Chem. 141: 627–669.

——, and E. Kirk. 1933. Comparison of gasometric colorometric and titrimetric determination of amino nitrogen in blood and urine. J. Biol. Chem. 102: 651–682.

Vestergaard, P., and R. Leverett. 1958. Constancy of urinary creatinine excretion. J. Lab. & Clin. Med. 51: 211–218.

Villee, C. A. 1961. Intracellular organization of enzyme systems. Pediatrics 27: 656–668.

Visakorpi, J. K. 1960. Aminoaciduria in various types of rickets. Ann. Paed. Fenniae. 6: (suppl. 16) 1–96.

Vries, A. de., S. Kochwa, J. Lazebnik, M. Frank, and M. Djaldetti. 1957. Glycinuria, a hereditary disorder associated with nephrolithiasis. Am. J. Med. 23: 408–415.

Waisman, H. A., H. L. Wang, H. Harlow, and R. R. Sponholz. 1959. Experimental phenylketonuria in the monkey. Proc. Soc. Exper. Biol. & Med. 101: 864–865.

——, ——, G. Palmer, and H. F. Harlow. 1960. Phenylketonuria in infant monkeys. Nature 188: 1124–1125.

Wallis, K., and A. Kalushiner. 1960. Oligophrenia in combination with congenital ichthyosis, spastic disorders and macular degeneration (Sjögren-Larsson syndrome). Ann. paediat. 194: 115–124.

Wallis, L. A., and R. L. Engle, Jr. 1957. The adult Fanconi syndrome. Am. J. Med. 22: 13–23.

Walshe, J. M. 1960. Treatment of Wilson's disease with penicillamine. Lancet 1: 188–192.

——. 1961. Pyruvate metabolism in Wilson's disease. Clin. Sc. 20: 197–203.

Wang, H. L., N. E. Morton, and H. A. Waisman. 1961. Increased reliability for the determination of the carrier state in phenylketonuria. Am. J. Human Genet. 13: 255–261.

Watts, R. W. E., and J. C. Crawhall. 1959. The first glycine metabolic pool in man. Biochem. J. 73: 277–286.

Webber, W. A. 1962. Interaction of neutral acidic amino acids in renal tubular transport. Am. J. Physiol. 202: 577–583.

——, J. L. Brown, and R. F. Pitts. 1961. Interactions of amino acids in renal tubular transport. Am. J. Physiol. 200: 380–386.

Weiner, I. M., J. A. Washington, and G. H. Mudge. 1960. On the mechanism of action of probenicid on renal tubular secretion. Bull Johns Hopkins Hosp. 106: 333–346.

Weissbach, H., W. King, A. Sjoerdsma, and S. Udenfriend. 1959. Formation of indole-3-acetic acid and tryptamine in animals. Method for estimation of indole-3-acetic acid in tissues. J. Biol. Chem. 234: 81–86.

Werner, G. 1954. Eine apparatur für Hochspannungs-Papierelektrophorese. Proc. 1st Eur. Cong. Clin. Chem. Page 28.

Westall, R. G. Personal communication.
——. 1950. Isolation of γ-amino butyric acid from Beetroot (Beta Vulgaris). Nature 165: 717–718.
——. 1952. The amino acids and other ampholytes of urine. 1. A general method of isolation. Biochem. J. 52: 638–642.
——. 1953. The amino acids and other ampholytes in urine. 2. The isolation of a new sulfur-containing amino acid from cat urine. Biochem. J. 55: 244–248.
——. 1955. The amino acids and other ampholytes of urine. 3. Unidentified substances excreted in normal human urine. Biochem. J. 60: 247–255.
——. 1960. Argininosuccinicaciduria: identification and reactions of the abnormal metabolite in a newly described form of mental disease, with some preliminary metabolic studies. Biochem. J. 77: 135–144.
——. 1961. In Atkins, H. J. B., Tools of Biological Research, 3rd series. Oxford: Blackwell.
——, E. Roitman, C. de la Peña, H. Rasmussen, J. Cravioto, F. Gomez, and L. E. Holt, Jr. 1958. The plasma amino acids in malnutrition. Arch. Dis. Child. 33: 499–504.
Weijers, H. A., J. H. van de Kamer, W. K. Dicke, and J. Ijsseling. 1961. Diarrhoea caused by deficiency of sugar splitting enzymes. I. Acta. paediat. 50: 55–71.
Weyers, H., and H. Bickel. 1958. Photodermatose mit Aminoacidurie, Indoleaceturie und Cerebralen Manifestationen (Hartnup syndrome). Klin. Wschnchr. 36: 893–897.
Widdas, W. F. 1952. Inability of diffusion to account for placental glucose transfer in the sheep and consideration of the kinetics of a possible carrier system. J. Physiol. 118: 23–39.
Wilbrandt, W. 1956. Carrier systems rate and affinity. J. Cell. Comp. Physiol. 47: 137–145.
——. 1959. Permeability and transport systems in living cells. J. Pharm. and Pharmacol. 11: 65–79.
Williams, R. D. B., and I. Lingtang. 1960. Mental defect, quadriplegia and ichthyosis. Am. J. Dis. Child. 100: 924–929.
Wilson, T. H., E. C. C. Lin. 1960. Active transport by intestines of fetal and new born rabbits. Am. J. Physiol. 199: 1030–1032.
——, and G. Wiseman. 1954. The use of sacs of everted small intestine for the study of the transference of substances from the mucosal to the serosal surface. J. Physiol. 123: 116–125.
——, E. C. C. Lin, B. R. Landau, and C. R. Jorgensen. 1960. Intestinal transport of sugars and amino acids. Fed. Proc. 19: 870–875.
Winberg, J. 1959. The 24-hour true endogenous creatinine clearance in infants and children without renal disease. Acta. paediat. 48: 1–10.
Wollaston, W. H. 1810. On cystic oxide, a new species of urinary calculus. Phil. Tr. Roy. Soc. (London) 100: 223–230.
Woolf, L. I. 1961. Aminoaciduria. Brit. Med. Bull. 17: 224–229.
——, and A. P. Norman. 1957. The urinary excretion of amino acids and sugars in early infancy. J. pediat. 50: 271–295.
Worthen, H. G. 1960. Effect of maleic acid on renal tubular enzyme activity and morphology (abstr.) Fed. Proc. 20: 408.
——, and R. A. Good. 1958. The de Toni-Fanconi syndrome with cystinosis. A.M.A. J. Dis. Child. 95: 653–688.
Wright, S. 1959. Genetics and the hierarchy of biological sciences. Science 130: 959–965.

Wright, S. W. 1960. Investigation on mentally defective sibs. Studies on urinary amino acids. J. Ment. Def. Res. 4: 32–41.

——, and G. Tarjan. 1957. Phenylketonuria. A.M.A. J. Dis. Child. 93: 405–419.

——, ——, R. W. Lippman, and T. L. Perry. 1958. Etiological factors in mental deficiency: errors in metabolism that may lead to mental deficiency. A.M.A. J. Dis. Child. 95: 541–562.

Wrong, O., R. B. I. Morrison, and P. E. Hurst. 1961. A method of obtaining faecal fluid by in-vivo dialysis. Lancet 1: 1208–1209.

Wyngaarden, J. B. Glycinuria. In Stanbury, J. B., Wyngaarden, J. B., and Frederickson, D. S., Eds.: The Metabolic Basis of Inherited Disease. New York: McGraw Hill, pp. 1295–1301.

Yeh, H. L., W. Frankl, M. S. Dunn, P. Parker, B. Hughes, and P. György. 1947. The urinary excretion of amino acids by a cystinuric subject. Am. J. Med. Sc. 214: 507–512.

Yuwiler, A., and R. T. Louttit. 1961. Effects of phenylalanine diet on brain serotonin in the rat. Science 134: 831–832.

Zannoni, V. G., and B. N. LaDu. 1961. Inhibition of p-hydroxyphenylpyruvic acid oxidase by excess substrate of phenylpyruvic acid. (abstr.) Fed. Proc. 20: 5.

Zellweger, H. 1961. Amino-aciduria and mental retardation. Clin. Chem. 7: 215–227.

Chapter 5

Antibody Synthesis

Seikichi Tokuda and Ray D. Owen
California Institute of Technology,
Pasadena, California.

Introduction

One of the most remarkable adaptive responses displayed by vertebrates is the elaboration of specific antibody following exposure to a foreign antigen. Particular cells differentiate, under the influence of a strange material, to devote their major synthetic activity to the elaboration of a protein capable of reacting, with a high degree of specificity, with that material. Antibody synthesis, therefore, is an aspect of cellular differentiation, on the molecular level. How is the genetic constitution of the cells involved in this differentiation?

An antibody (Ab) is a serum protein produced in the body of animals in response to a foreign substance, an antigen (Ag). In general antibodies are found in the γ-globulin fraction of the serum and have a molecular weight of approximately 160,000. Antibodies produced in response to a specific Ag are not identical molecular species. They are heterogeneous with respect to their immunological behavior and their physical-chemical properties (Cann, 1959; Talmage, 1957a).

Antibody combines specifically with the Ag that induced its formation. Each Ab molecule is believed to have two areas which have configurations complementary to the surface of the specific Ag (Eisen and Karush, 1949; Plescia, Becker and Williams, 1952; Singer, 1957; Singer and Campbell, 1952). The complementary area is called the Ab combining site of the Ab molecule, and has been described as a cavity whose van der Waal contour

Part of this review was written during the tenure of a Cancer Immunology Training Grant (CRT-5040) from the National Cancer Institute, USPHS and during the tenure of a Postdoctoral Fellowship from the National Institute of Allergy and Infectious Diseases, USPHS.

is closely complementary to the antigenic site (Karush, 1956). This site covers a small area of the total Ab molecule and the upper limit of the Ab combining site has been set at 700 A (Campbell and Bulman, 1952).

The term antigen is used in two senses: (a) to designate those substances that will cause the production of Ab as shown by subsequent tests, and (b) those substances that give a specific reaction with Ab, but are unable to induce Ab formation. Substances that induce Ab formation are called complete or functional Ag; substances which combine with Ab but are incapable of initiating Ab formation are called incomplete or haptenic Ag.

Complete Ag are complex molecular structures with molecular weight greater than 10,000 (Boyd, 1956; Campbell and Bulman, 1952; Edsall, 1957). Although lower molecular weight substances may sometimes become antigenic, it is assumed that they do so by reacting with proteins in vivo to form complete Ag (Landsteiner, 1945; Mayer, 1957). Proteins, carbohydrates and denatured DNA can be antigenic (Blix, Iland and Stacey, 1954; Boyd, 1956; Campbell and Bulman, 1952; Edsall, 1957; Levine, Murakami, Van Vunakis and Grossman, 1960; Phillips, Braun and Plescia, 1958). The antigenicity of lipid substances has not been clearly established.

The reaction between Ag and Ab is considered to take place in two stages. The division may not be sharp and it is likely that the second stage sets in before the first stage is completed. The specific combination between the Ag and Ab molecule takes place during the first stage of the reaction. The visible serological reactions (i.e., precipitation, agglutination, complement fixation, etc.) take place during the second stage of the Ag-Ab reactions. The initial reaction occurs rapidly and does not require the presence of salt. Although most of the energy is consumed during the first stage, there is no splitting or digestion of either of the reactants. The second stage proceeds more slowly and requires salt in the environment.

The specificity of the Ag-Ab reactions is determined by the complementary surface configurations of the interacting molecules. The complementariness in structure permits weak, nonspecific intermolecular forces (i.e., van der Waal forces, coulombic forces and hydrogen bonds) to come into their fullest operation to give a relatively stable bond (Pauling, 1940; Pauling and Pressman, 1945).

An animal injected with an Ag becomes "sensitized" to that substance. Following a single injection of Ag, a measurable Ab response is seen after a few days lag. The Ab concentration reaches its peak after a week or two and then begins to fall off slowly. A second injection of Ag administered before the clearance of circulating Ab results in a rapid removal of the

available Ab. Ab reappears earlier and at a faster rate after a second challenge of Ag than after the primary sensitization. The accelerated Ab response following a second challenge of Ag is called the anamnestic (recall) phenomenon (Cannon, 1942). During the anamnestic response, several changes take place. The Ab formed early in the immunization process has low avidity for the Ag molecule, and the antiserum shows little cross reaction with related Ag. Ab formed after continued sensitization is more avid and the antiserum shows a greater degree of cross reactivity with related Ag (Burnet, Keogh and Lush, 1937; Farr, 1958; Heidelberger and Kendall, 1935; Jerne, 1951).

Isotopically labeled protein Ag injected into normal rabbits disappears from the circulation in three stages (Dixon, Bukantz, Dammin and Talmage, 1953; Talmage et al., 1951; Weigle, 1957). The first stage is characterized by a rapid fall in concentration as the Ag equilibrates between the intravascular and extravascular plasma compartment. It usually takes two or three days for bovine serum albumin (BSA) and bovine gamma globulin (BGG) to equilibrate in the plasma protein pool. The second stage is characterized by a logarithmic clearance rate representing the natural nonimmune degradation of the Ag. This rate varies from species to species with a given protein and from Ag to Ag within species. The second stage of Ag elimination ends with the initiation of Ab production. The third stage is characterized by a final rapid clearance of the Ag. This is the result of Ab production. It has been suggested that the Ag combines with Ab as it is formed and the resulting soluble complex is removed from the circulation and rapidly catabolized. Studies tracing radioactive labeled protein Ag indicate a complete removal of Ag from the circulation before free Ab appears in the circulation.

The Ab response depends on a number of unrelated factors. Among these are: chemical state of the Ag, the dosage and route of Ag administration, and the physiological state of the animal (Boyd, 1956; Edsall, 1957). In hyperimmunized animals, the amount of Ab in the serum can be as high as 2.0 to 6.7 mg. Ab nitrogen per ml. of serum (Boyd, 1956).

The effect of sensitization can be increased by adsorbing the antigen to small particulate substances such as Kaolin, India ink and zymosan (Landsteiner, 1945; Malkiel and Hargis, 1959). Antigenicity can also be increased by precipitation with alum (Wright, Green and Kanode, 1954), incorporation in a water-in-oil emulsion (Freund, 1947, 1956) or by mixing with *B. pertussis* cells (Kind, 1957; Kind and Roffler, 1961). Agents that increase antigenicity of substances are called immunological adjuvants. Adjuvants enhance Ab response by bringing the Ag into a particulate form and influencing the uptake of Ag by phagocytes and the persistence of the Ag in

the body (Boyd, 1956). Freund's water-in-oil adjuvant increases the Ab response by altering the cellular response and by holding the Ag in a concentrated form and releasing it very slowly (Freund, 1956).

The Ab response to a protein Ag usually does not remain at a high level without constant stimulation. On the other hand, the response to a single injection of certain polysaccharide Ag is long-lasting (Heidelberger, 1953). It has been suggested that persistence of Ag or some determinant group of the Ag is necessary for Ab production (Campbell and Garvey, 1960; Haurowitz, 1949, 1952). The persistence of the Ab to pneumococci polysaccharide has been attributed to the stability of the Ag and the absence of the enzymes which are able to degrade it (Edsall, 1957).

Massive doses of Ag given to animals before they reach immunological maturity or to adults sometimes result in a specific block in the Ab-forming system. These phenomena have been designated as immunologic paralysis (Felton, 1949; Morgan, Watson and Cromartie, 1952), acquired immunological tolerance (Billingham, Brent, and Medawar, 1956; Cinader and Dubert, 1955; Hanan and Oyama, 1954; Terres and Hughes, 1959), and immunological unresponsiveness (Dixon and Maurer, 1955; Smith and Bridges, 1956, 1958). Chase (1959) sees little merit in differentiating among these conditions and uses the term immunologic tolerance to signify an "impressed diminution in immunologic responses below those which normally occur after known antigenic excitation." As in the case of Ab formation, it has been postulated that the persistence of the unresponsive state requires the persistence of Ag (Mitchison, 1959; Smith and Bridges, 1958). Dresser and Mitchison (1960) have postulated that two subcellular sites may be open for the Ag, or alternatively, the quantity of Ag at a single site may determine whether inhibition or initiation of the Ab response occurs. It is obvious that the mechanism of the inhibition of the Ab response must be explained by any theory of Ab formation.

Cellular Sites of Ab Synthesis

Since antibodies are γ-globulins, one would expect that cells involved in the synthesis of γ-globulins should also produce antibodies. Although there is little doubt that lymphoid tissues, lung, spleen and bone marrow are capable of producing γ-globulins and Ab (Askonas and Humphrey, 1958; McMasters, 1953; Thorbecke and Keuning, 1956), the definitive cell type involved in Ab synthesis has been difficult to identify. Macrophages, lymphocytes and plasma cells have been proposed by various investigators as cells taking part in Ab synthesis (McMasters, 1953; Rittenberg and Nelson, 1960; Wissler, Fitch, LaVia, and Gunderson, 1957; Wissler, Fitch, and LaVia, 1960). Part of the difficulty rests on a lack of precise identification

of the various cell types and their genesis (McMasters, 1953). Furthermore, several investigators have indicated that macrophages and/or lymphocytes give rise to plasma cells (Holub, 1960; Nossal, 1959b, 1960; Rebuck, Monto, Monaghan and Riddle, 1958; Roberts and Dixon, 1956; Roberts, Dixon and Weigle, 1957).

The evidence to implicate the macrophages has not been very convincing (McMasters, 1953; Rittenberg and Nelson, 1960). Although Sabin (1939) reported that macrophages exhibited cytoplasmic shedding which correlated with Ab formation and Hartley (1940) reported that macrophage-rich tissues contain large amounts of antiviral Ab, there has been no direct evidence to indicate that Ab is found in the cytoplasma of macrophages (Coons, Leduc and Connolly, 1955; Ehrich, Harris and Mertens, 1946; Roberts, 1955). Roberts and Dixon (1956), and Roberts, Dixon and Weigle (1957) noted that macrophages as well as lymphocytes taken from sensitized rabbits produced Ab in irradiated, nonsensitized animals. These authors concluded that Ab was synthesized by plasma cells which were derived from either macrophages or lymphocytes. Recently, McKenna and Stevens (1960) reported on the in vitro production of Ab by peritoneal exudate cells. Because the cells involved in Ab formation resembled monocytes more than macrophages, these workers attributed Ab formation to the monocytes.

The best evidence to indicate that lymphocytes produce Ab comes from both in vivo and in vitro tissue culture studies. Lymph node cells taken from nonsensitized rabbits give an Ab response when mixed with Ag in vitro and transferred to irradiated or newborn rabbits which are otherwise incapable of giving an Ab response (Harris and Harris, 1956). Examination of these cell populations indicate an average of 99 per cent lymphocytes in each preparation. Lymphocytes taken from sensitized animals also produce Ab in tissue cultures (Wesslen, 1952) and in microdroplets (Attardi, Cohn, Horibata and Lennox, 1959).

Convincing evidence favors the plasma cells as the main site of Ab synthesis. Antibodies have been demonstrated in extracts of tissue rich in plasma cells but low in lymphocyte or macrophage content (Bjornboe, Gormsen and Lundquist, 1947). Fagraeus (1948) noted a sharp increase of plasma cells in the spleens of sensitized rabbits. Explants of spleens from sensitized animals were rich in plasma cells and produced Ab in tissue cultures. Explants of lymph nodes rich in lymphocyte content did not yield Ab in tissue cultures. Histochemically, Ab has been found to be associated with plasma cells using fluorescein labeled Ag and Ab (Coons et al., 1955) as well as radioiodinated Ag (Berenbaum, 1958) in vitro. Whereas Nossal (1959, 1960) reported that only the plasma cells produce Ab in microdroplets, Attardi, Cohn, Horibata, and Lennox (1959) reported that both

the plasma cells and the lymphocytes produce Ab. The reason for the discrepancy in these findings is not readily apparent. However, it must be emphasized that different animals, methods of immunization and Ab detection, and Ag were employed by these workers. Aggammaglobulinemia in man is associated with a decrease or absence of plasma cells (Good, 1957). In germ-free animals (Thorbecke, Gordon, Wostman, Wagner and Reyniers, 1957), and in newborn animals (Bridges, Condie, Zak and Good, 1959), there is a good correlation between Ab formation and the appearance of plasma cells.

The above findings indicate at least two types of cells are involved in Ab production. Because various investigators have proposed that lymphocytes and/or macrophages differentiate into plasma cells (Holub, 1960; Nossal, 1959b, 1960; Rebuck, et al., 1958; Roberts and Dixon, 1956; and Roberts et al., 1957), and because other investigators have proposed that the lymphocyte is a multipotential cell capable of differentiating into other cell types (Harris, 1956; Yoffey and Courtice, 1956), the problem can be complicated by the design of any particular experiment. Most experiments are set up in such a manner that it is impossible to assay for Ab and simultaneously identify each Ab-producing cell. Because the rate of cell differentiation is not known, it is impossible to determine whether or not cellular changes took place during the interval between the identification of cells and the assay of Ab. However, if cell "A" differentiates into cell type "B" before initiating Ab synthesis, it would be a matter of semantics to say whether one or both cell types are capable of producing Ab.

The studies of Nossal (1959, 1960) indicate that plasma cells are involved in Ab synthesis. These cells were taken from immunized animals, and Nossal might therefore have been observing only the end of a sequence of events. Wissler, Fitch, LaVia and Gunderson (1957) and Wissler, Fitch and LaVia (1960) proposed that at least four cell types are involved in Ab formation. The macrophage ingests and digests particulate Ag. Another cell type not derived from macrophages proliferates, differentiates and initiates Ab synthesis. The cells which carry the newly formed Ab molecule are presumed to be either small lymphocytes or plasma cells. The conclusions of Wissler et al. were reached after studying the cellular response following a single intravenous injection of bacterial Ag. Studies from other quarters indicate that there are two phases in the Ab response. Dixon et al. (Dixon, Talmage and Bukantz, 1951; Dixon, Talmage and Bukantz, 1952; Talmage, 1955) observed that exposure of animals to x-rays 12 to 48 hours before Ag injection decreased both the ability to form Ab and the rate at which it was formed. The remainder of the Ab response, including the anamnestic response to protein Ag, was relatively unaffected by x-radiation. From these observations, the authors postulated two phases

in the Ab response, a short, initial radiosensitive adaptive phase and a later radioresistant productive phase. Indirect evidence for phases in Ab production has also been derived from studies of the transfer of Ab producing cells (Sterzl, 1960; Sterzl, 1961; Trnka and Sterzl, 1960), and from the use of agents that inhibit or destroy cells (Taliaferro, 1957). Although most studies indicate that changes take place following contact with Ag, the exact sequence of cellular and/or subcellular changes remains to be elucidated.

Subcellular Site of Antibody Synthesis

Several groups of investigators have established that serum globulin is not a direct precursor of Ab and that Ab is synthesized de novo from amino acids (Green and Anker, 1954; Stavitsky and Wolf, 1958; and Steiner and Anker, 1956). Because the formation of Ab represents new protein synthesis it has been compared to induced enzyme synthesis (Monod, 1959; Schweet and Owen, 1957). The rapid advances made in the studies on the subcellular site of protein synthesis have enabled immunochemists to study the subcellular site of Ab synthesis with renewed interest and information.

Specific proteins have been found in association with the microsomal fraction of the cell indicating synthesis at this site (Campbell, Greengard and Kernot, 1958; Peters, 1959). Other studies implicate the nuclei as well as the mitochondria as active sites of protein synthesis (Allfrey, Mirsky and Osawa, 1957; Kalf, Bates and Simpson, 1959; and McLean, Cohn, Brandt and Simpson, 1958). In cell-free systems, incorporation of amino acids is dependent on the presence of ribosomes, an energy source and soluble enzymes. Ribosomes are subcellular ribonucleoprotein particles associated with the endoplasmic reticulum. The endoplasmic reticulum consists of ribosomes attached to a lipoprotein membrane. Disruption and fractionation of the cell yields ribosomal particles attached to the lipoprotein membrane. The ribosomes can be isolated by dissolution of the membrane with agents such as desoxycholate (Hoagland, 1960; and Zamecnic, 1960). Electron micrograph studies reveal that the plasma cells possess a highly organized endoplasmic reticulum (Bernhard and Granboulan, 1960). This property is common to protein-secreting cells. Recent evidence indicates that Ab-producing cells do not store Ab, but release the Ab soon after synthesis (Askonas, 1960; Dineen and Perry, 1960; and Helmreich, Kern and Eisen, 1961).

In 1956, Sterzl and Hrubesova (1956) reported that nucleoprotein fractions obtained from spleens of immunized adult rabbits and injected into 5 day old rabbits resulted in Ab formation within 24 hours. These authors saw no evidence for direct transfer of either Ab or Ag. However, it was not certain whether the Ab was synthesized or merely released in the new en-

vironment. In a subsequent publication, Hrubesova, Askonas, and Humphrey (1959), indicated that Ab was not synthesized *de novo* and that the nucleoprotein fraction was contaminated with extraneous protein. Freidman (1959) also reported on the transfer of agglutinin formation to x-irradiated rabbits with the nuclei, mitochondria, and nucleoprotein rich fractions of lymph nodes taken from immunized adult rabbits. Heated or damaged cells did not transfer Ab formation. Although there was no detectable transfer of Ab or Ag, no evidence was given for *de novo* synthesis of Ab. Recently, Nester, Makela and Nossal (1961) reported that immunogenic amounts of Ag were present in nucleoprotein fractions obtained from normal rat spleens mixed with Ag in vitro.

Feldman, Elson and Globerson (1960) saw no in vitro activity associated with the purified ribonucleoprotein taken from the spleen of rabbits immunized with sheep RBC. However, when the ribonucleoprotein preparations were treated with ribonuclease prior to testing, Ab was released as evidenced by direct hemagglutination of sheep RBC or by the indirect antiglobulin test. Similar studies with horse globulin Ag revealed the dissociation of Ab from ribonucleoprotein preparations. On the basis of these findings, Feldman et al. (1960) suggested that Ab molecules form part of the ribonucleoprotein complex within the Ab-producing cell and that the complex may be the site of Ab synthesis. Kern, Helmreich and Eisen (1959) prepared lymph node microsomes from guinea pigs immunized with 2, 4-dinitrophenyl bovine gamma-globulin (DNP-BGG). DNP-bovine serum albumin (DNP-BSA) employed as a test Ag was adsorbed by the microsomes. The BSA was iodinated with I^{131}. The assay for Ab was based on the reversible binding of a soluble Ag by Ab which was insoluble due to association with microsomal particles. Epsilon DNP-lysine (E-DNP-lysine) dissociated I^{131} DNP-BSA from lymph node microsomes of animals immunized with DNP-BGG, but not from microsomes of animals immunized with BGG. (E-DNP-lysine has a high affinity for anti-DNP Ab and specifically dissolves precipitates formed by such Ab and homologous Ag.) Furthermore, the I^{131} DNP-BSA binding to microsomes was inhibited by prior incubation with E-DNP-lysine or DNP-BGG. In a later publication, Kern, Helmrich and Eisen (1961) indicated that the microsomal Ab activity is the result of a specific and reversible interaction between the microsomes and the segment of the Ab molecule corresponding to Porter's fraction III (Porter, 1958). Askonas (1960) reported that anti-type III pneumococci polysaccharide Ab is also associated with the microsomal fractions of spleen and bone marrow from immunized rabbits. Ab was dissociated from the the microsomes using various methods known to release proteins from ribonucleoprotein particles, such as ultrasonic disintegration or incubation at 37 C. with RNAse, trypsin, chymotrypsin, deoxycholate

and alkali (pH 9. 5). Ab was then assayed by the hemagglutination of sheep RBC sensitized with Ag.

Sussdorf (1961) noted a rise in hemolytic activity in homogenates of spleens from rabbits immunized with sheep RBC. He found an average net increase of 48 hemolytic units per Gm. spleen in the homogenates of immunized spleens. Normal spleen homogenates showed an average increase of only 5 hemolytic units per gram spleen. Four of 6 positive experiments showed a two-step rise in Ab titer during an 8 hour incubation period. Sussdorf suggested that the biphasic character of the hemolytic titer may represent the successive release of both preformed Ab and newly formed Ab.

The above studies all give strong evidence to associate Ab with the nucleoprotein fraction of the cell, but they do not give any real evidence for synthesis of Ab in these fractions. Moreover, the studies of Kern et al. (1961) and Sussdorf (1961) indicate that Ab can be adsorbed and released by subcellular particles in vitro. The relationship between the Ab associated with the microsomal particles and the "cytophillic" Ab is not clear. "Cytophilic" Ab is a term originated by Boyden and Sorkin (1960b) to describe an Ab which can be adsorbed on and eluted from spleen cells. This Ab is found in immune serum, but can be separated from the precipitating Ab. This finding suggests that Ab can be synthesized in one site and then transported to another site.

More direct evidence for the biosynthesis of γ-globulin in a cell-free system has been presented by Ogata, Omori and Hirokawa (1961). The incorporation of C^{14}-leucine into the γ-globulin fraction was achieved by incubating spleen cell microsomes from hyperimmunized rabbits with the pH 5 fraction, C^{14}-leucine, GTP, ATP and an ATP generating system. The incorporation of the amino acid was reduced by RNAase. On the basis of these findings, these investigators suggested that amino acid-activating enzymes, soluble RNA and microsomes provide the main pathway for γ-globulin synthesis in vivo. Although Ab was present in the γ-globulin fraction, no direct evidence was presented to indicate that C^{14}-leucine was incorporated into the Ab molecule.

The above findings implicate the microsomes as the site of Ab synthesis. Although it is not known what is involved in the formation of the Ab combining site, whether it is determined by a change of amino acid sequence (Edelman and Poulik, 1961; Gitlin and Merler, 1961) or a "folding" of a pre-existing amino acid chain (McFadden and Smith, 1955; Porter, 1950; and Smith, McFadden, Stockell and Buettner-Janush, 1955), it would be likely that the information which determines the sequence of the chain is carried by the microsomes. In ordinary protein synthesis, the ultimate source of the information is the DNA. But how does the Ag find expression in this kind of information and its transfer? Numerous theories of Ab for-

mation have been put forward; some of these will be discussed in another section of this review. The quality of the Ab as an Ag is under clear genetic control, as demonstrated by the observations of Oudin (1960a, 1960b) and others, and as suggested by genetic variations in the "Gm types" of human globulin (Linnet-Jepsen, Galatius-Jensen, and Hauge, 1958). How the quality of the combining sites of the Ab as Ab is controlled, however, is still the object of theory and speculation.

Antibody Synthesis Initiated in Vitro

Cell transfer studies on Ab formation have enabled immunologists to identify the cells involved in Ab synthesis. Most of these studies were performed on cells obtained from immunized donors. Considerable effort has been expended over the last 50 years to initiate Ab synthesis in vitro. Investigators from several groups have been successful in sensitizing normal Ab-forming cells in vitro and then obtaining Ab synthesis in immunologically incompetent hosts, such as newborn (Holub, 1958; Nossal, 1959a; Trnka, 1958) and x-irradiated animals (Harris and Harris, 1957; Sterzl, 1959). The attempts to initiate and produce Ab in an in vitro environment have met with some successes and many failures (Stevens and McKenna, 1958). In the modern literature, there have been only three claims of successes in initiating and producing Ab in vitro.

Stevens and McKenna (1958) reported that diced rabbit spleens incubated with Ag in vitro produced Ab in tissue culture medium. The formation of Ab was dependent on treatment of the rabbits with *Shigella* endotoxin prior to sacrifice and required large amounts of Ag during the in vitro induction process. Although the data presented by Stevens and McKenna indicate net synthesis of Ab, other investigators (Bussard, 1960; Dineen and Perry, 1960; Fagraeus, 1960; Sterzl, 1960) have been unable to repeat this observation. Recently, McKenna and Stevens (1960) presented evidence for the in vitro synthesis of Ab using peritoneal exudate cells sensitized in vitro.

Fishman (1959) reported in vitro synthesis of Ab using a unique two-step process. The antigens used were T_2 bacteriophage and haemocyanin. Ag was first incubated with rat peritoneal macrophages and then transferred to a tissue culture medium containing lymph node cells obtained from normal rats. Ab was observed during the fifth through the eleventh days of cultivation. It was found that treatment of the bacteriophage with macrophage extract was just as effective as treatment with whole macrophage. If the natural sequence of Ab production requires that Ag be digested by macrophages before being transferred to Ab-producing cells, then the system devised by Fishman would be relevant.

It appears that some progress has been made toward sensitizing Ab-forming cells in vitro. Further studies in this area would be important to the elucidation of the cellular mechanisms of Ab production.

Theories of Antibody Formation

Since the introduction of the side chain theory of Ehrlich (1900), many theories have been proposed for Ab formation. Although there are many aspects to consider in the immune response, Burnet (1958) contends that any theory worth considering must satisfactorily explain (a) the ability to distinguish between "self" and "foreign," (b) immunologic tolerance and paralysis and (c) the anamnestic reaction. Talmage and Cann (1961), however, add 8 other requirements which include the specificity and diversity of Ab and the change in the quality of Ab during the immune response. Because most investigators restrict their investigations only to certain phases of the immune process, the theories they propose would naturally be influenced by their experimental findings.

The various theories of Ab formation have been divided into two viewpoints: (a) the instructive theories which suppose that Ag causes the formation of globulins with new specificities and, (b) the selective theories which suppose that Ag induces an increased production of globulins with pre-existing specificities (Lederberg, 1959; Talmage, 1959a). The more important theories to be discussed will be presented in chronological order.

Ehrlich's (1900) side chain theory falls in the selective category. It postulated cells possessing "side-chains" which are necessary to take foodstuffs into the cells. These side chains are on the surfaces of cells; the side chains have a special affinity for particular substances analogous to a lock and key. Because it was observed that guinea pig brain tissue absorbed and neutralized tetanus toxin, Ehrlich postulated the existence of toxophile groupings on the brain cell surface. The toxophile groups were assumed to be side chains present by chance and only incidentally complementary to toxin molecules. Toxin molecules were presumed to fix to cell surfaces to exert their effect. The toxin molecule, being a complex substance, combines with side chains which normally attach to foodstuff necessary for cell growth. When these side chains become blocked, the cell produces more side chains of identical specificities to compensate for the neutralized side chains. The compensation proceeds beyond the necessary limit until an excess is produced. The overburdened cell then becomes a secretor cell. Thus, Ehrlich felt that "antitoxins represent nothing more than side chains reproduced in excess during regeneration, and therefore pushed off from the protoplasm, and so coming to exist in a free state." Once the side chains are blocked, these cells become "educated" or "trained" to produce the

necessary side chains in ever increasing quantities during continued immunization. Accordingly, Ehrlich believed that his theory explained the specificity of Ab and the long-lasting immune response.

Ehrlich's theory was discarded largely because of Landsteiner's (1945) findings that synthetic substances not found naturally could be antigenic. However, current thinking on this matter is that the number of possible determinant groups has been overestimated. The upper limit for the number of antigenic groups possible has been set at 5,000 by Talmage (1959b) and 50,000 by Haurowitz (1956). In recent years, the concept of pre-existing receptors has been revived and incorporated into new theories.

During the 1930's several groups of investigators proposed the direct Ag template theory which supposes that Ab-producing cells are directed to produce molecules with specificities complementary to the Ag (Alexander, 1931; Breinl and Haurowitz, 1930; Mudd, 1932). According to this theory, Ag must persist during Ab synthesis. It was assumed by Pauling (1940, 1945) that the amino acid sequence of the Ab molecule was identical to that of normal globulin, and that Ag influenced the manner in which the terminal amino acids were folded or coiled. Porter (1950) and Smith et al. (McFadden and Smith, 1955; Smith et al., 1955) have presented evidence that the terminal amino acid sequence of Ab molecules is identical to that in normal γ-globulin; on the other hand, Gitlin and Merler (1961) have evidence that the peptide distribution between Ab molecules is different, suggesting differences in both primary and tertiary structures among antibodies. Although the direct template theory satisfactorily explains the specificity as well as the heterogeneity of the Ab molecules, Burnet (1956, 1958, 1959) rejects this theory on the basis that it fails to account for tolerance and the anamnestic response.

Because it appeared unnecessary to postulate the persistence of Ag during Ab synthesis, Burnet and Fenner (1949) and Burnet (1956) suggested that Ag induces the formation of self-replicating units that continue to form Ab in the absence of Ag. According to this view, Ag alters the nucleus in a stem cell to produce a modified type of nucleoprotein which carries information to the cytoplasm. This "genocopy" of the Ag is inherited by the daughter cells and serves as the template for Ab synthesis. It was further postulated that body components carried self markers to prevent Ab formation against self components and that antigenic patterns were accepted as self-provided if the information is received before a certain critical period around the time of birth. Schweet and Owen (1957) postulated a similar theory which supposes that the initial action of Ag is on the nuclear DNA. It was further postulated that Ag was necessary to induce Ab formation in a system primed by the action of Ag on DNA.

The advantages of the indirect-template theories are: (a) they provide

for a replicating unit in Ab synthesis; (b) they provide for the distinction between self and non-self; (c) they provide for the anamnestic response and the changes in quality in the Ab during immunization. The main objection to these theories is the concept that Ag induces the equivalent of a directed mutation, a phenomenon for which no evidence exists.

In 1955, Jerne postulated the natural selection theory of Ab formation. He proposed that globulin molecules of a wide variety of specificities are continually being produced by the animal. Ag, by a chance affinity, combines with these natural antibodies and carries them to an appropriate cell. Once inside, the Ag is discarded, and the cell initiates the formation of globulin molecules with specificities like those of the introduced globulin molecule. Natural antibodies against self are presumed to be removed early in the development of the animal by the auto-Ag, and therefore would not be available for future reproduction. Thus, the body would recognize only foreign determinant groups. After the primary stimulus with Ag, a larger concentration of Ab will be in the circulation. A booster injection of Ag results in a larger concentration of globulins in the Ab-producing cells. Furthermore, with a larger concentration of Ab in the circulation, the Ag molecules select those Ab molecules with a better "fit," and thereby produce a change in the quality of Ab. This theory is similar to that proposed by Ehrlich (1900). Whereas Ehrlich proposed a replica on the cellular surface, Jerne proposes an extracellular protein in the circulation.

There are several advantages to this theory. It does not require a directed mutation, it does not require self markers, and it gives an answer to the diversity and differences in the quality of Ab. However, it is difficult to see how an Ag-Ab complex initiates Ab production; on the contrary, it has been observed that Ab suppresses Ab formation. (Talmage, Freter and Thomson, 1956). Furthermore, the autoimmune diseases remain a mystery under this theory (Jerne, 1960).

Talmage (1959a) suggested that the process of natural selection may require the selective multiplication of a few kinds of cells out a diverse population. It was postulated that cells selected for multiplication are those whose synthesized products have affinity for Ag. Thus, he favors selection on the cellular level. However, it was necessary to postulate that a different kind of cell was required for each kind of protein produced. Burnet (1958, 1959) and Lederberg (1959) presented a similar theory. Burnet called it the clonal selection theory, and adopted Jerne's idea that there is a wide variety of pre-existing γ-globulin patterns. But each pattern is ascribed to clones of mesenchymal cells and these patterns are inherited through successive generations. When an Ag enters the circulation it selects cells with the corresponding determinant group and initiates the se-

lective differentiation and multiplication of these cells. The shift in population results in an increased synthesis of the particular globulin carried by these cells. To account for the diversity of the Ab response, Burnet (1960) and Lederberg (1960) suggested mutation rates of 10^{-3} to 10^{-1} per cell division to provide for the variety of genotypes without depleting the existing clones. It was supposed that the immature Ab-forming cell is hypersensitive to the Ag-Ab reaction, and therefore self components as well as foreign Ag determinants introduced during a critical period in the developmental stage eliminate the corresponding cell types as they arise by mutation. It was assumed that mature cells proliferate extensively under Ag stimulation, but they are genetically stable. Clones of cells tend to persist after Ag is removed, and persisting cells are primed to give a vigorous secondary reaction.

The clonal selection theory was designed to answer most of the questions pertaining to the immune response. However, there appears to be an inherent weakness in a theory which postulates mutation rates of 10^{-3} to 10^{-1} per cell division. With the current experimental methods, it might be impossible to determine whether an event occurred by mutation at such high rates or by induction.

Recently, several other groups of investigators have also accepted the concept of the selective proliferation of clones of cells, but not on the basis of a selection of mutant cells. Monod (1959) postulated that the Ab forming cells have low efficiency for "capturing" Ag, but once a cell captures one or more Ag molecules, it is induced to synthesize the classical Ab as well as a cellular Ab. The cellular Ab has the property of capturing and accumulating the corresponding Ag in the cell. After the cell captures Ag, it produces more and more cellular as well as ordinary Ab and thereby increases its efficiency in capturing Ag. The fraction of responsive cells increases until Ag is exhausted; however, the cellular Ab becomes distributed among the daughter cells. When Ag is reinjected into the system, there is a rapid and greater Ab response. Pappenheimer, Scharff and Uhr (1959) have proposed a similar theory, but visualize the cellular Ab as the "cell-fixed" Ab of delayed hypersensitivity. They further postulate that the delayed hypersensitivity is an early phase of the Ab response.

Boyden (1960) and Boyden and Sorkin (1960a) have postulated the existence of an enzyme which they call accumulase. According to this theory, Ab molecules of many different specificities are continually being synthesized and broken down. If an Ag enters the Ab forming cell, it removes the corresponding Ab and accumulates these molecules in another part of the cell where they are not subject to breakdown. The cell thus becomes "modified" and some of the Ab molecules find their way to the cell surface.

The modified cell with the determinant groups on its surface has the capacity to pick up more Ag, which increases the intracellular Ab concentration. This in turn stimulates cell division and the redistribution of the "accumulase." Sooner or later an excess of Ab is produced and some of the Ab is released from the cell. Some of the released Ab is absorbed into another Ab-forming cell and acts as cellular receptors for accumulating more Ag to start the cycle over again. According to this theory Ag is necessary to initiate Ab synthesis, but the Ab forming cell does not undergo a "genetic change." Self components are not recognized because the "self-Ag" removes their corresponding "accumulase" before the cell matures. The continued presence of "accumulase" primes the cells for a secondary response. In effect, this theory is a natural selection theory at the subcellular level. The authors also suggest that the "cytophilic Ab" of Boyden and Sorkin (1960b) may be identical to the hypothetical accumulase. If such is the case, it should be possible to induce anamnestic response in normal animals by the prior treatment with cytophilic Ab.

The advantages of the permease-accumulase theories are that they explain clonal distribution of Ab forming cells without mutations. It is assumed that any cell can be induced to produce a variety of Ab.

Feedback control mechanisms have also been proposed for the Ab forming system. Talmage (1959a) suggests that Ab acts as its own inhibitor and that the role of Ag is to bind an inhibitor. The change in avidity during the immune response is related to Ab production—the greater the binding to Ag, the greater the stimulation to Ab production. The evidence for Ab being its own inhibitor is: (a) passively administered Ab inhibits Ab production and (b) the anamnestic response cannot be regularly initiated without the use of Ag.

Szilard (1961) postulates the existence of a coupling enzyme which is necessary for the formation of a repressor to Ab formation. Ag diffuses into the lymphocyte and combines with a specific coupling enzyme and therefore inhibits a specific Ab repressor. As the concentration of the repressor falls, the formation of Ab increases. The newly formed Ab also has the capacity to bind the repressor molecule and when the concentration of the repressor molecules falls below a certain level, the system becomes "locked." From then on the cell produces Ab at a high rate, even in the absence of Ag. The "locked" system represents some kind of memory, and it accounts for sustained immunity and the secondary response. It was postulated that there is one gene for each Ab, but each haploid set of chromosomes carries approximately 10,000 genes in this category. For each gene, there is one type of ribosome producing a specific type of Ab. In addition to repressors and coupling enzymes each Ab-forming cell con-

tains an enzyme "S" which suppresses cell division. "S," however, combines with an Ag-Ab precipitate and is produced in lesser quantities in "locked" cells. During the second injection, the Ag-Ab complexes in the "locked" cell deplete the concentration of "S" to the point where it can no longer inhibit cell division. This results in a selective multiplication of Ab-producing cells. According to Szilard, this explains the secondary response. Ag given in excess, or given before the animal matures, acts as an inhibitor of the system controlling the site of Ab synthesis. This theory attempts to explain Ab synthesis at the molecular level and assumes selection at a subcellular level. Recently, Weissman and Lustgraaf (1961) have also compared Ab formation to the repressor system in microorganisms.

Conclusion

Much has been learned of the nature of antibodies and their reactions, of gross aspects of the antibody response, and of the cellular and subcellular sites and processes of antibody synthesis. It remains for experimental work in the future, however, to distinguish among the various theories of antibody synthesis at hand or to provide a new and more compelling theory sustained by discriminating facts. The answer to the questions asked in our introduction still escapes us: How is the genetic constitution of the antibody-forming cell involved in the cellular differentiation that leads to the synthesis of specific combining sites on antibody molecules? Or is it?

References

Alexander, J. 1931. Some intracellular aspects of life and disease. Protoplasma 14: 296–306.

Allfrey, V. G., A. E. Mirsky and S. Osawa. 1957. Protein synthesis in isolated cell nuclei. J. Gen. Physiol. 40: 451–490.

Askonas, B. A. 1960. Studies on the mechanism of antibody secretion. In: Holub, M., and Jaroskova, L., Eds.: Mechanism of Antibody Formation. Prague: Czechoslovak Academy of Sciences, pp. 231–236.

——, and J. H. Humphrey. 1958. Formation of antibody by isolated perfused lungs of immunized rabbits. Biochem. J. 70: 212–222.

Attardi, G., M. Cohn, K. Horibata and E. S. Lennox. 1959. On the analysis of antibody synthesis at the cellular level. Bact. Rev. 23: 213–223.

Berenbaum, M. C. 1958. The antibody content of single cells. J. Clin. Path. 11: 543–547.

Bernhard, W., and N. Granboulan. 1960. Ultrastructure of immunologically competent cells. In CIBA Foundation Symposium on Cellular Aspects of Immunity. London: J. and A. Churchill, Ltd.

Billingham, R. E., L. Brent, and P. B. Medawar. 1956. Quantitative studies on tissue transplantation immunity. III. Actively acquired tolerance. Phil. Tr. Roy. Soc., London Series B. 239: 357–414.

Bjornboe, M., H. Gormsen, and F. Lundquist. 1947. Further experimental studies on the role of the plasma cells as antibody producers. J. Immunol. 55: 121–129.

Blix, U., C. N. Iland, and M. Stacey. 1954. The serological activity of deoxypentosenucleic acids. Brit. J. Exper. Path. 35: 241–251.

Boyd, W. C. 1956. Fundamentals of Immunology, ed. 3. New York: Interscience Publ.

Boyden, S. V. 1960. Antibody production. Nature 185: 724–727.

——, and E. Sorkin. 1960a. Discussion in Holub, M., and Jaroskova, L., Mechanisms of antibody Production. Prague: Czechoslovak Academy of Sciences, p. 216–222.

——, and ——. 1960b. The adsorption of antigen by spleen cells previously treated with antiserum in vitro. Immunol. 3: 272–283.

Breinl, F., and F. Haurowitz. 1930. Chemische Untersuchung des Prazipitates aus Hamoglobin und Anti-Hamoglobin-Serum und Bemerkungen uber Natur der Antikorper. Ztsch. f. physiol. Chemie. 192: 45–57.

Bridges, R. A., R.M. Condie, S. J. Zak, and R. A. Good. 1959. The morphologic basis of antibody formation development during the neonatal period. J. Lab. & Clin. Med. 53: 331–357.

Burnet, F. M. 1956. Enzyme Antigen and Virus. Cambridge University Press.

——. 1958. The clonal selection theory of acquired immunity. University Press, Cambridge, 209 pp.

——. 1959. The theories of antibody production. In Najjar, V. A., Ed.: Immunity and Virus Infection. New York: John Wiley & Sons, Inc., pp. 1–17.

——. 1960. Panel discussion on the clonal selection theory of antibody formation. In CIBA Foundation Symposium on Cellular Aspects of Immunity. London: J. and A. Churchill, Ltd.

——, and F. Fenner. 1949. Production of Antibodies, ed. 2. Melbourne: Macmillan.

——, E. V. Keogh, and D. Lush. 1937. The immunological reactions of the filterable viruses. Australian J. Exper. Biol. & M. Sc. 15: 227–278.

Bussard, H. E. 1960. Discussion. In Holub, M., and Jaroskova, L., Eds.: Mechanisms of Antibody Formation. Prague: Czechoslovak Academy of Sciences, p. 147.

Campbell, D. H., and N. Bulman. 1952. Some current concepts of the chemical nature of antigens and antibodies. Fortschr. chem. org. Naturstoffe 9: 443–484.

——, and J. S. Garvey. 1960. Factors involved in antibody formation. J. Infec. Dis. 107: 15–28.

Campbell, P. N., O. Greengard, and B. A. Kernot. 1958. Amino acid incorporation into serum albumin in microsome preparations from regenerating rat liver. Biochem. J. 68: 18 P.

Cann, J. R. 1959. The separation and purification of antibodies. In Najjar, V. A., Ed.: Immunity and Virus Infections. New York: John Wiley and Sons.

Cannon, P. R. 1942. Antibody production and the anamnestic response. J. Lab. & Clin. Med. 28: 127–139.

Chase, M. 1959. Immunologic tolerance. Ann. Rev. Microbiol. 13: 349–376.

Cinader, B., and J. M. Dubert. 1955. Acquired immune tolerance to human albumin and the response to subsequent injections of diazo albumin. Brit. J. Exper. Path. 36: 515–529.

Coons, A. H., E. H. Leduc and J. M. Connolly. 1955. Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyper-immune rabbit. J. Exper. Med. 102: 49–60.

Dineen, J. K., and B. T. Perry. 1960. Studies on the nature of antibody production during in vitro culture of lymphoid tissues. Australian J. Exper. Biol. 38: 363–374.

Dixon, F. J., S. C. Bukantz, G. J. Dammin, and D. W. Talmage. 1953. Fate of I^{131}-labelled bovine gamma globulin in rabbits. In Pappenheimer, A. M., Ed.: The

Nature and Significance of the Antibody Response. New York: Columbia University Press.
——, and P. H. Maurer. 1955. Immunologic unresponsiveness induced by protein antigens. J. Exper. Med. 101: 245–257.
——, D. W. Talmage, and S. C. Bukantz. 1951. Radiosensitive and radioresistant phases in antibody production. Fed. Proc. 10: 407.
——, ——, and P. H. Maurer. 1952. Radiosensitive and radioresistant phases in the antibody response. J. Immunol. 68: 693–700.
Dresser, D. W., and N. A. Mitchison. 1960. The cellular basis for the immunological memory. In CIBA Foundation Symposium on Cellular Aspects of Immunity. London: J. and A. Churchill, Ltd., pp. 227–238.
Edelman, G. M., and M. D. Poulik. 1961. Studies on structural units of the γ-globulins. J. Exper. Med. 113: 861–884.
Edsall, G. 1957. The nature of the antigen and the basic characteristic of the immune response. J. Allergy 28: 1–17.
Ehrlich, P. 1900. On immunity with special reference to cell life. Roy. Soc. Proc. 66: 424–448.
Ehrich, W. E., T. N. Harris, and E. Mertens. 1946. The absence of antibody in the macrophages during maximum antibody formation. J. Exper. Med. 83: 373–381.
Eisen, H. N., and F. Karush. 1949. The interaction of purified antibody with homologous hapten. Antibody valence and binding constant. J. Am. Chem. Soc. 71: 363–364.
Fagraeus, A. 1948. Antibody production in relationship to the development of plasma cells. Acta med. scandinav. Suppl. 204.
——. 1960. Discussion. In Holub, M., and Jaroskova, J., Eds.: Mechanisms of Antibody Formation. Prague: Czechoslovak Academy of Sciences, p. 147.
Farr, R. S. 1958. A quantitative immunochemical measure of the primary interaction between I°BSA and antibody. J. Infec. Dis. 103: 239–262.
Feldman, M., D. Elson, and A. Globerson. 1960. Antibodies in ribonucleoproteins. Nature 185: 317–319.
Felton, L. D. 1949. The significance of antigen in animal tissues. J. Immunol. 61: 107–117.
Fishman, M. 1959. Antibody formation in tissue culture. Nature 183: 1200–1201.
Freidman, H. 1959. Transfer of agglutinin formation by immune lymph node cell fractions. Fed. Proc. 18: 567.
Freund, J. 1947. Some aspects of active immunization. Ann. Rev. Microbiol. 1: 291–308.
——. 1956. The mode of action of immunologic adjuvants. Adv. Tuberc. Res. 7: 130–148.
Gitlin, D., and E. Merler. 1961. A comparison of the peptides released from related rabbit antibodies by enzymatic hydrolysis. J. Exper. Med. 114: 217–230.
Good, R. A. 1957. Morphological basis of the immune response and hypersensitivity. In Felton, H. M., Ed.: Host-Parasite Relationship in Living Cells. Springfield, Ill.: Charles C. Thomas.
Green, H., and H. S. Anker. 1954. On the synthesis of antibody protein. Biochem. et biophys. acta 13: 365–373.
Hanan, R., and J. Oyama. 1954. Inhibition of antibody formation in mature rabbits by contact with antigen at an early age. J. Immunol. 73: 49–53.
Harris, P. F. 1956. Quantitative examination of bone marrow in guinea pigs after gamma irradiation. Brit. M. J. 2: 1032–1035.
Harris, S., T. N. Harris, C. A. Ogborn, and M. B. Farber. 1956. Studies on the transfer

of lymph node cells. VII. Transfer of cells incubated in vitro with filtrates of trypsin-treated suspensions of Shigella paradysenteriae. J. Exper. Med. 104: 645–661.

Harris, T. N., and S. Harris. 1956. The genesis of antibodies. Am. J. Med. 20: 114–132.

——, and ——. 1957. Formation of agglutinins to Shigella paradysenteriae by transfer of lymph node cells. Fed. Proc. 16: 643–649.

Hartley, G., Jr. 1940. The local formation of antiviral antibodies by the skin. J. Infec. Dis. 66: 44–52.

Haurowitz, F. 1949. Biological problems and immunochemistry. Quart. Rev. Biol. 24: 93–101.

——. 1952. The mechanism of the immunologic response. Biol. Rev. 27: 247–280.

——. 1956. The nature of the protein molecule: problems of protein structure. J. Cellular Comp. Physiol. 47, Supp. 1: 1–16.

Heidelberger, M. 1953. Persistence of antibodies in man after immunization. In Nature and Significance of the Antibody Response. Pappenheimer, A. M., Jr., Ed.: New York: Columbia Univ. Press.

——, and F. E. Kendall. 1935. A quantitative theory of the precipitin reaction. III. The reaction between crystalline egg albumin and its homologous antibody. J. Exper. Med. 62: 697–720.

Helmreich, E., M. Kern, and H. N. Eisen. 1961. The secretion of antibody by isolated lymph node cells. J. Biol. Chem. 236: 464–473.

Hoagland, M. 1960. The relation of nucleic acids and protein synthesis as revealed by studies in cell-free systems. In Chargaff, E., and Davidson, J., Eds.: The Nucleic Acids. New York: Academic Press, vol. 3.

Holub, M. 1958. Antibody production by lymphocytes after in vitro contact with bacterial antigens and transfer to new-born rabbits. Nature 181: 122.

——. 1960. Morphological changes in lymphocytes cultivated in diffusion chambers during the primary response to a protein antigen. In Holub, M., and Jaroskova, L., Eds.: Mechanisms of Antibody Formation. Prague: Czechoslovak Academy of Sciences.

Hrubesova, M., B. A. Askonas, and J. H. Humphrey. 1959. Serum antibody and γ-globulin in baby rabbits after transfer of ribonucleoprotein from adult rabbits. Nature 183: 97–99.

Jerne, N. K. 1951. A study of avidity. Acta path. et microbiol. scandinav. suppl. 87.

——. 1955. The natural-selection theory of antibody formation. Proc. Nat. Acad. Sc. U.S. 41: 849–857.

——. 1960. Immunological speculations. Ann. Rev. Microbiol. 14: 341–358.

Kalf, G. F., H. M. Bates, and M. V. Simpson. 1959. Protein synthesis in intact and sonically disrupted mitochondria. J. Histochem. Cytochem. 7: 245–247.

Karush, F. 1956. The interaction of purified antibody with optically isomeric haptens. J. Am. Chem. Soc. 78: 5519–5526.

Kern, M., E. Helmreich, and H. N. Eisen. 1959. A demonstration of antibody activity on microsomes. Proc. Nat. Acad. Sc. U.S. 45: 862–867.

——, ——, and ——. 1961. The solubilization of microsomal antibody activity by the specific interaction between the crystallizable fraction of γ-globulin and lymph-node microsomes. Proc. Nat. Acad. Sc. 46: 767–778.

Kind, L. 1957. Relationship of the anaphylaxis sensitizing and adjuvant properties of H. pertussis. J. Immunol. 79: 238–242.

Kind, L. S., and S. K. Roffler. 1961. Reduction of the anaphylaxis enhancing and adjuvant effects of Bordetella pertussis by prior injection of B. pertussis. J. Immunol. 86: 324–330.

Landsteiner, K. 1945. The Specificity of Serological Reactions, ed. 2. Cambridge, Mass.: Harvard U. Press.

Lederberg, J. 1959. Genes and antibodies. Science 129: 1649–1653.

——. 1960. Panel discussion on the clonal selection theory of antibody formation. In CIBA Foundation Symposium on Cellular Aspects of Immunity. London: J. and A. Churchill, Ltd.

Levine, L., W. T. Murakami, H. Van Vunakis, and L. Grossman. 1960. Specific antibodies to thermally denatured deoxyribonucleic acid of phage T4. Proc. Nat. Acad. Sc. U.S. 46: 1037–1043.

Linnet-Jepsen, P., F. Galatius-Jensen, and M. Hauge. 1958. On the inheritance of the Gm serum group. Acta Genet. 8: 164–196.

McFadden, M. L., and E. L. Smith. 1955. Free amino groups and N-terminal sequence of rabbit antibodies. J. Biol. Chem. 214: 185–196.

McKenna, J. M., and K. M. Stevens. 1960. Studies on antibody formation by peritoneal exudate cells in vitro. J. Exper. Med. 111: 573–600.

McLean, J. R., G. L. Cohn, I. K. Brandt, and M. V. Simpson. 1958. Incorporation of labelled amino acids into the protein of muscle and liver mitochondria. J. Biol. Chem. 233: 657–663.

McMasters, P. D. 1953. Sites of antibody formation. In Pappenheimer, A. M., Jr., Ed.: The Nature and Significance of the Antibody Response. New York: Columbia University Press, pp. 13–45.

Malkiel, S. and B. J. Hargis. 1959. The use of adjuvants in the sensitization of the mouse. J. Allergy 30: 387–393.

Mayer, R. L. 1957. The role of carrier in the formation of complete antigens. J. Allergy 28: 191–205.

Mitchison, N. 1959. Blood transfusion in fowl: an example of immunological tolerance requiring the persistence of antigen. In Albert, F., and Lejeune-Ledant, G., Eds.: Biochemical Problems of Grafting. University of Liege.

Monod, J. 1959. Antibodies and induced enzymes. In Lawrence, H. S., Ed.: Cellular and Humoral Aspects of the Hypersensitive States. New York: Hoeber-Harper, pp. 628–650.

Morgan, P., D. W. Watson, and W. J. Cromartie. 1952. Immunization of rabbits with type II pneumococcal polysaccharide. Proc. Soc. Exper. Biol. & Med. 80: 512–516.

Mudd, S. 1932. A hypothetical mechanism of antibody formation. J. Immunol. 23: 423–427.

Nester, M., O. Makela, and G. J. V. Nossal. 1961. Studies on the transfer of nucleoprotein fractions from immunized spleens. Trans. Bull. 28: 98–99.

Nossal, G. J. V. 1959a. Studies on the transfer of antibody producing capacity. I. The transfer of antibody producing cells to young animals. Immunol. 2: 137–147.

——. 1959b. Antibody production by single cells. III. The histology of antibody production. Brit. J. Exper. Path. 40: 301–311.

——. 1960. Antibody production by single cells. IV: Further studies on multiply immunized animals. Brit. J. Exper. Path. 41: 89–96.

Ogata, K., S. Omori, and R. Hirokawa. 1961. Studies on the biosynthesis of antibody and other γ-globulin in microsomes of immunized spleen cells. J. Biochem. (Tokyo) 49: 660–662.

Oudin, J. 1960a. Allotypy of rabbit serum proteins. I. Immunochemical analysis leading to the individualization of seven main types. J. Exper. Med. 112: 107–124.

——. 1960b. Allotypy of rabbit serum proteins. II. Relationship between various allo-

types. Their common antigenic specificity, their distribution in a sample population; genetic implication. J. Exper. Med. 112: 125–142.

Pappenheimer, A. M., Jr., M. Scharff and J. W. Uhr. 1959. Delayed hypersensitivity and its possible relation to antibody formation. In Schafer, J. H., Lo Grippo, G. A., and Chase, M. W., Eds.: Mechanisms in Hypersensitivity. Boston: Little, Brown & Co., pp. 417–434.

Pauling, L. 1940. A theory of the structure and process of formation of antibodies. J. Am. Chem. Soc. 62: 2643–2657.

——, and D. Pressman. 1945. The serological properties of simple substances. IX. Hapten inhibition of precipitation of antisera homologous to the o-, m-, and p-azophenylarsonic acid groups. J. Am. Chem. Soc. 67: 1003–1012.

Peters, T. Jr. 1959. Cytoplasmic particles and serum albumin synthesis. J. Histochem. Cytochem. 7: 224–234.

Phillips, J. H., W. Braun, and O. J. Plescia. 1958. Antigenicity of a bacterial deoxyribonucleic acid. Nature 181: 573–575.

Plescia, O. J., E. L. Becker, and J. W. Williams. 1952. The valence of precipitating antibody. J. Am. Chem. Soc. 74: 1362–1363.

Porter, R. R. 1950. A chemical study of rabbit antiovalbumin. Biochem. J. 46: 473–478.

——. 1958. Separation and isolation of fractions of rabbit gammaglobulin containing the antibody and antigenic combining sites. Nature 182: 670–671.

Rebuck, J. W., R. W. Monto, E. A. Monaghan, and J. M. Riddle. 1958. Potentialities of the lymphocytes. Ann. New York Acad. Sc. 73: 8–38.

Rittenberg, M. B., and E. L. Nelson. 1960. Macrophages, nucleic acids and the induction of antibody formation. Amer. Natur. 94: 321–342.

Roberts, J. C., and F. J. Dixon. 1956. The morphology of antibody-producing lymph node and peritoneal cells transferred to immunologically inert recipients. Am. J. Path. 32: 625–626.

——, ——, and W. O. Weigle. 1957. Antibody-producing lymph node and peritoneal exudate cells. Morphologic studies of transfer to immunologically inert rabbits. A.M.A. Arch. Path. 64: 324–332.

Roberts, K. B. 1955. The failure of macrophages to produce antibodies. Brit. J. Exper. Path. 36: 199–204.

Sabin, F. R. 1939. Cellular reactions to a dye-protein with a concept of the mechanism of antibody formation. J. Exper. Med. 70: 67–82.

Schweet, R. S., and R. D. Owen. 1957. Concepts of protein synthesis in relation to antibody formation. J. Cell. Comp. Physiol. 50: suppl. 1, 199–228.

Singer, S. J. 1957. Physical-chemical studies on the nature of antigen-antibody reactions. J. Cell. Comp. Physiol. suppl. 1, 50: 51–78.

——, and D. H. Campbell. 1952. Physical chemical studies of soluble antigen-antibody complexes. I. The valence of precipitating antibody. J. Am. Chem. Soc. 74: 1794–1802.

Smith, E. L., M. L. McFadden, A. Stockell, and V. Buettner-Janusch. 1955. Amino acid composition of four rabbit antibodies. J. Biol. Chem. 214: 197–207.

Smith, R. T., and R. A. Bridges. 1956. Response of rabbits to defined antigens following neonatal injections. Trans. Bull. 3: 145–147.

——, ——. 1958. Immunological unresponsiveness in rabbits produced by neonatal injection of defined antigens. J. Exper. Med. 108: 227–250.

Stavitsky, A. B., and B. Wolf. 1958. Mechanism of antibody globulin synthesis by lymphoid tissue in vitro. Biochim. et biophys. acta 27: 4–11.

Steiner, D. F., and H. S. Anker. 1956. On the synthesis of antibody protein in vitro. Proc. Nat. Acad. Sc. U.S. 42: 580–586.

Stevens, K. M., and J. M. McKenna. 1958. Studies on antibody synthesis intiated in vitro. J. Exper. Med. 107: 537–559.

Sterzl, J. 1959. Maintenance of the ability of cells cultivated in vitro to commence formation of antibodies. Exper. 15: 62–64.

——. 1960. Inhibition of the inductive phase of antibody formation by 6-mercaptopurine examined by transfer of isolated cells. Nature 185: 256–257.

——. 1960. Discussion. In Holub, N., and Jaroskova, L., Eds.: Mechanisms of Antibody Formation. Prague: Czechoslovak Academy of Sciences, p. 147.

——. 1961. Effect of some metabolic inhibitors on antibody formation. Nature 189: 1022–1023.

——, and M. Hrubesova. 1956. The transfer of antibody formation by means of nucleoprotein fractions to non-immunized recipients. Folia Biol. 2: 21–28.

Sussdorf, D. H. 1961. Rise of antibody titer in cell-free spleen preparations incubated in vitro. Nature 191: 170–172.

Szilard, L. 1960. The molecular basis of antibody formation. Proc. Nat. Acad. Sc. U.S. 46: 293–302.

Taliaferro, W. H. 1957. Modification of the immune response by radiation and by cortisone. Ann. New York Acad. Sc. 69: 745–764.

Talmage, D. W. 1955. Effect of ionizing radiation on resistance and infection. Ann. Rev. Microbiol. 9: 335–346.

——. 1957a. Diversity of antibodies. J. Cell. Comp. Physiol. (suppl. 1) 50: 229–246.

——. 1957b. Allergy and immunology. Ann. Rev. Med. 8: 239–256.

——. 1959a. Mechanism of the antibody response. In Zirkle, R. E., Ed.: A Symposium on Molecular Biology. Chicago: U. of Chicago Press.

——. 1959b. Immunological specificity. Science 129: 1643–1648.

——, and J. R. Cann. 1961. The Chemistry of Immunity in Health and Disease. Springfield, Ill.: Charles C. Thomas, 178 pp.

——, F. J. Dixon, S. C. Bukantz, and G. J. Dammin. 1951. Antigen elimination from the blood as an early manifestation of the immune response. J. Immunol. 67: 243–255.

——, G. G. Freter, and A. Thomson. 1956. The effect of whole body X radiation on the specific anamnestic response in the rabbit. J. Infect. Dis. 99: 246–252.

Terres, G., and W. L. Hughes. 1959. Acquired immunological tolerance to crystalline bovine serum albumin. J. Immunol. 83: 459–467.

Thorbecke, G. J., H. A. Gordon, B. Wostman, M. Wagner, and J. A. Reyniers. 1957. Lymphoid tissue and serum gamma globulin in young germ free chickens. J. Infect. Dis. 101: 237–251.

——, and F. J. Keuning. 1956. Antibody and gamma globulin formation in vitro in hemopoietic organs. J. Infect. Dis. 98: 157–171.

Trnka, Z. 1958. Antibody formation by isolated cells of hen spleen after mixing with antigen in vitro and transfer to chicks. Nature 181: 55.

——, and J. Sterzl. 1960. Conditions for realizing the inductive phase of antibody production in isolated cells transferred to newborn recipients. In Holub, M., and Jaroskova, L., Eds.: Mechanism of Antibody Formation. Prague: Czechoslovak Academy of Sciences, pp. 190–194.

Weigle, W. O. 1957. Elimination of I^{131} labelled homologous and heterologous serum proteins from blood of various species. Proc. Soc. Exper. Biol. & Med. 94: 306–309.

Weissman, I. L., and E. C. Lustgraaf. 1961. Antibody formation and repressor systems. Trans. Bull. 26: 134–135.

Wesslen, T. 1952. Studies on the role of lymphocytes in antibody production. Acta dermat.-venereol. 32: 265–273.

Wissler, R. W., F. W. Fitch, M. F. LaVia, and C. H. Gunderson. 1957. The cellular basis for antibody formation. J. Cell. Comp. Physiol. (suppl. 1) 50: 265–301.

——, ——, and ——. 1960. The reticuloendothelial system in antibody formation. Ann. New York Acad. Sc. 88: 134–148.

Wright, G. W., T. W. Green, and R. G. Kanode, Jr. 1954. Studies of immunity in anthrax. V. Immunizing activity of alum-precipitated protective antigen. J. Immunol. 73: 387–391.

Yoffey, J. M., and F. C. Courtice. 1956. Lymphatics, Lymph and Lymphoid Tissue. London: E. Arnold publishers, Ltd.

Zamecnic, P. C. 1960. Historical and current aspects of the problem of protein synthesis. The Harvey Lectures, Series 54: 256–281.

Chapter 6

Clinical and Immunologic Evidence for a Predisposition to Rheumatic Disease in Certain Families

Halsted R. Holman
Stanford University School of Medicine,
Palo Alto, California

Under the heading of rheumatic diseases clinicians generally include rheumatic fever, rheumatoid arthritis and the diseases often referred to as connective tissue diseases, such as systemic lupus erythematosus (SLE), dermatomyositis, polymyositis, scleroderma and polyarteritis. Erythema nodosum and erythema multiforme which may exist alone or accompany rheumatic diseases are frequently considered in the same category. The etiology of rheumatic disorders is obscure. The association between streptococcal infection and the appearance of rheumatic fever is the only known causal relationship, and its mechanism is not understood.

Historically, a relationship between these diseases has been accepted because of similar clinical and pathological features and because, under certain experimental conditions of apparent hypersensitivity in animals, lesions appear which resemble those seen in rheumatic disorders. In recent years, various immunologic abnormalities have been identified in patients suffering rheumatic diseases. The principal abnormalities have an autoimmune character in which the serum or cells of the patient are capable of reacting with autologous tissue. As a consequence, rheumatic diseases are sometimes referred to as "autoimmune diseases." These recent discoveries provide additional evidence for a relationship between the different clinical entities in the rheumatic category and, more important, raise the possibility that an immunologic disorder underlies these diseases.

The identification of immunologic aberrations in the rheumatic diseases

has also enlarged the list of disorders which may bear some relationship to one another, at least insofar as an immunologic derangement is present in each. Notable in this regard are multiple myeloma, macroglobulinemia, agammaglobulinemia, thyroiditis, glomerulonephritis, acquired hemolytic anemia and idiopathic thrombocytopenia, lymphatic leukemia, Hodgkin's disease, and possibly even certain peculiar illnesses associated with the presence of thymoma, such as myasthenia gravis and aregenerative red cell anemia.

No biological evidence is at hand which links closely such a wide spectrum of human disease. However, the similarities in certain of the immunologic abnormalities in these illnesses, and the fact that two or more of these illnesses may occur in the same individual or the same family (see below) underscore the possibility that fundamental abnormalities of the reticuloendothelial or lymphoid system may express themselves in a variety of ways leading to a group of clinical syndromes.

For many years data have accumulated suggesting that both rheumatic fever and rheumatoid arthritis occur more commonly in certain families than in the population as a whole. However the data purporting to indicate a familial predisposition to rheumatic fever, for example, has been the subject of considerable debate and can not yet be considered conclusive (Schweitzer, 1961). More recently, studies of relatives of patients with other rheumatic diseases suggest that both clinical rheumatic diseases and immunologic abnormalities are more common in these relatives than in other members of the population. These data are fragmentary, but are strongly suggestive of a hereditary predisposition to both an abnormal immunologic reactivity and clinical rheumatic disease.

The justification for a brief review of these early data appearing in a genetic publication lies in a desire to entice geneticists into lending a guiding hand to the efforts of clinicians and immunologists who seek to explore this problem. Possibly these investigations will open new vistas in understanding of the relationships between human constitution and susceptibility to disease. They might also lead to early identification of susceptible individuals and ultimately to application of preventive medical measures in a group of diseases which are among the most prevalent of human disorders and which have defied most measures designed for their cure.

Major Approaches to the Study of Rheumatic Disease

I. Immunologic Study of the Human Disease State

The rheumatic diseases are chronic, febrile, relapsing disorders with wide variation in the intensity of the disease process. Many different organ systems may be involved with joints and connective tissue structures so

commonly affected as to provide the name rheumatic. Hypertrophy of lymphoid tissue is characteristic and hypergammaglobulinemia is common. Inflammation of organs containing connective tissue is typical and not infrequently an increased number of plasma cells or other cells in the lymphoid series group may be seen.

The constellation of clinical and pathological findings has long suggested a form of immunologic abnormality. The most fruitful recent studies have been designed to test this hypothesis through exploration of (1) the abnormal gamma-globulins, (2) possible abnormal skin sensitivities and (3) the immune response to known bacterial infections or to provocative immunizations.

A. Abnormal gamma-globulins. Study of the abnormal gamma-globulins in rheumatic disease has borne fruit in two main areas. The first of these is the identification of the rheumatoid factor and its characterization (Kunkel, 1959). This factor, commonly present in the serum of patients with rheumatoid arthritis, is a gamma-globulin with a molecular weight of approximately a million which is capable of reacting with the typical major constituent of gamma-globulin which has a molecular weight of 160,000. This reaction may take the form of direct precipitation when the low molecular weight gamma-globulin has been slightly altered, or of agglutination of inert carrier particles such as latex or red blood cells when the latter are coated with the low molecular weight gamma-globulin. This reaction provides the basis of the rheumatoid serologic tests which are used diagnostically. The reaction between these two gamma-globulin species is presumably an immune reaction and, because it can occur between the two types of gamma-globulin from the same individual, is presumably a form of autoimmune reaction.

The presence of the rheumatoid factor is not unique for rheumatoid arthritis. It is not only found in a certain number of other patients with different rheumatic diseases, but it is also present frequently in the sera of patients with hypergammaglobulinemia of diverse causes and is found in approximately 5 per cent of sera collected at random. Thus, while the reactive high molecular weight gamma-globulin is frequently increased in rheumatoid arthritis, this increase is not specific for this disease or even for the existence of any detectable clinical disorder.

The second major form of immunologic abnormality consists of the presence of antitissue autoantibodies in various rheumatic disorders. These take many forms. For example, the studies of Moore and his associates established the high incidence of benign false positive Wasserman reactions in patients with rheumatoid arthritis and systemic lupus. They also demonstrated that persons with a false positive Wasserman who do not have clinical disease at the time the reaction is identified have a higher

chance of developing rheumatoid arthritis or SLE during their subsequent years of life than do other members of the population (Moore and Lutz, 1955). Perhaps most striking has been the identification in sera from patients with active SLE of serum factors which react with many different constituents of the cell cytoplasm and nucleus (Holman, Deicher and Kunkel, 1959). At least two of these serum factors have been isolated in highly purified form and possess all the characteristics of typical human antibody gamma-globulin (Deicher, Holman and Kunkel, 1959; Holman and Deicher, 1959). These two factors, interestingly, react with deoxyribonucleic acid (DNA), one reacting with DNA alone and the other reacting with nucleohistone requiring both the DNA and the histone for the reaction. The latter antibody is the one which is responsible for the formation of LE cells, the test which is in wide use clinically in the diagnosis of systemic lupus. These antitissue antibodies possess no organ or species specificity and are capable of reacting with the autologous cells of the patient. It is also pertinent that despite the existence of circulating autoantibodies able to react with virtually any cell of the patient's body in vitro, there is evidence that these antibodies can not react with or injure normal cells in vivo (Holman and Robbins, 1959).

Sera from patients with chronic thyroiditis or Hashimoto's disease have also been found to contain antitissue autoantibodies, one of which reacts specifically with purified thyroglobulin while the other reacts with a constituent of the thyroid cell cytoplasm (Doniach, Hudson and Roitt, 1960).

Antitissue antibodies such as those described above may be found in other members of the rheumatic disease group. Frequently, many types of antibodies including both the rheumatoid factor and antitissue antibodies may be found in an individual who suffers from only one type of clinical disease, such as SLE or Sjogren's syndrome, a variant of rheumatoid arthritis (Deicher, Holman and Kunkel, 1960; Bloch, Wohl, Ship, Oglesby and Bonim, 1960).

Such antitissue antibodies have not been demonstrated in the disease rheumatic fever, though they have been sought by a variety of methods. The mechanism whereby streptococcal infection gives rise to rheumatic fever remains obscure though many have suspected that in some way the streptococcal infection initiates a form of autoimmune reaction. Recently, Kaplan has presented data supporting this view in that he can demonstrate by fluorescent antibody methods localization of gamma-globulin in myocardial muscle fibriles. Furthermore, antibodies to streptococcal cell walls will react with human heart tissue (Kaplan and Meyeserian, 1961). This suggests that an immune response to the streptococcus can under certain circumstances give rise to antibodies capable of reacting with the host's own tissue.

Thus abnormal gamma-globulins of both high and low molecular weight have been discovered which appear to be autoantibodies. While a preponderance of one or another of these types of autoantibodies characterizes certain clinical syndromes, autoantibodies of many types may be found in many of the rheumatic disorders and, as will be seen, also in apparently well individuals.

A reservation must be placed on the designation of the rheumatoid factors and antitissue antibodies as autoantibodies. In a few instances these factors have appeared to react with homologous but not with autologous tissues. If confirmed, this distinction in reactivity would indicate that some of the serum factors more closely resemble isoantibodies.

B. Skin reactivity to autologous cells. Another type of possible immunologic abnormality in rheumatic diseases lies in the skin reactivity demonstrated by patients to their own cells (Holman, 1960; Friedman, Bardawil, Merrill and Hanau, 1960; Bennett and Holley, 1961). When patients with systemic lupus are inoculated intradermally with whole autologous white cells or with homogenates of autologous white cells, they develop an erythematous and indurated reaction around the site of inoculation starting at approximately 10 hours and achieving its maximum around 18 hours. In most but not all instances, the reaction has faded by 48 hours. In many patients, it is indistinguishable from a tuberculin reaction except in timing. It is also characteristically unrelated to the presence or absence of circulating antitissue antibodies. The reactivity exists when the disease is active or in remission. A similar reaction has been observed in patients with rheumatoid arthritis, rheumatic fever and other connective tissue disorders. It is occasionally seen in patients with other types of tissue damage. Rarely has the reaction been positive in normal individuals on the first inoculation. Interestingly, however, normal individuals will develop a positive reaction on the second or third inoculation of their own white cells (Holman, unpubl. results). Thus, patients with rheumatic diseases differ from normal only in that they respond the first time they are inoculated with autologous cells. The reaction in its appearance and timing is similar to that found on intradermal inoculation with brain extracts of animals previously immunized with autologous or heterologous brain who developed antibrain antibodies and/or allergic encephalomyelitis (Waksman, 1956). An analogous reaction is also found in individual animals, including human beings, who have been grafted with skin from an unrelated donor (Brent, Brown and Medawar, 1958; Merrill, Friedman, Wilson and Marshall, 1961). Following the rejection of the skin graft the recipient demonstrates a skin reaction to leukocytes of the donor which appears to be identical in timing and characteristics to that found in patients with SLE. A similar skin reactivity has also been described in patients with thyroid-

itis upon intradermal inoculation with thyroid extract (Buchanan, Anderson and Goudie, 1958).

In preliminary experiments, it has been possible to demonstrate a similar skin reaction in guinea pigs after two or more inoculations of autologous white cells (Raitt, unpubl. results). However, studies of this skin reactivity have been relatively recent and have not been thorough. The appearance of a similar reaction in normal individuals after one or two inoculations appears to preclude serial studies in the same individual, thus hampering investigation.

C. Response to provocative immunization with foreign antigens. The existence of immunologic abnormalities in patients with rheumatic diseases has led many investigators to explore the possibility that these patients are hyperreactive to immunization with foreign antigens in general. The suspicion that this might be so has been heightened by the clinical correlation between allergic reactions to drugs and the appearance of such diseases as systemic lupus and polyarteritis (Harvey, Shulman, Tumulty, Conley and Schroenrich, 1954) and also by the fact that patients with SLE have been demonstrated to form antibodies to rare blood group substances in greater profusion following transfusion than other members of the human population (Waller and Race, 1951). However, in general, antibody titers following provocative immunization in these patients have not confirmed the existence of a generalized hyper-reactivity. In the most instances, the antibody titers have not differed significantly from those found in normal controls (Miller, Kibrick and Nassel, 1953; Quinn, Seastone and Dickie, 1953; Kuhns and McCarty, 1954; Deicher and Holman, unpubl. results). Furthermore, skin reactivity to dermal sensitizing agents has not been significantly greater in rheumatic disease subjects than in control subjects (Epstein and Jessar, 1959). Exceptions to these conclusions exist, however (Rantz, Creger and Choy, 1952; Meiselas, Zingale, Lee, Richman and Siegel, 1961). For example, patients with rheumatic fever show a higher titer of antistreptococcal antibodies than nonrheumatic subjects following streptococcal infections (McCarty, 1952). Waldenstrom (Waldenstrom and Winblad, 1958) has reported antistreptolysin and antistaphylolysin titers to be higher in patients with various rheumatic diseases than in controls. However, in his study, active immunization was not conducted and it is impossible to know whether the high titers were due to a heightened immune response or simply to the general elevation of gamma-globulin levels in the patients. There is considerable evidence suggesting that at least two drugs in fairly common clinical usage, hydralazine (Dammin, 1959) and hydantoin derivatives (Ruppli and Vossen, 1957), can produce a syndrome similar to systemic lupus in persons who have been taking these drugs for protracted periods of time. Presumably, this

is a hypersensitivity reaction but this is not certain, because there has been no other evidence of an immunologic reaction to the drugs in these patients.

The data at hand therefore do not support the contention that patients with rheumatic diseases are in general hyperreactive to antigenic stimulation. Significant exceptions to this generalization exist, but where they do the hyperreactivity appears to be to only one antigen and not to foreign antigens in general. Here too, however, the experimental evidence is fragmentary and can not be considered definitive.

D. The role of immunologic abnormalities in disease pathogenesis. The immunologic aberrations mentioned above, plus the existence of low serum complement levels in acute systemic lupus and acute nephritis (Williams and Law, 1958), support the view that major immunologic abnormalities occur in the rheumatic diseases. It might be presumed that circulating autoantibodies or a delayed hypersensitivity to autologous tissue constituents would play a major role in disease pathogenesis. Data do not support this contention, however. Not only do autoantibodies appear in individuals who are not clinically ill but there is a paucity of evidence indicating a cytopathogenic effect of these antibodies. For example, despite the existence of circulating antitissue antibodies prior to death, the tissues of patients dying with SLE do not demonstrate widespread localization of gamma-globulin on or within cells (Mellors, Ortega and Holman, 1957; Vazquez and Dixon, 1957). Furthermore, tissue culture studies of cells grown in the presence of large amounts of anticytoplasmic or antinuclear antibodies have not indicated a cytopathogenic effect of these antibodies (Holman and Nemes, unpubl. data; Scheffer, 1961). Infants born of mothers with SLE have been found to have in their circulation antitissue antibodies which crossed the placenta, but no damage has been identified in these infants which can be attributed to the circulating antibodies. Thus cells functioning normally in vivo may not be affected significantly by antitissue antibodies. This does not preclude the possibility that such antibodies enter and alter cells which have been partially damaged, but experimental data bearing on this is fragmentary and inconclusive. The interesting possibility persists that once tissue damage has been initiated, the reaction of antitissue antibodies with constituents of damaged cells helps to perpetuate the tissue injury.

Similarly, there is no evidence to suggest that the skin reactivity to autologous cells is an indicator of a cellular hypersensitivity which can damage tissues. Though this possibility has not been thoroughly explored, the ability to provoke such a reaction in normal patients without evident deleterious effects speaks strongly against this reaction itself being an indicator of a pathogenically significant delayed type of autoimmunity.

The inability to incriminate directly the demonstrable immunologic abnormalities in the pathogenesis of rheumatic diseases forces the conclusion that the currently measurable immunologic aberrations are a byproduct of another, more fundamental underlying abnormality. The nature of this latter abnormality is unknown. It is possible that in some as yet unexplained way this abnormality is directly related to disease pathogenesis and that, as a secondary consequence, the autoantibodies and peculiar skin reactivity arise.

II. Animal Experimentation

With minor possible exceptions, the rheumatic diseases occur spontaneously only in the human species; there are few if any natural analogues of these clinical conditions in other animals. Furthermore, efforts to create precise analogues in animals by experimental means have met with very limited success.

The most notable initial experimental successes were in the study of serum sickness and hypersensitivity to certain chemicals such as sulfonamides. In the former instance, an acute disease which affects the joints, the small blood vessels, the kidneys and the lungs can be induced following immunization with a foreign protein (Dixon, Feldman and Vazquez, 1959). In the latter instance, a vasculitis with glomerulonephritis appears in the animals after chronic exposure to certain chemical compounds (Rich, 1947). While in both cases the lesions resemble in many ways those seen in the rheumatic diseases, in neither instance is the disease induced a chronic relapsing type with the serologic abnormalities found in the human disease. In the case of serum sickness, the provocative agent is known and the disease can be ascribed to a reaction involving antigen and antibody. In the case of the sulfonamide-induced vasculitis, neither an antigen-antibody reaction nor a delayed type of hypersensitivity can be clearly incriminated. The hydralazine and hydantalin derivatives which will provoke syndromes resembling rheumatic diseases in certain human beings have not been found to do so with consistency in normal experimental animals.

The greatest experimental success has been in investigations of experimental thyroiditis (Rose and Witebsky, 1956) and experimental autoimmune encephalomyelitis (Waksman, 1959). In both of these instances, immunization of certain animals with heterologous, homologous or autologous thyroid or brain gives rise to clinical and pathological results which are difficult to distinguish from the human diseases of chronic relapsing thyroiditis in the former instance or multiple sclerosis in the latter instance. In experimental thyroiditis, circulating autoantibodies arise which are similar to those which appear in the human disease. Furthermore, in both

experimental thyroiditis and experimental encephalomyelitis, the animals demonstrate a skin reactivity to the offending antigen which is similar in nature and timing to the reaction to autologous cells in the human rheumatic diseases. Also, in these experimental conditions, there is sizable evidence which suggests that the circulating antibodies are not the pathogenic agents. The analogy between experimental thyroiditis and human thyroiditis is good, though in both instances the nature of the tissue-damaging reaction is unknown. The analogy between experimental encephalomyelitis and multiple sclerosis is less exact because of the absence of demonstrable serologic or other immunologic abnormalities in multiple sclerosis. There is as yet no similar experimental analogue to rheumatic fever, rheumatoid arthritis, systemic lupus or the other connective tissue diseases.

The direct role of the lymphoid system in causing allergic encephalomyelitis has been elegantly demonstrated by Paterson who has been able to transfer the disease from one animal to another through passage of spleen cells from a diseased donor (Paterson, 1960). Recently, Hess and Ziff (Hess, Ashworth and Ziff, 1961) have also succeeded in transferring rat nephrosis from one animal to another by the passage of sensitized lymphoid cells.

Dixon and his co-workers have demonstrated another way in which the immune system may be incriminated in connective tissue disease (Dixon, Feldman and Vazquez, 1961). They have shown that chronic exposure to a foreign antigen can give rise in certain animals to a chronic glomerulonephritis indistinguishable morphologically from the disease which is seen in human beings. Of great pertinence is the fact that this nephritis develops in those animals who are relatively poor antibody formers, and does not appear in those animals who have a vigorous immune response to the immunogen. They suggest that the nephritis is due to circulating antigen-antibody complexes which are continuously renewed because the susceptible animal is in a constant state of antigen excess, not having enough antibody to clear the antigens from the circulation. The vigorous antibody formers on the other hand are able to eliminate the antigen and are not exposed continuously to circulating antigen-antibody complexes.

Thus far, animal experimentation may be said to have failed to provide an exact model of most of the rheumatic and connective tissue diseases. It has, however, yielded evidence (1) that immune reactions can produce diseases of connective tissue which affect the organs also involved in many of the human rheumatic diseases, (2) that at least in the instance of thyroiditis a model apparently indistinguishable from the human disease can be achieved through immunization of the subject animal with autologous or homologous thyroid, (3) that in the latter instance as well as in allergic encephalomyelitis (which may ultimately be shown to be analogous to

human multiple sclerosis) the currently demonstrable abnormal reactions cannot be clearly identified as the pathogenic mechanisms, (4) that transferred, immunologically competent, sensitized cells can induce certain disease states in animals similar to the human disorders of demyelinating disease and nephrosis and (5) that antigen-antibody complexes may under certain circumstances induce lesions resembling those seen in rheumatic diseases. There is no doubt therefore that immune reactions are capable of inducing major pathological alterations in tissues and consequent clinical syndromes. In a majority of instances, however, it is uncertain whether classic immediate or delayed types of hypersensivity are directly or solely responsible for the disease states. Thus in these induced animal disorders, as well as in the human rheumatic diseases, a form of immunologic reaction as yet unidentified may be responsible for the tissue lesions and disease state.

III. Studies of Relatives of Patients with Rheumatic Diseases

For many years physicians have observed an increase in the incidence of rheumatic fever in certain families. Numerous studies have been conducted to determine whether this increased incidence is the result of a genetic predisposition to rheumatic fever or of an increased exposure to streptococcal infections. It has proved very difficult to gauge the intensity of the exposure to the streptococcus and therefore the data are not conclusive. The studies, recently summarized by Schweitzer (1961) and by Stollerman (1960), have consisted of an evaluation of disease incidence coupled with efforts to obtain appropriate controls which take into account both the exposure to and the incidence of streptococcal infection. They have not been designed to explore the existence of immunologic abnormalities in the relatives of the patients. Taranta (Taranta, Torosdag and Uchida, 1959) recently studied 37 sets of twins one of whom had rheumatic fever. In 14 sets, the evidence indicated monozygosity, and 3 sets were concordant for rheumatic fever. In another 14 couples with the same sex but considered dizygotic, there was no rheumatic fever in the other twin. The remaining 9 couples had a different sex and were considered dizygotic. Only one set was concordant for rheumatic fever. This incidence of concordance was less than that observed for cases of tuberculosis and poliomyelitis in twins. Taranta suggests that the genetic predisposition to rheumatic fever, if it exists, is no greater than that seen with certain other infectious diseases and therefore may reflect not a specific predisposition to rheumatic fever but rather a general state of susceptibility to infection. This view is not shared by others, but they have not presented data involving large groups of twins.

Because infection directly influences the incidence of rheumatic fever

and because only negligible data are available on immunologic abnormalities in relatives of patients with rheumatic fever, these data will not be examined further. With other rheumatic diseases, no relationship with streptococcal or other infection has been found. Here too, however, clinical studies suggest that there is a higher incidence of rheumatic disease in certain families (Stecher, 1955). These impressions, coupled with the evidence of immunologic abnormalities in the patients, has led investigators to combine clinical and immunologic investigation in families of diseased individuals. The results, while preliminary, are quite interesting.

Lawrence and Ball (1958) found a fourfold increase, over matched controls, of clinical, radiological and serologic rheumatoid arthritis in relatives of patients suffering from serologically positive rheumatoid arthritis. They also found a lower incidence of clinical rheumatoid arthritis and no increase in serologic abnormalities in relatives of propositi who had clinical disease without serologic abnormalities. Ziff and his co-workers (Ziff, Schmid, Lewis and Tanner, 1958) similarly explored matched groups of patients and relatives, one group consisting of relatives of patients with rheumatoid arthritis and the other of relatives of persons who were normal. They found a considerably higher incidence of the rheumatoid factor in both clinically sick and normal relatives of patients with rheumatoid arthritis. These data indicate that there is a higher incidence of clinical rheumatoid arthritis in certain families and that in these families many asymptomatic relatives may have the serologic aberrations characteristic of rheumatoid arthritis. Rheumatoid arthritis has been reported in both members of pairs of uniovular twins (Kaufmann and Scheerer, 1928).

Instances of systemic lupus have been recorded in both monozygotic and dizygotic twins (Brunjes, Zike and Julian, 1961). Numerous instances of the disease have been reported in parents and offspring (McKusick, 1959). Leonhardt reported an interesting family of 14 children (Leonhardt, 1959). Three of the children suffered from systemic lupus and had the characteristic hypergammaglobulinemia of this disease. One other sibling had hypergammaglobulinemia without clinical disease and 4 additional siblings possessed mild elevations of gamma-globulin without evident clinical disease. Thus, there were 8 siblings in this family with hypergammaglobulinemia or clinical systemic lupus erythematosus. Other relatives in the same family were found to have a statistically significant increase in gamma-globulin over control groups (Larsson and Leonhardt, 1959).

In another study of relatives of patients with unequivocal systemic lupus, more detailed abnormalities were uncovered (Holman and Deicher, 1960). The relatives were examined for clinical and radiographic evidence of disease and their blood was studied for the presence of hypergammaglobulinemia, rheumatoid serologic factors and antitissue antibodies of the

type which occur in SLE, including a positive Wasserman reaction. In all, 27 families were studied with 106 randomly selected relatives. A complete survey of existing family members in each family was not accomplished; only available relatives were studied. As controls a group of randomly selected individuals and some of their relatives were also studied. However, the controls were not matched. Consanguinity was denied in all families. The results show that in 18, or 66 per cent of the families, at least 1 other family member either had unequivocal disease of the rheumatic type or had an abnormality in 1 of the serologic parameters. Of the 106 relatives who were examined, 38, or 36 per cent, possessed at least 1 distinct abnormality. An additional 3 persons had probable but not unequivocal abnormalities. Most interesting of all, of the 38 relatives with definite abnormalities, 26 were free of signs or symptoms of clinical disease. The most common serologic abnormality in the relatives was a positive test for the presence of rheumatoid factor, occuring in 23 of the 38 abnormal persons. The second most common abnormality was an elevated serum gammaglobulin level and the third was clinical or radiographic rheumatoid arthritis. Four persons had a positive Wasserman reaction and only 1 showed the abnormal antitissue antibodies commonly found in SLE. Each of the above-mentioned abnormalities was far more frequently encountered in the relatives of the patients than in the control individuals studied. Pedigrees of 2 of these families are presented in Figures 1 and 2.

Similar studies to this one have been conducted by other workers with essentially similar results using the families of patients with SLE and scleroderma (Morteo, Franklin, McEwen, Phythyon and Tanner, 1961; Rodnan, Maclachlan and Creighton, 1960; Corcos, Robbins, Rogoff and Heiner, 1961; Siegel et al., 1961; Siegel and Lifshay, 1962). In these latter studies as well, the striking feature was the presence of hypergammaglobulinemia or abnormal serologic reactions in apparently well relatives. Exploration of families of patients with thyroiditis have demonstrated a higher than normal incidence of antithyroid antibodies in well relatives (Hall, Owen and Smart, 1960). Two most interesting studies of children with agammaglobulinemia and their families has led to the finding not only of hypergammaglobulinemia in the relatives, but also of rheumatoid arthritis and systemic lupus (Fudenberg, German and Kunkel, 1960; Wolf, 1962).

The limitation of these studies are great. The experimental design has been neither consistent nor thorough. The use of paired and matched controls has varied extensively from one study to another. Though in certain instances questions concerning intermarriage have been asked, there has been no search through family pedigrees to establish with certainty the existence or absence of consanguinity. The reports of disease in twins has

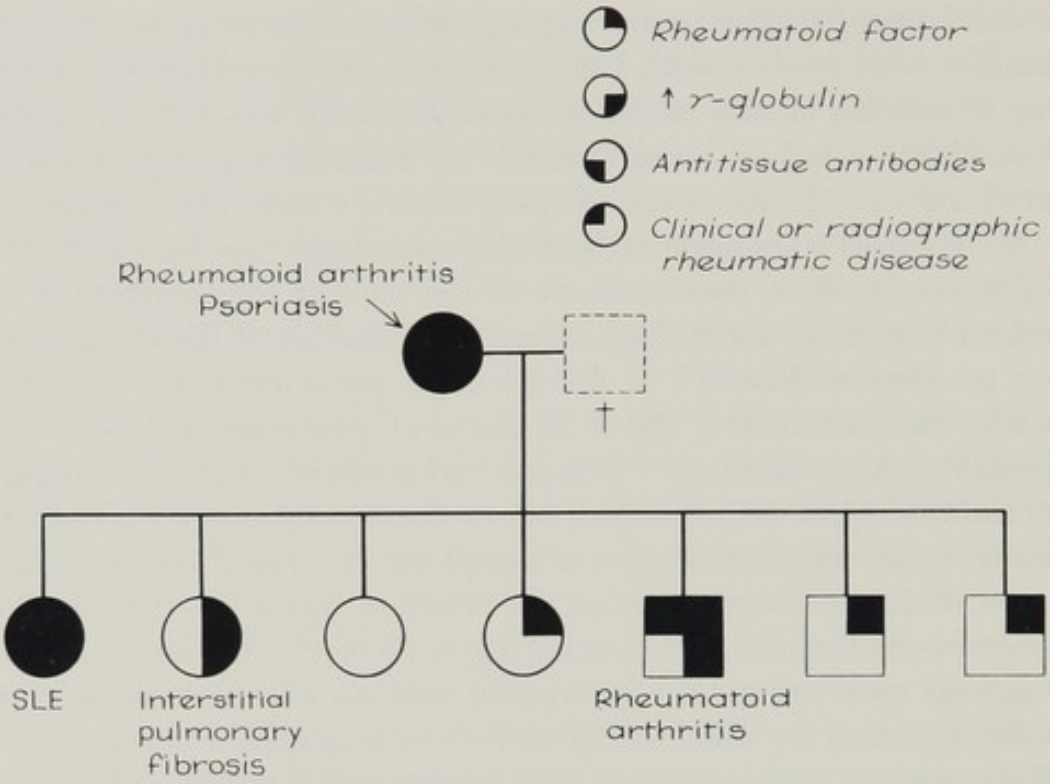


FIG. 1—Mother of proband had extensive serologic abnormalities and clinical rheumatoid arthritis. Five of six siblings are abnormal. Two siblings have clinical disease, in one instance pulmonary fibrosis which may be a part of the rheumatic disease picture. Individuals indicated by a dash line were not studied.

been of the clinical nature often without serologic evaluation and no one has attempted to collect a group of twins and analyze for concordance. There has been almost no study of special environmental factors including, for example, exposure to and reaction to allergens, chemicals and drugs. There has been no analysis of social status and the general environmental circumstances of the families. In one instance, however, spouses and relatives of spouses of propositi have been examined, and this group has less evidence of disease or serologic abnormality than has the group of relatives of the propositus (Larsson and Leonhardt, 1959). Thus, a study thoroughly evaluating both the genetic and environmental circumstances of families and comparing them to similar controls has not been conducted.

The specific methods for evaluating immunologic abnormalities have differed. The only relatively uniform feature has been the general application of standard criteria for diagnosis of rheumatoid arthritis. The methods used for detecting rheumatoid factor, gamma-globulin levels and various types of other autoantibodies have varied from study to study. Lawrence and Ball (1958) explored the existence of sheep cell agglutination reaction characteristic for the rheumatoid factor but no other agglutination reac-

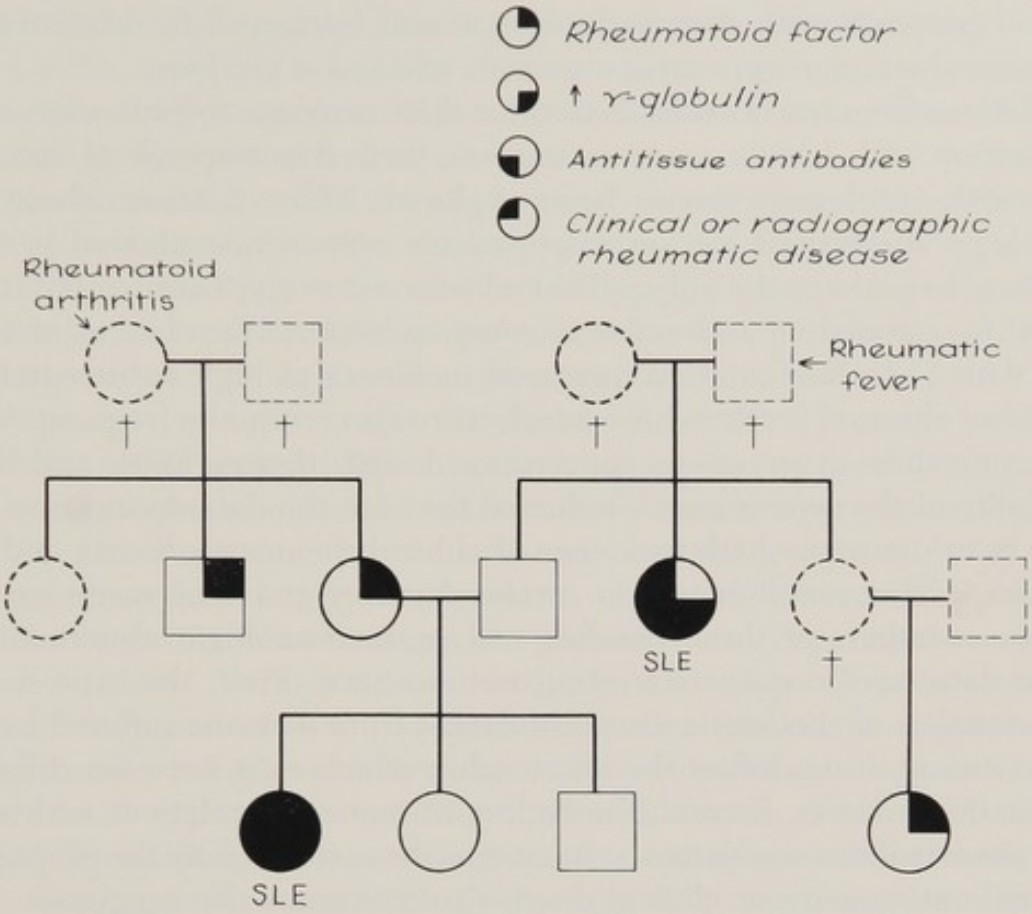


FIG. 2—Father of proband has a borderline elevation of the serum gammaglobulin which is not indicated in the pedigree. One paternal aunt has systemic lupus and the other paternal aunt died of an unexplained renal disease. The daughter of the paternal aunt was considered to have systemic lupus by other physicians but had only a circulating rheumatoid factor at the time of testing. The paternal grandfather was alleged to have had rheumatic heart disease. The mother of the proband has morning stiffness and arthralgia in addition to a circulating rheumatoid factor. The maternal grandmother was alleged to have had typical rheumatoid arthritis. Individuals indicated by a dash line were not studied.

tions and did not include a study of gamma-globulin levels among relatives. Certain of the groups searching for antitissue antibodies have employed the fluorescent antibody method while others have used complement fixation techniques, though these methods do not parallel one another in antitissue antibody identification (Friou and Holman, unpubl. data). Estimation of gamma-globulin levels have been done by different methods and, indeed, no clear normal range has yet been established. It is known that gamma-globulin levels differ in various ethnic groups and generally increase with age. However, data on the ethnic origin of patients and the age of the individuals involved in the studies has been lacking. Thus, a thorough and standardized immunologic evaluation has not been made of all living rela-

tives of propositi with rheumatic disease and compared in detail with a similar evaluation of appropriate controls selected at random.

Relatives have not been evaluated for their response to provocative immunization with known antigens nor has their skin response to inoculations with autologous tissues been explored. Thus, 2 areas where immunologic aberrations may exist in patients remain unevaluated in their relatives. In perhaps the only published relevant study, Leonhardt (1959) looked for circulating antibodies to common bacteria in relatives of 1 patient with SLE. He found an increased incidence of high antistreptolysin titers, but elevated levels in his controls were also unusually frequent.

Despite these reservations concerning design, thoroughness and comparability of the experiments conducted thus far, the data do indicate that there is an unusually high incidence of clinical rheumatic disease and immunologic abnormalities within certain families and that many asymptomatic members of these families will possess serologic abnormalities. These data have certain interesting ramifications. First, the existence in these families of rheumatic disease different from that one suffered by the propositus again underlies the relationship which exist between different rheumatic disorders. Second, the finding of numerous relatives with serologic abnormalities sometimes as intense as those suffered by the propositus but without concurrent clinical disease substantiates the suggestion that the serologic factors themselves are not pathogenic agents but are rather by-products of a deranged immunologic reactivity. Third, the increased incidence of both clinical disease and serologic abnormality in these families supports the contention that there is some form of susceptibility to rheumatic diseases which is influenced by hereditary factors.

An additional conclusion may be advanced which bears upon the investigation of these disorders. The apparent limitation of the rheumatic diseases to the human species and the possible existence of genetic factors predisposing to these diseases suggest that the search for animal models of rheumatic diseases is likely to be difficult or fruitless unless strains of animals are found which possess a qualitatively similar genetic predisposition. Thus, the difficulty in obtaining lesions similar to those of rheumatic fever in experimental animals infected with streptococcus and the great difficulty in provoking syndromes similar to systemic lupus in animals with the use of chemical agents which do induce such syndromes in human beings may in part be explained. Two recent developments are significant in this regard. Burnet and his co-workers have found an inbred strain of mice which characteristically suffers an autoimmune hemolytic anemia with positive Coomb's test beginning at about the fifth or sixth month of life (Holmes, Gorrie and Burnet, 1961). In preliminary studies of this strain, these workers have found considerable evidence of immunologic abnormality. Also, a genetic hypergammaglobulinemia with lesions of various

reticuloendothelial organs has been described in the Aleutian mink (Henson, Leader and Gorham, 1961). Perhaps these diseases in animals will prove to be the first truly useful animal analogues of human diseases characterized by the existence of autoimmunity.

While the family studies already conducted underscore the relationship between certain of the more common rheumatic disorders, they do not relate these disorders to other diseases with major gamma-globulin abnormalities such as myeloma, macroglobulinemia or to the lymphomas. Fragmentary evidence available today, however, indicates that when such relationships are carefully sought they may be found. Thus, for example, Lindholm (1959) has published information on a family in which one child had hypogammaglobulinemia and 4 or 5 siblings had hypergammaglobulinemia. The mother of the child had an elevated gamma-globulin level with a narrow band suggesting myeloma while the father had hypergammaglobulinemia with a broad gamma-globulin band. A paternal uncle had typical myeloma. Various studies have shown that typical macroglobulins obtained from patients with Waldenstrom's macroglobulinemia possess the biologic and serologic characteristics of rheumatoid factors obtained from patients with typical rheumatoid arthritis (Kritzman, Kunkel, McCarthy and Mellors, 1961). Many examples are available of more than 1 of these diseases in the same individual, suggesting that they also might be found in the family. Patients with agammaglobulinemia who have been kept in relatively good health by gamma-globulin replacement have been found to develop frequently rheumatoid arthritis, dermatomyositis, scleroderma and lymphatic leukemia (Janeway, Gitlin, Craig and Grice, 1956). Numerous individual patients have been reported who suffered simultaneously or in sequence polyarteritis and lymphosarcoma; systemic lupus and lymphoma; thymoma and agammaglobulinemia; lymphoma and macroglobulinemia; and thymoma and aregenerative red cell anemia with positive LE cell preparations. Waldenstrom (1961) was presented a number of patients who experienced as many as three disorders of the lymphoid system.

One can not close this brief review without mentioning an intriguing additional possibility. It is now known that gamma-globulins in human beings and in animals can be typed into serologic groups which are inherited (Fudenberg and Kunkel, 1961). An attempt to correlate the types of gamma-globulin in any individual or family with the abnormalities of gamma-globulin which may appear might prove most fruitful.

Summary

Numerous immunologic abnormalities exist in patients with rheumatic diseases and many of their relatives. These abnormalities are frequently

of an autoimmune character. The patterns of abnormalities which are emerging in the patients and their relatives suggest that there is a hereditary predisposition to an immunologic aberration which may exist in some individuals without clinical disease and may express itself clinically in other individuals as one of a variety of different rheumatic or related disease states. The studies reported, however, do not provide consistently useful data for genetic analysis. Current abilities to recognize disease states and to conduct immunologic studies on human beings are sufficient to permit well designed epidemiologic, genetic and immunologic investigations which can clarify the suggested relationships. This will require standardization of both the subject material and the methods used to analyze clinical status and immunologic abnormalities. Thorough evaluation of all types of immunologic response are indicated. In order to accomplish this most effectively, clinicians and geneticists should combine forces; if they do, most useful and exciting new relationships between human constitution and human disease will almost certainly emerge.

References

Bennett, J. C., and H. L. Holley. 1961. Intradermal hypersensitivity in systemic lupus erythematosus. Arth. & Rheumat. 4: 64.

Bloch, K. J., M. J. Wohl, I. I. Ship, R. B. Oglesby, and J. J. Bunim. 1960. Sjorgren's syndrome. I. Serologic reactions in patients with Sjogren's syndrome with and without rheumatoid arthritis. Arth. & Rheumat. 3: 287.

Brent, L., J. Brown, and P. Medawar. 1958. Skin transplantation immunity in relation to hypersensitivity. Lancet 2: 561.

Brunjes, S., K. Zike, and R. Julian. 1961. Familial systematic lupus erythematosus. Am. J. Med. 30: 529.

Buchanan, W., J. R. Anderson, and R. B. Goudie. 1958. A skin test in thyroid disease. Lancet 2: 928.

Corcos, J. M., W. C. Robbins, B. Rogoff, and R. Heimer. 1961. Some serum protein abnormalities in patients with progressive systemic sclerosis and their relatives. Arth. & Rheumat. 4: 107 (abstract).

Dammin, G. 1959. Serum sickness and related states. In Lawrence, S. H., Ed.: Cellular and Humoral Aspects of Hypersensitive States. New York: Paul B. Hoeber, Inc.

Deicher, H., H. Holman, and H. G. Kunkel. 1959. The precipitin reaction between DNA and a serum factor in systemic lupus erythematosus. J. Exper. Med. 109: 97.

——, ——, and ——. 1960. Anti-cytoplasmic factors in the sera of patients with systemic lupus erythematosus and certain other diseases. Arth. & Rheumat. 3: 1–15.

——, and ——. Response of patients with systemic lupus erythematosus to immunization with diphtheria and typhoid antigens. Unpublished results.

Doniach, D., R. Hudson, and I. Roitt. 1960. Human autoimmune thyroiditis: clinical studies. Brit. M. J. 1: 365.

Dixon, F. J., J. Feldman, and J. Vazquez. 1959. Immunology and pathogenesis of experimental serum sickness. In Lawrence, S. H., Ed.: Cellular and Humoral Aspects of Hypersensitive States. New York: Paul B. Hoeber, Inc.

——, ——, and ——. 1961. Experimental glomerulonephritis. J. Exper. Med. 113: 899.

Epstein, W. L., and R. A. Jessar. 1959. Contact-type delayed hypersensitivity in patients with rheumatoid arthritis. Arth. & Rheumat. 2: 178.

Friedman, E. A., W. A. Bardawil, J. P. Merrill, and C. Hanau. 1960. "Delayed" cutaneous hypersensitivity to leukocytes in disseminated lupus erythematosus. New England J. Med. 262: 486.

Friou, G., and H. Holman. Unpublished results.

Fudenberg, H., and H. G. Kunkel. 1961. Specificity of the reaction between rheumatoid factors and gammaglobulin. J. Exper. Med. 114: 257.

——, J. L. German, and H. G. Kunkel. 1960. Occurrence of rheumatoid factor and other gammaglobulin abnormalities in families of patients with agammaglobulinemia. J. Clin. Invest. 39: 987 (abstract).

Hall, R., S. G. Owen, and G. A. Smart. 1960. Evidence for genetic predisposition to formation of thyroid autoantibodies. Lancet 2: 187.

Harvey, A. M., L. E. Shulman, P. A. Tumulty, C. L. Conley, and E. H. Schoenrich. 1954. Systemic lupus erythematosus: Review of the literature and clinical analysis of 138 cases. Medicine 33: 291.

Henson, J. B., R. Leader, and J. Gorham. 1961. Hypergammaglobulinemia in mink. Proc. Soc. Exper. Biol. & Med. 107: 919.

Hess, E., G. Ashworth, and M. Ziff. 1961. Transfer of autoimmune nephrosis in rats by means of lymph node cells. Arth. & Rheumat. 4: 114 (abstract).

Holman, H. 1960. Systemic lupus erythematosus. J. Pediat. 56: 109–119.

——. Unpublished results.

——, and H. Deicher. 1959. The reaction of the LE cell factor with deoxyribonucleoprotein of the cell nucleus. J. Clin. Invest. 38: 2059.

——, ——, and H. G. Kunkel. 1959. The L. E. cell and the L. E. serum factors. Bull. New York Acad. Med. 35: 409–418.

——, and ——. 1960. The appearance of hypergammaglobulinemia, positive serologic reactions for rheumatoid arthritis and complement fixation reactions with tissue constituents in the sera of relatives of patients with systemic lupus erythematosus. Arth. & Rheumat. 3: 244 (abstract).

——, and Nemes, M. Unpublished data.

——, and Robbins, W. 1959. Antinuclear antibodies in systemic lupus erythematosus. Arth. & Rheumat. 2: 468.

Holmes, M., J. Gorrie, and F. Burnet. 1961. Transmission by splenic cells of an autoimmune disease occurring spontaneously in mice. Lancet 2: 638.

Janeway, C. A., D. Gitlin, J. Craig, and D. Grice. 1956. Collagen disease in patients with congenital agammaglobulinemia. Tr. A. Am. Physicians 69: 93.

Kaplan, M., and M. Meyeserian. 1961. An immunologic cross-reaction between group A streptococcal cells and a constituent of human heart tissue. Presented to the American Rheumatism Association, December 8 & 9.

Kaufmann and Scheerer. 1928. See Falls, H. F. 1953. Clinical Genetics, edited by A. Sorsby, London: Butterworth.

Kritzman, J., H. Kunkel, J. McCarthy, and R. Mellors. 1961. Studies of a Waldenstrom-type macroglobulin with rheumatoid factor properties. J. Lab. & Clin. Med., 57: 905.

Kuhns, W. J., and M. McCarty. 1954. Studies of diphtheria antitoxin in rheumatic fever subjects: Analysis of reactions to the Schick test and of antitoxin responses following hyperimmunization with diphtheria toxoid. J. Clin. Invest. 34: 759.

Kunkel, H. G. 1959. The rheumatoid factors. Arch. Int. Med. 104: 832–836.

Larsson, O., and T. Leonhardt. 1959. Hereditary hypergammaglobulinaemia and systemic lupus erythematosus. Acta med. scandinav. 165: 371.

Lawrence, J. S., and J. Ball. 1958. Genetic studies on rheumatoid arthritis. Ann. Rheumat. Dis. 17: 160.

Leonhardt, T. 1957. Familial hypergammaglobulinemia and systemic lupus erythematosus. Lancet 2: 1200.

Leonhardt, T. 1959. Hereditary hypergammaglobulinaemia and systemic lupus erythematosus. Acta med. scandinav. 165: 395.

Lindholm, H. 1959. Hypogammaglobulinemia. Nord. Med. 62: 1552.

McCarty, M. 1952. Immune Response in Rheumatic Fever. In Thomas, L., Ed.: Rheumatic Fever. Minneapolis: University of Minnesota Press.

McKusick, V. A. 1959. Genetic factors in diseases of connective tissue. Am. J. Med. 24: 283.

Meiselas, L. E., S. B. Zingale, S. L. Lee, S. Richman, and M. Siegel. 1961. Antibody production in rheumatic diseases. The effect of brucella antigen. J. Clin. Invest. 40: 1872.

Mellors, R. C., L. G. Ortega, and H. R. Holman. 1957. Role of gamma globulins in pathogenesis of renal lesions in systemic lupus erythematosus and chronic membranous glomerulonephritis, with an observation on the lupus erythematosus cell reaction. J. Exper. Med. 106: 191.

Merrill, J. P., E. A. Friedman, R. E. Wilson, and D. C. Marshall. 1961. The production of "delayed type" cutaneous hypersensitivity to human donor leukocytes as a result of the rejection of skin homografts. J. Clin. Invest. 40: 631.

Miller, J. M., S. Kibrick, and B. F. Massel. 1953. Antibody response of non-streptococcal antigens as related to rheumatic fever susceptibility. J. Clin. Invest. 32: 691.

Moore, J., and W. Lutz. 1955. Natural history of systemic lupus erythematosus: An approach to its study through chronic biologic false positive reactors. J. Chron. Dis. 1: 297.

Morteo, O. G., E. C. Franklin, C. McEwen, J. Phythyon, and M. Tanner. 1961. Studies of relatives of patients with systemic lupus erythematosus. Arth. & Rheumat. 4: 356.

Paterson, P. 1960. Transfer of allergic encephalomyelitis in rats by means of lymph node cells. J. Exper. Med. 111: 119.

Quinn, R. W., C. V. Seastone, and H. A. Dickie. 1953. Antibody production and tuberculin sensitivity in individuals with a history of rheumatic fever. J. Immunol. 70: 493.

Raitt, J. Unpublished results.

Rantz, L. A., W. P. Creger, and S. H. Choy. 1952. Immunologic hyper-reactivity in the pathogenesis of rheumatic fever and other diseases. Am. J. Med. 12: 115 (abstract).

Rich, A. R. 1947. Hypersensitivity in disease. The Harvey Lectures 42: 106.

Rodnan, G. P., M. J. Maclachlan, and A. S. Creighton. 1960. Study of serum proteins and serologic reactions in relatives of patients with systemic lupus erythematosus. Paper delivered at Ann. Meeting, Am. Rheumat. Assoc., Miami, Florida, June 9–11.

Rose, N. R., and E. Witebsky. 1956. Studies on organ specificity. V. Changes in the thyroid glands of rabbits following active immunization with rabbit thyroid extracts. J. Immunol. 76: 417–427.

Ruppli, Von H., and R. Vossen. 1957. Reaction to hydantoin therapy resembling visceral lupus erythematosus. Schweiz. Med. Woch. 87: 1555.

Scheffer, E. 1961. An investigation into the effect of lupus erythematosus serum on living cells. The finding of so-called "purple bodies." Koninkl. Nederl. Akademie Van Wetenschappen-Amsterdam 64: 501.

Schweitzer, M. D. 1961. Finding genetic determinants of communicable disease in human populations. Ann. New York Acad. Sc. 91: 595.

Siegel, M., et al. 1961. The epidemiology of systemic lupus erythematosus (S.L.E.): Preliminary results in New York City. Arth. & Rheumat. 4: 439 (abstract).

——, and A. Lifshay. 1962. The epidemiology of systemic lupus erythematosus: An analysis of birth order. Arth. & Rheumat. 5: 121 (abstract).

Stecher, R. M. 1955. Hereditary factors in arthritis. Med. Clin. North America 39: 499.

Stollerman, G. H. 1960. Factors that predispose to rheumatic fever. Med. Clin. North America. 44: 17.

Taranta, A., S. Torosdag, and I. Uchida. 1959. Rheumatic Fever in twins. Presented at the Second Pan-American Congress on Rheumatic Diseases. June 3.

Vazquez, J. J., and F. J. Dixon. 1957. Immunohistochemical study of lesions in rheumatic fever, systemic lupus erythematosus, and rheumatoid arthritis. Lab. Invest. 6: 205.

Waksman, B. H. 1956. Further study of skin reactions in rabbits with experimental allergic encephalomyelitis. J. Infect. Dis. 99: 258.

——. 1959. Experimental allergic encephalomyelitis and "auto-allergic" diseases. Internat. Arch. Allergy. suppl. 14.

Waldenstrom, J., and S. Winblad. 1958. Some observations on the relationship of certain serological reactions in various diseases with hypergammaglobulinemia. Acta rheum. scandinav. 4: 3.

——. 1961. Studies on conditions associated with disturbed gamma globulin formation (gammopathies) The Harvey Lectures. 1960–61.

Waller, R., and R. Race. 1951. Six blood group antibodies in the serum of a transfused patient. Brit. M. J. 1: 225.

Williams, R., and D. Law. 1958. Serum complement in connective tissue disorders. J. Lab. & Clin. Med. 52: 273.

Wolf, J. K. 1962. Primary acquired agammaglobulinemia, with a family history of collagen disease and hematologic disorders. New England J. Med. 266: 473.

Ziff, M., F. R. Schmid, A. J. Lewis, and M. Tanner. 1958. Familial occurrence of the rheumatoid factor. Arth. & Rheumat. 1: 376.

CHAPTER 7

Chromosome Aberrations in Experimental Mammals

Liane Brauch Russell
Oak Ridge National Laboratory,
Oak Ridge, Tennessee

TREMENDOUS ADVANCES HAVE BEEN MADE IN THE LAST FEW YEARS IN CORrelating a variety of abnormal human phenotypes with certain specific chromosome aberrations. In man, the observed population numbers in the millions, the investigators are numerous, and the acuity with which abnormal phenotypes are recognized is of a high degree. It is therefore not surprising that recent advances in cytological technique have been able to trigger off a number of useful chromosomal studies in man. The already tremendous body of literature concerned with this subject was reviewed in the first volume of this series (Ferguson-Smith, 1961).

In experimental mammals, on the other hand, progress has been spread over decades and has come along different avenues of approach, with the primary interest usually being in the fields of genetics, reproductive physiology, embryology, or radiobiology. Because of this difference in approach, and because of the possibility for experimentation, work on laboratory mammals—while admittedly lagging in many aspects behind work on human beings—has been able to furnish a few of the answers to some of the more fundamental questions. A review of this field may therefore be useful to those whose primary interest lies in medical genetics.

In general, the present paper will concentrate on the work of the past 10 years. Publications covered in earlier reviews are discussed where necessary; but in most cases reference will only be made to the review in question, which may be consulted for more details and for literature citations. Only chromosomal aberrations in germ cells, or arising from the circumstances of fertilization, are discussed. The review therefore excludes studies done purely on somatic cells, both in vivo and in vitro. Work on *normal*

mammalian cytology will not be covered, except where this is necessary for a better understanding of the chromosomal abnormalities.

In general, the order of discussion of the various topics will proceed along the following outline: Changes that affect entire chromosomes (heteroploidy), including both those involving entire chromosome sets (euploid heteroploidy) and those involving individual chromosomes (aneuploid conditions either of a known or of an unspecified nature); changes that involve additions or deletions of portions of individual chromosomes (duplications and deficiencies); and rearrangements of chromosome material (inversions and translocations). Certain groups of experiments do not fit neatly into any of these classifications, either because the exact nature of the chromosomal aberration involved is unknown in specific cases (e.g., dominant lethals which, in most cases, are associated with losses of entire chromosomes but may also result from deficiencies), or because information on several topics is provided by the same aberration (e.g., segregation from translocation heterozygotes produces duplication and deficiency types). Such experiments have been placed in a topic according to the bulk of the information provided, and as many cross references as possible have been made with other topics.

I. Heteroploidy

Heteroploidy in mammals may be defined as the possession of chromosome numbers other than the haploid (1n) set in the gametes, and the diploid (2n) complement from fertilization onwards. Heteroploid states involving multiples of the haploid set other than the normal 2n are termed euploid and will be discussed in section IA; those involving not exact multiples of the haploid number are termed aneuploid. Special cases of aneuploidy are those in which specific known chromosomes are present in single or triple, rather than the normal, double, dose. This will be taken up in section IB. The dominant lethals, discussed in section IC, include cases of aneuploidy of an unknown nature, more specifically, those resulting from artificially induced chromosome losses.

Since, as will be discussed, certain heteroploid and parthenogenetic mammals have been shown to be viable—some through certain embryonic stages, a few into adulthood—the study of the mechanism by which these types originate is of potential interest for questions of evolution, gene dosage effects, sex determination and the effect of chromosome number and chromosome balance on viability and fertility. Certain aneuploid states (dominant lethals, monosomy of X) have also been employed as an index for induced chromosome breakage and have been useful in determining the effects of a number of variables on the induction of genetic damage.

A. Euploid Heteroploidy

1. Outline of causes. Work published through 1955 on spontaneous and induced heteroploidy in mammals is covered in a thorough and logically organized review by Beatty (1957). Much of the mammalian evidence cited in that review comes from the work of the author done in collaboration with M. Fischberg, as well as from investigations by Austin, Braden, Edwards, Thibault, Pesonen and Chang. Early studies, such as those of Pincus and his group, are also discussed. Beatty has organized most of the mammalian data into 16 major routes of development which result from the operation or nonoperation, either singly or in combination, of 4 cytological variables: suppression of first polar body, suppression of second polar body, suppression of first cleavage division and activation without paternal-genome contribution. These variables and several additional ones which are mentioned by Beatty but do not enter into his scheme of 16 routes—and concerning some of which considerable evidence has been collected since the time of his review—are all combined in a diagrammatic checkerboard of causes in Figure 1.

Figure 1 summarizes virtually all the described abnormalities of egg maturation and fertilization that can potentially lead to euploid heteroploidy. Each one separately has been observed to occur, although not all of the combinations shown have been seen. The normal course of events is shown in row 3 and column b. In almost all mammals, the first meiotic division takes place shortly before ovulation; the ovulated secondary oocyte remains in metaphase of the second division until sperm entry, which triggers the budding off of the second polar body.

Various abnormalities involving the whole chromosome set can occur or be induced at either of the maturation divisions of the oocyte. Thus, *all* of the chromosomes may go into the polar body, with none left in the vitellus. This is illustrated for first and second divisions respectively in rows 1 and 2 of Figure 1. Secondly, budding off of the polar body may be suppressed, leaving in the vitellus the chromosomes which would normally have been shed. Depending on the stage of division at which this suppression occurs, the twice-normal number of chromosomes will be contained in 1 or 2 nuclei. Suppression of the first polar body is illustrated in rows 4, 6 and 7; of the second polar body in rows 5, 7, 10 and 11. The third abnormality is of a slightly different nature in that it involves abnormal division of the cytoplasm. Instead of only a small piece of cytoplasm being budded off for the polar body (which eventually degenerates), division is into two equal halves; and the two cells thus formed both participate in formation of the early embryo. When this division into equal cells occurs at the second meiotic division it has been named "immediate cleavage" and this is

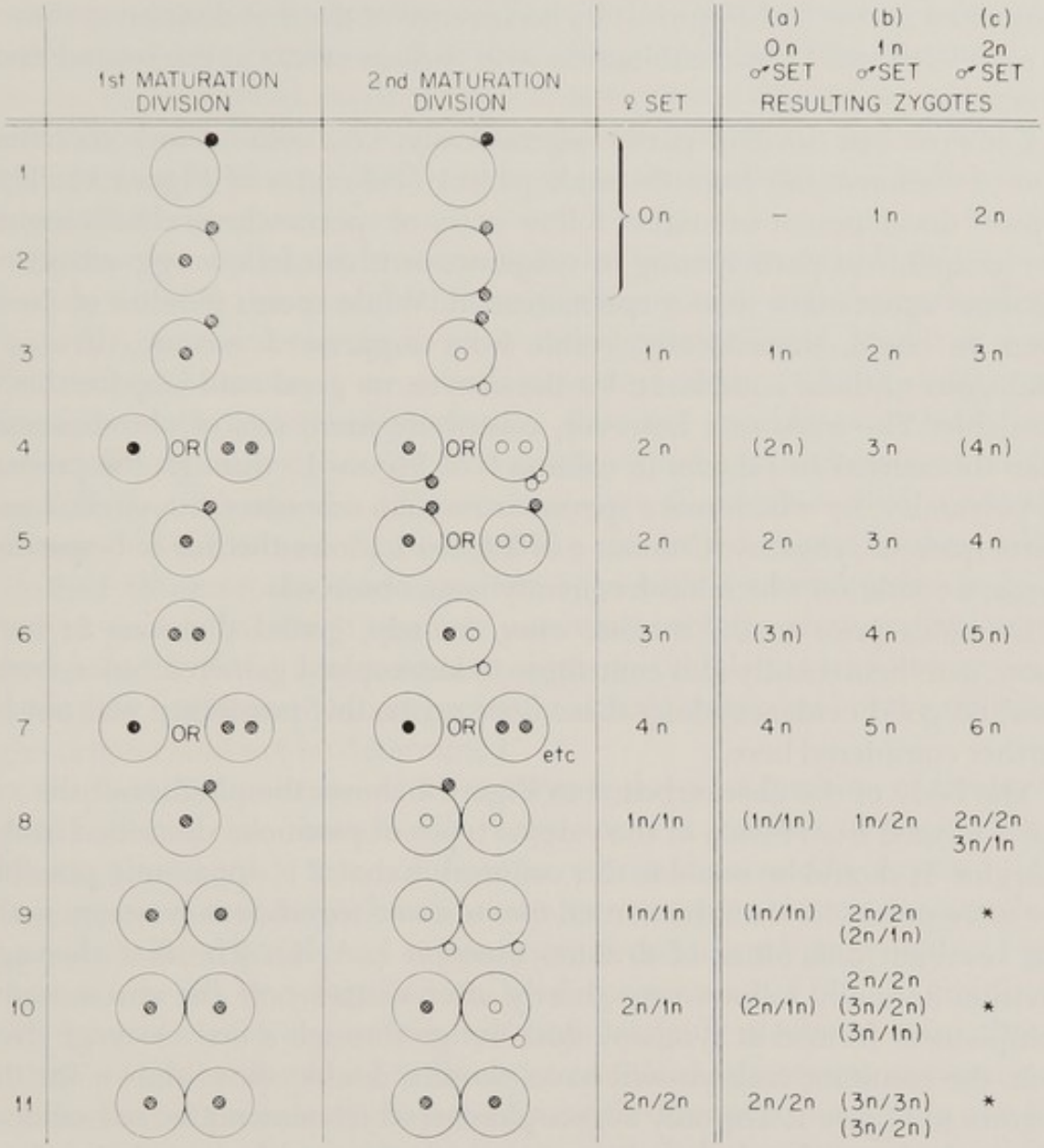


FIG. 1—Zygote ploidies resulting from combinations of various possible maternal and paternal chromosome sets (see text, section I, A, 1). Unlikely combinations are in parentheses. Inhibition of the first cleavage in any particular case would give a zygote ploidy double that shown. The designation 1n/2n, 2n/2n, etc., indicates mosaics. * = several possible but improbable types of mosaics. Contribution from paternal chromosome sets is shown in columns (a)–(c) as follows: (a) activation without functional paternal chromosomes; (b) fertilization with 1 normal sperm; (c) dispermy. Fertilization with more than 2 spermatozoa, though possible, is not considered here. Derivation of the maternal set of chromosomes is shown in the left portions of lines 1–11, with the normal situation represented in line 3. Shading of oocyte nuclei and polar bodies indicates number of sets: ● = 4n; ⊙ = 2n; ○ = 1n. For diagrammatic purposes, the first polar body is shown on top of the vitellus, the second at the bottom. Possible division of the first polar body has been ignored.

illustrated in row 8 of Figure 1. Its occurrence at the first division is shown in rows 9, 10 and 11, in combination with various events at the second division.

Embryos can develop parthenogenetically, i.e., without any contribution of chromosomes from the male parent (column a of Figure 1). This type of development can either follow entry of sperm whose chromosomes are incapable of participating in syngamy; or it can follow egg activation by some agent other than a spermatozoon. While sperm ploidies of 2n or even 4n could, theoretically, result from suppressed meiotic divisions, analogous to those considered for the oocyte, no good evidence for this is available. The male can, however, contribute more sets of chromosomes than the normal 1n (shown in column b of Figure 1) through the process of polyandry, by which more spermatozoa than one enter the vitellus and participate in syngamy. Column c of Figure 1 shows this for two spermatozoa, a condition which has frequently been observed.

Divisions prior to the meiotic ones, namely, gonial divisions in both sexes, can theoretically also contribute to heteroploid gametes. Since, however, no good evidence along these lines exists, this possibility will not be further considered here.

The body of the checkerboard in Figure 1 shows the ploidies of the zygotes formed from fusion of the various types of pronuclei described in the margins. It should be noted in this connection that it is apparently possible for more pronuclei than the normal two to come together at syngamy without resultant difficulties of division (section I, A, 4). The first cleavage division normally follows very shortly after syngamy. If the chromosome complement formed at syngamy does not go through a first cleavage division, the resultant embryo will have ploidies double those shown for the zygotes in Figure 1. The only zygote ploidies which may not be self-evident are those shown for the condition in which the female contributes 2 attached, equal-sized cells instead of 1 egg (rows 8, 9, 10, 11). In such cases either or both of these cells may be fertilized.

At first glance, it may seem meaningless to fill in all of the squares of the checkerboard, since it might be supposed that, with some of the marginal events already being infrequent, the combinations would be too rare to be even considered. However, as will be shown, some of the events are apparently correlated. Most striking among these is polyandry and second polar body suppression, a relation that is not surprising in view of the fact that completion of the second meiotic division of the oocyte is normally dependent on sperm entry. Another example of correlation in the finding is the fact that agents that cause egg activation in the absence of sperm can also cause suppression of the second polar body and may thus lead to diploid parthenogenesis.

2. *Spontaneous occurrence.* a. The Types. Spontaneously heteroploid early mammalian embryos of the following types have been reported: 1n, 3n, 4n, 6n, aneuploid, euploid/euploid mosaics and euploid/aneuploid mosaic. The bulk of the data come from the work of Beatty and collaborators summarized by Beatty (1957), and most of this was collected in mouse embryos 3½ days postcoitus.

Spontaneously haploid mouse embryos have been found, but it is not known whether these resulted from parthenogenetic development (Fig. 1, lines 3, 8, or 9, and row a), or from androgenesis (Fig. 1, lines 1 or 2, row b). A haploid embryo in an uninseminated ferret (Chang, 1950) must have developed parthenogenetically. Rats are capable of at least "rudimentary parthenogenesis," i.e., completion of the second maturation division without the stimulus of sperm entry into the vitellus which is normally required (Austin and Braden, 1954b). In mated mice, Braden (1957) studied 85 unipronucleate eggs, i.e., eggs with a second polar body but only 1 pronucleus. Such eggs are potentially able to produce haploid embryos. In 20 of the unipronucleate eggs, the single nucleus was probably of female origin, in 16 others probably of male origin, and in the remainder the origin could not be determined.

Triploid, 3n, embryos, which constitute the most frequent of the heteroploid classes, can, theoretically, originate in a number of ways (Fig. 1: 3c, 4b, 5b, 6a, 11b). Some of these have been discussed by Austin (1960). Among the possible mechanisms, the two considered most frequently responsible for triploidy are suppression of the second polar body (Fig. 1, line 5, row b) and fertilization by 2 spermatozoa (Fig. 1, line 3, row c). In a study of one of Beatty's high-triploidy stocks of mice, Braden (1957) found polyspermy to be about one-third as frequent as suppression of the second polar body. Suppression of the first polar body may also have occurred with low frequency in the same stock, but practically no evidence for this abnormality was found in a number of other strains of mice.

Spontaneously tetraploid, 4n, mouse embryos of 3½ days gestation have been reported by Beatty and co-workers (summarized 1957) and by Edwards (1958b). Their incidence is in the order of tenths of per cents, depending on strain. Even lower was the incidence of hexaploid, 6n, embryos. These 2 types are presumed to have arisen as follows: 4n from suppression of the first cleavage; and 6n from suppression of second polar body followed by suppression of first cleavage. Ploidies higher than 6n have not been recorded in any mammal. The spontaneous aneuploid 3½ day mouse embryos are ascribed by Beatty (1957) to "fortuitous misdivision" in meiosis or mitosis.

Possible mechanisms that might lead to euploid mosaicism are considered by Beatty (1957). The most plausible among these involve division

into 2 equal-sized cells at the maturation divisions of the oocyte, in lieu of abstriction of polar bodies (Fig. 1, lines 8–11). If this event occurs at the first meiotic division, then 1, neither, or both of the resultant 2 cells might be fertilized by either X- or Y-bearing sperm, and extrusion of the second polar body would presumably follow in the penetrated cell(s) (but perhaps also the adjacent one). Braden (1957) discusses this mechanism as a possible cause of the mouse gynandromorphs that have been reported in the literature. In his examination of several thousand tubal eggs, he observed, however, only 2 cases of "immediate cleavage" at first meiosis: in one, no sperm had penetrated and each of the 2 cells contained a spindle of the second meiotic division; in the other, only 1 of the 2 cells had been penetrated. A less likely route for the production of gynandromorphs is the much more frequently observed "immediate cleavage" at the *second* meiotic division (Fig. 1, line 8). Braden (1957) has found this phenomenon to occur spontaneously in several strains of mice. Since the "immediate cleavage" presumably follows sperm penetration(as would normally be the case for abstriction of second polar body), one would expect to find only one of the two equal-sized cells to contain a sperm. This situation, which potentially leads to a haplo-diploid chromosomal mosaic, was, indeed, found in all 16 of the cases of "immediate cleavage" analyzed by Braden. Only in the unlikely event that "immediate cleavage" were to follow dispermy, and that the 2 male pronuclei came to lie in separate cells after "cleavage," would one expect this mechanism to produce gynandromorphs or other 2n/2n mosaics.

b. Genetic Control. Spontaneous incidence of heteroploidy in mammalian embryos is apparently under genetic control, as is the incidence of the various abnormalities of egg maturation and fertilization which can lead to heteroploidy. Beatty (1957) summarizes a number of investigations, done in collaboration with M. Fischberg, that show great differences in percentage of spontaneous heteroploidy between certain *silver* stocks of mice on the one hand, and a variety of non-*silver* strains on the other. While euploid heteroploidy other than triploidy is virtually absent in non-*silver* strains, the *silver* stocks had a low, but measurable, incidence of 1n, 4n, 6n, 1n/2n, 2n/3n, 2n/4n and a high incidence of triploids. The incidences for triploidy in various matings are as follows: non-*silver* ♀ × non-*silver* ♂, 0.25 per cent; non-*silver* ♀ × *silver* ♂, 1.8 per cent; *silver* ♀ × *silver* ♂, 2.4 per cent; *silver* ♀ × non-*silver* ♂, 5.7 per cent. It is interesting that it is the outcross of *silver* females that gave the highest incidence, rather than matings within the *silver* stocks; and Beatty notes that between-strain matings in general showed a slightly higher frequency of heteroploidy than within-strain matings. This tendency was also noted by Braden (1957) for "immediate cleavage," but not for some of the other abnormalities of

egg maturation and fertilization. The factor causing high incidence of heteroploidy was probably not the *silver* gene itself, although it may have been a gene linked to it. Beatty also found that the high-heteroploidy factor(s) are apparently not dominant, since F_1 females from *silver* outcrosses had a low incidence of heteroploid embryos.

Braden (1957), in a comparison of various mouse strains and crosses between them, found a stock derived from Beatty's *silver* stocks to have a relatively high incidence of a number of abnormalities of egg maturation. In eggs derived from females of this stock, the frequency of first-polar body suppression was 2.3 per cent (this abnormality was virtually absent in all the other strains), second-polar body suppression 4.4 per cent (other strains 0–0.8 per cent), and sub-nuclei, which are probably the result of disturbances in the maturation divisions, 7.2 per cent (other strains 0–0.2 per cent). In addition to the abnormalities just mentioned, Braden also studied the effect of strain on "immediate cleavage," uninucleate eggs and polyspermy. For 5 of these 6 abnormalities, a significant variation in incidence was found between strains. Suppression of first- and second-polar body, and the presence of sub-nuclei are controlled by the genotype of the female. For "immediate cleavage" and the occurrence of uninucleate eggs, there may be an interaction between male and female genotypes. The incidence of polyspermy, i.e., the penetration of the vitellus itself with more than 1 sperm, did not vary significantly in the different strain matings of this study (range 0.2 to 1.7 per cent). However, it was found in another study (Braden, 1958a) that sperm penetration of the zona pellucida (not vitellus) was strongly under control of strain of male in the mouse. There is some evidence that it may be under control of the strain of the female in the rat (Braden, 1958b), but the delayed mating used in this study may complicate the picture (section I, A, 3). Austin (1960) points out that the mammalian egg has 4 main lines of defense against polyspermy and summarizes evidence from his own work and that of others on several mammalian species (mouse, rat, hamster and field vole) which shows that the efficiency of the various mechanisms varies with the genetic constitution of the animal.

3. *Experimental induction.* The frequency of various abnormalities of egg maturation and fertilization can be markedly increased by not allowing coitus until a late stage in estrus has been reached. This delay, which may be involved in some of the "spontaneous" occurrences of these abnormalities also, results in ageing of the egg before it comes in contact with sperm. Delayed mating has been found to produce great increases in the incidence of polyspermy in the rat (Austin and Braden, 1953; Odor and Blandau, 1956; Braden, 1958b; Pikó, 1958) and in the pig (Hancock, 1959; Thibault, 1959), some increase in the rabbit (Austin, 1960), but

none in the mouse (Braden and Austin, 1954). The frequency of polygyny resulting from second-polar body suppression is also increased by delayed mating in the rabbit (Austin, 1960) and, to a very high degree, in the pig (Thibault, 1959). As Austin (1960) has pointed out, it is not surprising to find polyspermy and second-polar body suppression to be correlated in occurrence, since both are functionally dependent on properties of the vitelline surface.

Among experimental treatments which have been very successful in inducing various types of heteroploidy are cold and heat shock. Beatty (1957) reviews the evidence on parthenogenetic development induced by cold treatment. Chilling is apparently able to activate unfertilized mammalian eggs. In at least some species (rabbit and perhaps sheep), it also causes suppression of the second maturation division. When the 2 effects occur together, development of a diploid organism can proceed (see Fig. 1, line 5, row a). Several workers have, by this method, obtained development, at least as far as implantation; and Pincus and co-workers managed to obtain 1 living rabbit at term following this procedure. It should here be noted, that the same route of development (activation of unfertilized egg, second-polar body suppression) probably resulted from the hypo- and hypertonic treatments of rabbit eggs by means of which Pincus was able to obtain parthenogenetic development of rabbits to the adult stage.

Heat shock has been used successfully in various ways. In the mouse it can result in a high incidence of triploidy (Fischberg and Beatty, 1952) which is apparently due to second-polar body suppression (Fig. 1, line 5, row b) (Braden and Austin, 1954). In the rat, second-polar body suppression as a result of heat treatment is only transitory; the main effect of the treatment is to produce a high incidence of polyspermy, particularly when heat shock is used in conjunction with delayed mating (results summarized by Austin, 1960, review; Pikó and Bomsel-Helmreich, 1960). Heat shock applied at the time of the first cleavage (Beatty and Fischberg, 1952) resulted in a high incidence of tetraploid mouse embryos, presumably as a result of inhibition of the first cleavage.

Colchicine has been used successfully for the induction both of heteroploidy and of conditions that would presumably lead to heteroploidy. Studies of pronuclear-stage eggs show a strong effect of colchicine on the second maturation division of the oocyte. In rats given colchicine intraperitoneally shortly after mating, Austin and Braden (1954a) found that 90 per cent of the penetrated eggs had no second polar body but were otherwise normal. Presumably such eggs would produce 3n zygotes (line 5 of Fig. 1). The time of injection has been shown by Pikó and Bomsel-Helmreich (1960) to have a marked effect on the type of second-maturation abnormality in the rat. Thus, injection 2 hours after mating caused

mostly suppression of the second polar body, as in Austin and Braden's work; but injection only half an hour later led, in a large proportion of cases, to expulsion of all of the female chromatin into the polar body (Fig. 1, line 2). Because of this time effect, the rat results are not strictly comparable with those obtained in the mouse, where Edwards has injected colchicine into the uterus just prior to mating (Edwards, 1958a, 1961; Edwards and Sirlin, 1959). In the mouse, as in the rat, the effect was on the second maturation division, but destruction of the spindle had variable end results—from 100 per cent retention to 100 per cent extrusion of chromatin. The extruded chromosomes were usually contained in one to several polar-body-like structures, while the retained chromosomes formed from 1 to 17 pronuclei (but usually no more than 4). With optimum concentration of colchicine, 30 to 40 per cent of the eggs had abnormal pronuclear content. The most frequent abnormality was that in which only 1 pronucleus was found (instead of the normal 2), the next most frequent category being that where there were 3 (Edwards, 1958a, 1961; Edwards and Sirlin, 1959). Radioactive labeling of sperm (Edwards and Sirlin, 1959) showed that 1 of the pronuclei, and *only* 1, was provided by the male, proving correct the earlier suppositions (a) that colchicine-treated eggs with a single pronucleus were potentially androgenetic haploids and (b) that excessive numbers of pronuclei in many of the treated eggs are of female origin. Occasionally, the colchicine treatment may cause extrusion of all pronuclear material, including that provided by the father (Edwards and Sirlin, 1959).

Further development up to 3½ days postfertilization following colchicine treatment has also been studied by Edwards (1958a, b). About 90 per cent of the expected androgenetic haploids are missing by that stage and are presumed to have been lost in the first cleavage. Pikó and Bomsel-Helmreich (1960) also report early death of this type. On the other hand, only occasionally does a multi-pronucleate state result in amitotic cleavage and, therefore, presumably, degeneration (Edwards and Sirlin, 1959). Where several pronuclei are present, these can apparently come together in a compact syngamy (Edwards, 1958a, 1961; Edwards and Sirlin, 1959) (with the first cleavage, however, being somewhat delayed as a result of the colchicine treatment). As a result, there are found, at 3½ days, a number of heteroploid embryos of the types and relative frequencies predicted from the pronuclear-stage studies. With optimum concentrations of colchicine the incidence of heteroploids at 3½ days is 19 per cent, with about half of these being triploids. Chromosome numbers between 1n and 3n (other than the normal 2n) are also encountered, as is to be expected from the variable extrusion of maternal chromatin that often results from destruction of the second maturation spindle.

Colchicine applied at stages other than that of the second maturation di-

vision can also produce heteroploidy by different routes. Edwards (1958b) believes that the first maturation division can be affected. Even more certain is the effect of this agent on cleavage. This has been shown both in experiments where it was applied 15 hours after mating (Edwards, 1958b) and in experiments where it was applied before mating but where its effect apparently persisted (Edwards, 1958a, b) occasionally as long as 3 weeks. The effects on cleavage were evident in the formation of tetraploids (effect on first cleavage), mosaics (effect on later cleavages), mitoses with contracted chromosomes and slight delays in cleavage.

Even though colchicine has thus been shown effective in producing heteroploidy in a variety of ways, the claim by Häggqvist and collaborators to have produced adult triploid rabbits and a triploid pig by insemination with spermatozoa suspended in colchicine solution has been strongly criticized by Beatty (1957) on a number of valid grounds. In this connection, it is of interest that Edwards (1958a) failed to produce heteroploidy in the mouse when colchicine was applied to sperm suspension, although this chemical proved highly effective when injected into the uterus prior to mating.

The chemical diacetylmethylcolchicine ("Colcemid") has been found even more effective than colchicine, of which it is a less toxic derivative, in producing destruction of the second maturation spindle of the oocyte (Edwards, 1961). The percentage of eggs with unusual number of pronuclei was as high as 93 per cent in one of the treated groups. In contrast to colchicine, however, complete loss of maternal chromosomes is very rare. Most frequent, by far, is the condition where there are 3 pronuclei (potentially triploid embryos), although a very large number of eggs with more than 3 pronuclei was also observed.

Two types of radiation have been tested for their ability to produce euploid heteroploidy. These are x-rays (Edwards, 1957a) and ultraviolet (Edwards, 1957b). X-ray doses up to about 1,000 r to sperm produced aneuploidy as a result of breakage of individual chromosomes and are therefore discussed under dominant lethals (section IC). Only with doses of 10,000 r and above is there any evidence of damage to sperm activity. This damage manifests itself in failure to stimulate the second maturation division of the oocyte and in polyspermy. Gynogenetic haploid zygotes may also occasionally be formed, but they are very rare and almost invariably perish prior to the first cleavage. More efficient than x-rays in the production of gynogenetic haploids is ultraviolet applied to sperm suspension. Following this treatment, the chromatin contributed by the male may fail to participate in syngamy, even though it has apparently not lost its ability to stimulate the second maturation division and cleavage. Ultraviolet can also cause loss of individual chromosomes (and thus aneuploidy), and in

this connection it will be discussed again under dominant lethals (section I, C, 3).

4. Viability of euploid heteroploid and parthenogenetic mammals. Parthenogenetic rabbits, diploid as a result of second polar body suppression, have, as already discussed (section I, A, 3), been observed to develop to adulthood. Other parthenogenetic mammals have been shown capable of at least partial development. Beatty's review (1957) should be consulted for a fuller treatment of this subject. Haldane (1959) has proposed a method for testing presumed cases of parthenogenesis by tissue transplantation. There has, to date, been no well-substantiated report of adult polyploid or haploid experimental mammals.

As has already been mentioned in various connections, haploids, triploids, tetraploids, hexaploids, mosaics and aneuploids can be found among mouse embryos of 3½ days gestation, by which time an early blastocyst stage has normally been achieved. Edwards (1958d) has analyzed the state of development at 3½ days of various heteroploids reported in a number of separate experiments. He finds that tetraploids lag only one cleavage behind diploids. Since the first cleavage was suppressed in order to induce tetraploidy, their cleavage rate is, therefore, normal. Triploids are only very slightly retarded, and this, again, may be due to a slight delay in first cleavage, rather than to a subsequently reduced cleavage rate. Those triploids which were induced by heat are retarded less than those induced by colchicine, which may indicate that part of the retardation in the latter case is due to persistence of the colchicine effect, rather than to ploidy. The development of androgenetic haploids produced by colchicine treatment of secondary oocytes stops at very early stages (Pikó and Bomsel-Helmreich, 1960). Edwards (1958a) found only 10 per cent to survive to 3½ days; but those that did were only slightly retarded. On the other hand, none of the gynogenetic haploids studied was capable of normal cleavage rate. However, this latter group was induced by treatment of spermatozoa with x-rays, ultraviolet, or nitrogen mustard, and Edwards believes that it does not show the effect of ploidy alone. These treatments can slow down cleavage as a function of dose and independently of ploidy (acting even in diploids). Therefore, retardation may be caused by some other mechanism, possibly damage to the sperm centriole (Edwards, 1957a, 1958d). Two spontaneous 6n-embryos reported by Beatty (1957) were retarded at 3½ days of gestation.

No evidence of euploid heteroploid states other than triploid exists for stages later than 3½ days gestation. Triploids have been found as late as midterm or slightly after, namely, at 9½ days gestation in the mouse, when they appeared to be normal in development, although slightly smaller than their diploid littermates (Fischberg and Beatty, 1951); and at 12 days ges-

tation in the rat, when almost all were retarded (Pikó and Bomsel-Helmreich, 1960). Dissections at 13 to 15 days in the rat experiments yielded no triploids and only a small percentage of mosaics possibly derived from triploids. In the mouse, it has been determined that triploids probably do not survive to term: Edwards (1958a, b), in various colchicine experiments, examined 109 young at term which, on the basis of the 3½ day incidence of triploidy, should have included between 8 and 9 triploids. None was found. Edwards suggests that lethality of triploids after the age of 9½ days gestation is not an absolute phenomenon, and that some could perhaps survive to term if competition from diploid littermates could be reduced through the induction of higher proportions of triploids. Since there is no obvious genetic cause for the failure of triploids to survive to term, Stern (discussion of paper by Austin, 1960) suggests that, perhaps, interaction of the fetal tissues with those of the mother depends on both being diploid.

A special problem bearing on formation and survival of mammalian heteroploids concerns the role of the centrosome of cleavage. It has been shown repeatedly that when more than the normal two pronuclei are present, these nevertheless come together in syngamy just prior to the first cleavage division. Thus, the three pronuclei resulting from dispermy join in a triploid mitosis which results in triploid embryos (Pikó, 1958; Pikó and Bomsel-Helmreich, 1960; Austin, 1960). The several pronuclei of maternal origin formed after colchicine treatment also may join with each other and with the male pronucleus to form one compact group of chromosomes at syngamy (Edwards and Sirlin, 1959; Edwards, 1961). It might have been expected that multiple pronuclei would lead to a multipolar spindle, especially in the case of polyspermy, since it is assumed that the spermatozoon provides the centriole instrumental in forming the first cleavage spindle. Austin (1960) suggests that there may be, in mammals, some kind of regulatory mechanism that ensures the formation of a bipolar spindle at syngamy, possibly by suppression of supernumerary centrosomes. Such a regulatory mechanism may exist in later cleavages also. Thus, Edwards (1958b) has noted that even though centrosomes have probably been doubled in the course of colchicine-induced cleavage suppression, there is no evidence of multipolar spindles in subsequent cleavages.

B. Monosomy and Trisomy of Specific Chromosomes

There is, to date, very little information concerning monosomy or trisomy involving specific autosomes of experimental mammals. On the other hand, a considerable amount of progress has been made with the sex chromosomes in the past 2 years.

1. Autosomes. a. Trisomy. No record exists, so far, of an experimental mammal trisomic for an autosome. Beatty (1957) reports one 15-celled

mouse embryo with 41 (i.e., 2n + 1) chromosomes, but it is not known whether the extra chromosome was an autosome or an X. Several methods —other than routine cytological screening of large numbers of animals— may be suggested to detect autosomal trisomics. Thus, it is possible that the existence of supernumerary autosomes would manifest itself by some generalized anomaly, analogous to Down's syndrome (mongolism) in man. Such an anomaly, provided it did not interfere with fertility, should be transmitted somewhat like a dominant gene. In the mouse, several rather generalized anomalies are known which are transmitted in a dominant fashion. Thus, it would be of interest to make a cytological study of such inherited anomalies, as well as of sporadically abnormal animals which are usually attributed to "accidents of development." Other methods involve the use of genetic markers. One scheme utilizes intermediate alleles or hypomorphs in heterozygous stocks. For example, in stocks of the type c^{ch}/c, exceptional animals of the types $c^{ch}/c^{ch}/c$ or $c^{ch}/c/c$ might be detectable. A second scheme uses closely linked recessive markers. Thus, in a mating of *d* +/+ *se* × *d se*/*d se*, wild-type animals would normally not occur, except as a result either of rare crossing-over (yielding ++/*d se*), or of trisomy from nondisjunction in one parent (yielding *d* +/+ *se*/*d se*). The distinction between the two can easily be made genetically and cytologically.

b. Monosomy. Evidence available to date indicates that monosomy of autosomes is probably lethal before birth. In fact, the bulk of the "dominant lethal" category (section I, C) is thought to consist of monosomics. Monosomy could be detected by the uncovering of the recessive in an animal which would normally be heterozygous for this recessive and, therefore, wild-type in appearance. The same phenotype is, of course, expected also from the occurrence of deletions or point mutations involving the locus. This is the basis for the so-called "specific locus method" for measuring mutation rates which has been used in very large scale experiments in the mouse by W. L. Russell (W. L. Russell, 1951; W. L. Russell, L. B. Russell and Kelly, 1958, 1960). While it might seem, at first glance, that any of the specific locus "mutations" obtained in these experiments could be due to monosomy, this condition can actually be ruled out in the great bulk of them. Thus, monosomy can be excluded for mutations in which the linked marker is not involved, mutations which are homozygous viable or semiviable, and mutations giving a phenotype intermediate between the test-stock marker and wild-type. These classifications cover more than three-quarters of the spontaneous mutations as specific loci.

The most likely candidates for monosomy in the specific locus experiments are double mutations involving two linked markers (no other markers present on that chromosome). In a study made of a large number of

double mutations of *d* and *se* (crossover frequency = 0.16 per cent) in linkage group II (L. B. Russell and W. L. Russell, 1960), we found that the *induced d se*-mutants were the result of deletions involving these loci rather than of monosomy (see section II, B). Spontaneous *d se* "mutants," on the other hand, behaved as if they were the result of nondisjunction of the chromosome containing linkage group II in the test-stock parent, combined with loss of that chromosome in the wild-type parent. The "mutant" animal thus carries two doses of normal *de se* contributed by the test stock. Since nondisjunction of linkage group II thus apparently occurs spontaneously, the *dse*/0 type should be considerably more frequent than the *dse*/*dse*/0 type. The former, however, was never found, indicating that monosomy for this chromosome is lethal. Since, according to Griffen's (1960) pachytene map, linkage group II occupies one of the shorter chromosomes of the mouse, it may be tentatively concluded that monosomy for autosomes would always be lethal.

Supporting evidence for this conclusion also comes from an experiment in which loss of a sex chromosome was induced by irradiation between sperm entry and the first cleavage (L. B. Russell and Saylors, 1960; L. B. Russell, 1961). The same treatment presumably also induces loss of individual autosomes, but although 5 autosomes were genetically marked, no such losses were detected.

2. *Sex chromosomes.* While the autosomes of experimental mammals have to date yielded only fragmentary information on monosomy and trisomy, the sex chromosomes of the mouse have contributed much to our knowledge of these phenomena.

a. The Phenotypes. Combined genetic and cytological evidence indicated that animals with the XO sex-chromosome constitution were normal and fertile females (W. L. Russell, L. B. Russell and Gower, 1959; Welshons and L. B. Russell, 1959). These females were detected because they exhibited the phenotypes normally produced by certain sex-linked marker genes in males only. The results of breeding experiments pointed strongly to the XO explanation and ruled out a number of possible alternative hypotheses. The XO hypothesis was finally verified by the cytological evidence which indicated that the exceptional females had 39, instead of 40, chromosomes. XO mice are phenotypically normal females which are usually fertile and are only slightly less viable than their XX littermates. Since their original discovery, they have been reported by other workers also (McLaren, 1960; Cattanach, 1961a, b; Kindred, 1961).

More recently, Russell and Chu (1961) have described the much rarer type, XXY. An XXY mouse, which was detected by sex-linked markers and studied cytologically, was viable, of normal size, and, externally, of male phenotype, though sterile. The male copulated regularly, but there was no

sperm in the ejaculate, and histological examination of a biopsy (Oakberg, private communication) indicated that the testis did not contain any spermatogenic elements. Another XXY male has been produced (L. B. Russell, unpublished) in experiments designed to determine whether nondisjunction can be induced by radiation (section I, B, 2d). Kindred (1961) has recently reported two presumed XXY mice, for one of which confirmatory cytological evidence was obtained. Both were sterile. Cattanach (1961a, b) observed two males, one or both of which were probably near-XXY. These animals presumably resulted from nondisjunction of X and Y centromeres in males bearing a rearrangement (section III, B, 5). The near-XXY type had, in addition to an intact X and Y, a translocated chromosome consisting of the bulk of the X and a small piece of autosome. Like the true XXY mice, the near-XXY lacked spermatogenic cells but copulated readily. The recent combined genetic and cytological evidence strengthens an earlier report of an XXY male (McLaren, 1960) for which no cytological proof could be obtained because of the animal's untimely death.

In cats, the rare "tortoise-shell" or "calico" males, most of which are sterile, may be XXY. This coat-color pattern is the result of heterozygosis for an X-linked gene and, therefore, could presumably not be exhibited unless the animal had two X chromosomes. Earlier interpretations for the tortoise-shell males had invoked either a newly arisen *partial* sex linkage for the gene (Jude and Searle, 1957), or the occurrence of crossing-over between X and Y, whereby the Y gained the color-gene but lost its male-fertility factors (Komai and Ishihara, 1956). Two tricolor male cats (of which only one was a typical "calico") have recently been reported by Thuline and Norby (1961) to have 2n + 1 chromosomes and chromatin-positive buccal smears. One lacked the later stages of spermatogenesis, resembling, in this respect, Komai and Ishihara's sterile cases. The other, however, completely lacked testes as well as all internal reproductive structures. It seems doubtful that this could have been the result of the XXY constitution alone. Whether the occasionally fertile tortoise-shell males are also XXY needs still to be determined. Komai and Ishihara interpret them on the basis of incomplete epistasis (i.e., not truly of tortoise-shell genotype); but the male reported by Jude and Searle had many tortoise-shell sons.

In the mouse, in addition to XO and XXY, we know the fate of YO, which has been shown to be a prenatal lethal (W. L. Russell, L. B. Russell and Gower, 1959). Males carrying a deficient X and a Y are very poorly viable and small (Cattanach, 1961b). Neither XXX nor XYY have been reported to date. Both of these types would be difficult to detect by phenotype with the genetic markers available in the mouse at this time; but, if they are fertile, they could reveal themselves by the production of XXY sons.

The evidence on XO and XXY mice has provided information and stimulated research on a number of topics, such as sex determination, the genetic activity of X and Y, mechanisms that spontaneously produce abnormal numbers of sex chromosomes, and the induction of nondisjunction and chromosome loss using the sex chromosomes as markers.

b. Sex Determination; Genetic Activity of X and Y. While the Y chromosome is strongly male-determining, it is not necessary, at least in the mouse, to assume any female determiners on the X, since neither XO nor XXY are intersexes. XO is a fertile female. While it is true that XXY is sterile, its sterility could be due to dosage phenomena of genes not necessarily directly concerned with sex determination; or, it could result from the possible presence in X and Y of a short homologous region, with trisomy for this region leading to certain defects which result in infertility (L. B. Russell and Chu, 1961).

The Y chromosome affects neither the hemizygous expression of the X-linked markers *scurfy* (W. L. Russell et al., 1959), *Tabby* (Welshons and Russell, 1959), or *Blotchy* (L. B. Russell, 1960); nor the heterozygous expression of *Tabby* (L. B. Russell and Chu, 1961). Presence or absence of V-type position effects in X-linked translocations are also not determined by the Y (L. B. Russell and Bangham, 1960, 1961; Cattanach, 1961a, b). On the other hand, it has been shown that two X's must be present in order to produce position-effect variegation. This finding is discussed in detail in a later section (section III, B, 5).

c. Causes of Spontaneously Abnormal Sex-Chromosome Constitutions. L. B. Russell (1961) has summarized data on spontaneous incidence of abnormal sex-chromosome constitutions in the mouse and has examined these in terms of theoretical expectations from nondisjunction, chromosome loss, or chromatid loss, in meiosis or in cleavage. Since no instances of X^MX^MY have as yet been described (the superscripts M or P designate maternal or paternal derivation of the X), there is to date no incontrovertible evidence for spontaneous nondisjunction in meiosis of the female mouse. (OX^P can have a different origin, as shown below.) The X^MX^PY mouse described by Russell and Chu (1961) showed, by virtue of its sex-linked marker genes, that nondisjunction must have occurred in the first meiotic division of a presumably normal male. (Such proof is not available for XXY individuals in man.) Meiotic nondisjunction in the male must also have accounted for McLaren's (1960) presumed (but unconfirmed) XXY and for Cattanach's two cases of XXY (1961a, b). In the latter case, though, the frequency of nondisjunction is very probably increased by the presence of a rearrangement.

Events that can produce the XO type have been outlined (L. B. Russell, 1961). They include nondisjunction at either the first or second meiotic division of either parent; chromosome loss (other than that resulting from

nondisjunction) at any time in gametogenesis; and nondisjunction or loss at the first cleavage. A very striking finding in the mouse is that absence of the paternal sex chromosome is very much more frequent than absence of the maternal—even after allowance has been made for the fact that, while all cases of the former type are potentially detectable as X^MO, half the cases of the latter type would be OY and, therefore, lethal. Dozens and dozens of spontaneously occurring X^MO's have by now been reported (W. L. Russell et al., 1959; Welshons and Russell, 1959; Russell, 1961; Russell and Saylors, 1962; McLaren, 1960; Kindred, 1961; and Cattanach, 1961b), and its frequency varies from 0.1 to 1.7 per cent according to stock (L. B. Russell and Saylors, 1961). On the other hand, there is only one substantiated case of OX^P (Cattanach, 1961b). (Claims of primary OX^P, if not substantiated by test, must be viewed with reservation, since it is possible that an OX^P animal, instead of being the result of primary loss of X^M, could be a segregant from a previously arisen X^MO mother (L. B. Russell, 1961). This reservation, as well as another strong one that cannot be detailed here, applies to Kindred's report (Kindred, 1961) of OX^P. However, in McLaren's (1960) case, a recent personal communication, to the effect that the mother yielded a normal sex ratio, makes it likely that the OX^P was indeed primary.) While it is true that X^MO would be detectable in more of the usually made matings than would OX^P, results of crosses in which *simultaneous* detection is possible (L. B. Russell, 1961, and unpublished) confirm the relative rarity of OX^P.

Is X^MO the result of meiotic nondisjunction in the male, as has been proved to be the case for X^MX^PY? Results from a number of crosses (involving several sex-linked markers) in which *simultaneous* detection of X^MO and X^MX^PY was possible, yielded a frequency of 0.76 per cent for the former, but of only 0.02 per cent for the latter (L. B. Russell, 1961). The low frequency of XXY is also confirmed by Kindred's finding of 0.02 per cent. A source other than nondisjunction in the first meiotic division of the father—which should provide XY and O sperm in equal numbers—must, therefore, be sought for the great excess of X^MO. It has been suggested (L. B. Russell, 1961) that the bulk of X^MO individuals are the result of events taking place *after* sperm penetration of the vitellus. This conclusion is based on the findings that the spontaneous occurrence of X^MO is non-random with respect to litters and sibships (W. L. Russell et al., 1959; Welshons and Russell, 1959); that the incidence of X^MO can easily be increased by irradiation after sperm penetration of the vitellus (section I, B, 2d); and that, on cytological grounds, there is no evidence for the formation of O-sperm with frequencies that would account for the spontaneous incidence of X^MO (Ohno, Kaplan and Kinosita, 1959). It has been suggested (L. B. Russell, 1961) that the relatively high spontaneous incidence of X^MO can be explained by the existence of a vulnerable state

of the paternally contributed nuclear material some time between sperm penetration and completion of the first cleavage. Losses of this vulnerable material could, perhaps, occur in response to certain vitelline conditions which might be under maternal control or traceable to circumstances of fertilization.

d. Induced Nondisjunction or Loss of Sex Chromosomes. The induction of abnormal sex-chromosome constitutions has been achieved in a number of ways. Irradiation with only 100 r following sperm penetration of the vitellus (L. B. Russell and Saylors, 1960; L. B. Russell, 1961) markedly increases the incidence of X^MO and also leads to the production of the rare OX^P type, which did not occur in the controls. A marked sensitivity difference, both with respect to induced XO frequency and to mortality, was found over a span of 4½ hours within the interval between sperm penetration and cleavage. The most sensitive time was 11:00 a.m. following the night of copulation, with sensitivity decreasing thereafter. No evidence for XO-mosaicism was found and it was therefore concluded that the effective loss occurred prior to the first cleavage.

Irradiation of males prior to copulation (stage irradiated = spermatozoa) has also been shown to be effective in inducing loss of a paternal sex chromosome (L. B. Russell and Saylors, 1961, 1962). With the method of detection used, the events scored could be either loss of an entire paternal sex chromosome, X or Y; or deficiencies in X, involving the marked loci; or deficiencies in Y, involving all male-determining regions. Preliminary cytological analysis indicates that loss of an entire paternal sex chromosome is produced in at least some instances. Comparison between the induction of paternal sex-chromosome loss (either total or partial) in spermatozoa, on the one hand, and in the male pronucleus shortly after sperm entry into the oocyte, on the other, indicates the latter stage to be more sensitive by approximately one order of magnitude. Thus, with 600 r to spermatozoa, the induced rate is 1.2 per cent; while with only 100 r delivered after sperm entry (11:00 a.m. postcopulation), the induced rate for paternal losses is 2.3 per cent.

Recent attempts to induce nondisjunction in meiotic divisions of both sexes have already met with success in the male (Russell and Saylors, 1962b). Matings made five weeks after irradiation of the male (sperm derived from irradiated early primary spermatocytes) have yielded XXY as well as XO. This procedure will be used to test for the induction of trisomy in autosomes by the methods outlined above.

C. Dominant Lethals

Under the term "dominant lethals" are grouped genetic changes, whatever their exact nature, that cause early embryonic death of some of the

first-generation offspring of animals treated with certain agents. Strictly speaking, this category should include the euploid heteroploidy induced in various ways and the scattered aneuploidy produced by the same treatments (particularly colchicine and colcemid), since these conditions are invariably lethal before birth (sections I, A, 3 and 4). However, the term "dominant lethals" has, more specifically, come to be applied to embryonic death resulting from chromosome breakage in parental germ cells. Any induced changes which affect the germ cells themselves or render the gametes incapable of participating in fertilization are excluded from the dominant-lethal category.

Chromosome breakage in parental germ cells is, in most cases, assumed to result in elimination of the broken chromosome(s) during cleavage divisions (section I, C, 2). Dominant lethals have therefore been included under the main topic of aneuploidy. However, it is conceivable that in some cases chromosome breaks will result in deficiencies which also can cause embryonic death.

1. Early studies. The evidence published prior to 1952 for dominant lethals in mammals has been summarized and analyzed by W. L. Russell (1954). Some of the fundamental findings published in the 1930's in a number of papers by Strandskov, Snell, Hertwig and Brenneke, may be briefly listed here. Litters sired during the presterile period of irradiated males were found to be of reduced size. Since there was no effect on sperm motility, and since the number of fertilized eggs was normal, it was concluded that the reduced littersize was due to death of embryos after fertilization. The finding of various nuclear and chromosomal abnormalities in cleavage led to the conclusion that embryonic death was due to chromosomal abnormalities induced by irradiation in spermatozoa; and the approximately straight-line relationship between log littersize and dose was interpreted as meaning that dominant lethals were single-hit events, at least within the range of doses used. It was also found that death from dominant lethals occurred either before implantation of the embryo into the uterus or very shortly after; and W. L. Russell (1954), by comparing the results of various workers, concludes that some of the embryos showing nuclear abnormalities in cleavage must be able to implant. Some of the early evidence suggested that the ratio of preimplantation to postimplantation death goes up at higher doses, and Lea (1947) suggests that the relatively greater death in early stages could be attributed to more of the sperm carrying more than one lethal chromosome change. The early work also showed that dominant lethals are not recovered from irradiated spermatogonia, possibly because there may be selection against them during the course of spermatogenesis.

Dominant-lethal experiments done since the time of W. L. Russell's re-

view, in addition to amply confirming the findings of the pioneer workers, have been extended into several new fields. The bulk of the earlier work dealt with the offspring derived during the first two weeks following acute x-irradiation of males, and much of it was based on littersize determinations. In the new work, a number of inducing agents, other than acute x-rays, have been investigated, namely, chronic and acute gamma rays, x-rays in conjunction with hypoxia, neutron irradiation of various types and energies, and mutagenic chemicals. Offspring derived over a period of several weeks following treatment of the male have been studied in an attempt to determine the effect of gametogenic stage treated on dominant-lethal frequency. Some investigations and speculations have been made concerning the time of death of embryos bearing dominant lethals, and alternative expressions have been derived for calculating dominant-lethal frequencies. Finally, dominant-lethal investigations have been extended to the female, where a number of complications had to be overcome before effect of gametogenic stage could be investigated.

2. *Mechanisms of the dominant lethal effect.* Recent findings extend the pioneer studies on mechanisms of the dominant-lethal effect following irradiation of the male. The effects of very high doses of x-rays, employed in an attempt to produce gynogenetic haploids, have already been discussed in the section on euploidy (section I, A, 3). Following less extreme doses, namely, up to and including 1,000 r. administered to sperm suspensions (Edwards, 1957a), entry of the spermatozoon into the vitellus, formation of the pronuclei, and initiation of cleavage are normal. With doses of 100 or 500 r., cleavage proceeds with perfectly normal speed through 3½ days of gestation, at which time the mouse embryo has, on the average, about 40 cells and is assuming the blastocyst stage of development. Beginning, however, with the lowest dose investigated by Edwards, 100 r., subnuclei ("micronuclei") are found in early cleavages, and hypodiploid embryos are observed at 3½ days gestation. These results confirm the earlier ones of Hertwig and Brenneke already mentioned. The subnuclei, which are the visible manifestations of injured paternal chromatin, increase in number as well as size with increasing dose.

The mechanisms of dominant lethality may briefly be examined. Breakage of a single chromosome in the sperm is probably followed by formation of an acentric fragment and a dicentric. The acentric presumably fails to be incorporated into one of the nuclei resulting from the first cleavage and may lie naked in the cytoplasm, or form a micronucleus, or be lost from the cells altogether. The dicentric may also form a micronucleus; or it may form a bridge either at the first cleavage or—if it is included whole in one blastomere nucleus—at a later cleavage. Such bridges can theoretically produce mechanical interferences with cleavage divisions; or they

may initiate bridge-breakage-fusion cycles which eventually lead to loss of the chromosome. Breakage of two chromosomes that leads to an asymmetrical exchange also results in fragments and usually dicentrics at cleavage, with the eventual loss of two chromosomes from the nuclear complement of the blastomeres. (For symmetrical exchanges, see section III, B.)

As shown in the pioneer studies mentioned above and fully confirmed later (W. L. Russell, L. B. Russell and Kimball, 1954; Bateman, 1958a), death from radiation-induced dominant lethals occurs no later than shortly following implantation of the embryo into the uterus. The actual time span over which the deaths are distributed is probably very short. Thus, Edwards (1957a) has shown that following doses up to and including 500 r, all embryos are still alive and of the normal stage of development by 3½ days gestation. Bateman (1958b) states that on the "4th day" (this may be equivalent to day 3½) all ovulated eggs can still be found; but that, by the "5th day," sections of deciduomata show no signs of embryos. Therefore, a deciduomatal reaction is probably invoked by some of the dying embryos within this 24 hour period; other embryos dying during the same interval do not evoke such a reaction.

There have been indications in a number of separate investigations (W. L. Russell et al., 1954; Hollander and Gowen, 1955; and Bateman, 1958c) that the proportion of deaths occurring before implantation increases with dose. Bateman (1958c) tests the hypothesis first suggested by Lea (1947) that the greater the number of chromosome breaks induced in the parental germ cells, the earlier will be the death of the offspring. The first assumption made is that the presence of more than one break causes preimplantation death and that only those embryos having but one break are able to evoke a deciduomatal reaction. Bateman (1958c), however, finds that a better fit of the data is obtained if it is assumed that not only all single-lethal embryos, but also one-quarter of the multi-lethal embryos, can form deciduomata.

In determinations of dominant lethals induced in males, Russell and coworkers (W. L. Russell, L. B. Russell, Gower and Sheppard, 1953; W. L. Russell et al., 1954; W. L. Russell, L. B. Russell and Oakberg, 1958; W. L. Russell, L. B. Russell and Kelly, 1958; L. B. Russell, W. L. Russell, Major and Coffman, 1956) have dissected the mates at about 2 weeks of gestation and counted corpora lutea (assumed to be a measure of ovulated eggs), living embryos, recently dead embryos (whose death is not due to dominant lethals), and deciduomata. The last class plus the preimplantation loss (calculated as the difference between corpora lutea and all implants, living or dead) measures—after allowance for controls has been made—the frequency of animals dying as a result of induced dominant lethals. Calculations are based on the ratios of living (plus recently dead)

embryos to corpora lutea in the irradiated group, as compared to the control. Bateman (1958c) has derived several expressions for measuring dominant-lethal mutation rate. In one of his publications (Bateman, 1958a) he has used the ratio of deciduomata to other implants, which is based on the assumption that only single-break lethals evoke a deciduomatal reaction. Actually, this ratio gives an over-estimate at higher doses. In a later publication, Bateman (1960) uses the ratio of survivors from dominant-lethal-induced death to implants of all types. It should be noted that both of his measures ignore dominant-lethal deaths occurring preimplantation, a very sizable proportion of the total deaths. This, however, is considered an advantage by Bateman (1958a) since the preimplantation-loss category, as measured, can also include failure of fertilization; and, while this is known not to be a factor in most dominant-lethal experiments, there are certain circumstances (e.g., treatment of certain gametogenic stages), when it can play a role. However, in experiments where there is known to be no effect on fertilization, it would seem preferable to calculate dominant lethality on the basis of all deaths, rather than only those occurring postimplantation.

3. *Agents that induce dominant lethals.* For many years, acute x-irradiation in air was the only method used for inducing dominant lethals in mammals. More recently, several agents and conditions of applying them have been compared with this standard treatment.

Russell and co-workers applied x-rays under conditions of hypoxia, which, in other organisms, have been found to protect against a number of genetic changes. Hypoxia, however, had no effect on the incidence of dominant lethals induced in mouse spermatozoa (W. L. Russell, Kile, and L. B. Russell, 1951), even though oxygen levels used for comparison with air were as low as 5 and 2 per cent. It is possible that the normal state of sperm is already a hypoxic one, in which case the experimental reduction in oxygen tension would have caused little or no change. Hypoxia probably does protect against dominant lethals induced in oocytes (L. B. Russell and Major, 1953).

A comparison of acute gamma-rays (Co^{60}, dose rate about 24 r/min.) and acute x-rays revealed little or no difference in frequency of dominant lethals induced in spermatozoa (W. L. Russell, L. B. Russell and Kelly, 1958). Thus, survival of embryos conceived during the first nine days after irradiation was 61 and 57 per cent of control survival, respectively, following a dose of 564 rad (L. B. Russell, unpublished). It was also shown (W. L. Russell, L. B. Russell and Kelly, 1958) that the rate of administration of gamma rays has little effect on dominant-lethal frequency, even though the two dose rates being compared differed by a factor of almost 3,000. Since the animals irradiated at the low intensity had to accumulate their

dose over a period of three weeks, this experiment was complicated somewhat by the fact that dominant-lethal frequency varies according to gametogenic stage irradiated (section I, C, 4). Comparison, however, was made with the average of a number of acutely irradiated groups conceived at various intervals during that period (L. B. Russell, unpublished).

Russell and co-workers have compared neutrons with x-rays, and various types of neutron irradiation with each other, for effectiveness in producing dominant lethals in mice. With neutrons, as with x-rays, deaths due to dominant lethals occur both before and at (or shortly after) implantation of the embryo (W. L. Russell et al., 1953; W. L. Russell et al., 1954). In all neutron experiments, log survival was linearly related to dose; but, since it is known from work on other material that a single neutron track is able to induce more than one break, this straight-line relationship does not necessarily indicate that dominant lethals were due to single breaks. The effectiveness of dominant-lethal induction, measured at the 50 per cent survival point, of fast neutrons from a cyclotron (about 1 Mev average) was about six times that of x-rays (W. L. Russell et al., 1953). (The factor of 6 is derived from recent revisions in dose estimates.) Neutrons from a nuclear detonation, although delivered at very high dose rate, were found to be equal to cyclotron neutrons in frequency of dominant lethals induced (W. L. Russell et al., 1954). On the other hand, monoenergetic 14-Mev neutrons from a Cockcroft-Walton accelerator produced only about one-third as many dominant lethals as did the cyclotron or detonation neutrons (L. B. Russell, W. L. Russell, Major and Coffman, 1956), indicating an inverse relation between neutron energy and efficiency in breaking chromosomes.

The effect of ultraviolet irradiation (2,100 to 3,200 A, with sharp peak between 2,500 to 2,600 A) of spermatozoa on the subsequent development of embryos has been studied by Edwards (1957b). Some of the results are similar to those found by him for x-rays (Edwards, 1957a) and typical for dominant lethals in general, namely, the occurrence of embryos with subnuclei, and the death of embryos before or shortly after implantation. Ultraviolet is, however, a more efficient agent for the production of gynogenetic haploids and has already been discussed in that connection (section I, A, 3).

A number of chemicals have been tested with respect to their ability to induce dominant lethals. Insemination with spermatozoa treated in vitro with trypaflavine or toluidine blue gave negative or confusing results (Edwards, 1958c). Nitrogen mustard, applied in a similar manner, (Edwards, 1958c), caused extreme chromosome damage, but the chemical acted on maternal as well as paternal chromosomes by damaging the second-maturation spindle of the oocyte. In contrast to the 100 per cent

preimplantation death of embryos resulting from Edwards' in vitro treatment of sperm, Falconer, Slizynski and Auerbach (1952) found that injection of males prior to mating, with dosages approximately equivalent to Edwards' lowest, but still effective, dose, produced no evident effect on littersize during the first week after mating, i.e., no marked dominant lethality in mature spermatozoa. It is, however, possible that a period of subsequent sterility of the males may have indicated induction of a high frequency of dominant lethals in earlier germ-cell stages.

The chemical most carefully investigated with respect to dominant-lethal induction is triethylenemelamine, TEM. This chemical, when injected into male rats (Bock and Jackson, 1957; Steinberger, Nelson, Boccabella and Dixon, 1959; Bateman, 1960) or mice (Cattanach and Edwards, 1958; Bateman, 1960), causes death of embryos subsequently sired. Even after TEM treatments so severe that they cause 100 per cent death of embryos by day 6½ of gestation and severe developmental retardation by day 3½, the frequency of fertilization is normal for matings made at short intervals following injection (Bock and Jackson, 1957; Cattanach and Edwards, 1958). At lower doses, a fair proportion of the embryos survive to implantation. Survival beyond that stage is exceedingly rare after a total dose of 0.8 mg./Kg. TEM, but relatively frequent after 0.4 mg./Kg. (Cattanach and Edwards, 1958). The 0.4 mg./Kg. dose can be approximately equated to 500 r x-rays on the basis of survival (Bateman, 1960). the 0.8 mg./Kg. dose (two fractions of 0.4 mg./Kg. given a day apart), which can be equated to 600 or 700 r x-rays on the basis of induced frequency of translocations (see section III, B, 3), is very much more effective than that dose of x-rays in inducing dominant lethals in spermatozoa and spermatids (Cattanach and Edwards, 1958). On the other hand, TEM, in contrast to x-rays, produces next to no dominant lethals in spermatocytes of mouse or rat (Bateman, 1960). The comparison of TEM with x-rays is, therefore, not a simple one.

4. Effect of gametogenic stage on dominant-lethal frequency. Matings made immediately after irradiation utilize spermatozoa which were mature at the time of the treatment; progressively later matings utilize spermatozoa which, at the time of treatment, were in progressively earlier stages of spermatogenesis. Even the pioneer workers in the field were able to show that there is a change in dominant-lethal frequency with time after irradiation of the male; specifically, that the dominant-lethal frequency is higher in litters sired toward the end of a two-week period after irradiation (see review by W. L. Russell, 1954). Hertwig attributed this increase to a greater sensitivity of prespermatozoal stages. In more recent years, extensive studies have been carried out on time relations in spermatogenesis.

On the basis of careful analysis of the cycle of the seminiferous epithe-

lium and of radiation effects on the various germ cell stages, Oakberg (1956a, b) has tabulated for each spermatogenic cell type the minimum and maximum interval to release of mature spermatozoa from the seminiferous tubule (Oakberg, 1957). (His papers may be consulted for other references on timing of spermatogenesis; detailed discussion of this field is outside the scope of this review.) To this interval must be added a minimum of 7.5 days required for passage of spermatozoa from the tubule to the ejaculate (Sirlin and Edwards, 1957; Oakberg and DiMinno, 1960). During the first week after irradiation (or any other treatment that does not affect the time relations of spermatogenesis), the ejaculate consists of spermatozoa treated in the vas and epididymis; during the second and third weeks, cells treated as testicular sperm and spermatids are utilized. Treated spermatocytes do not reach the ejaculate until at least twenty-two days after exposure to the experimental agent have elapsed. Type B and A spermatogonia can be represented in the ejaculate after five and six weeks, respectively; but even relatively low doses of radiation kill all type B and a large proportion of type A and thus cause a period of oligospermia or aspermia, representative of the time required for replenishment of the basic spermatogonial population.

There are some indications that testicular sperm is slightly less sensitive to the induction of dominant lethals than is sperm present in the vas (W. L. Russell, L. B. Russell, and Oakberg, 1958; Bateman, 1956b, 1958a, 1960). A good deal of independent evidence points to spermatids as yielding the highest incidence of recoverable dominant lethals. Thus, following each of seven doses of neutrons, the number of surviving embryos was lower for matings made from 19 to 23 days after irradiation than for matings made two to six days after irradiation (W. L. Russell et al., 1954). In a 300 r x-ray experiment, also, Russell and co-workers found a marked rise in dominant-lethal frequency in spermatids (W. L. Russell, L. B. Russell and Oakberg, 1958). The peak dominant-lethal incidence found in the third week of mating after x irradiation was originally attributed by Bateman (1956b) to a later stage in spermiogenesis; but following a criticism by Auerbach (1957) and Sirlin and Edwards (1957), Bateman (1957) modified his original conclusion and agreed that the peak sensitivity was in spermatids, a result which makes the mouse comparable to *Drosophila*. Later data of Bateman's (1958a) show a similar relationship. Following injection with TEM, Cattanach and Edwards (1958) using 0.4 mg./Kg., and Bateman (1960) using 0.2 mg./Kg., find strikingly similar time relations in sensitivity: survival of embryos is lowest 11 to 14 days postinjection, and almost as low for 8 days before that; from 15 days on, there is a rapid return to normal. The interpretations made in the two papers, however, are somewhat different. Thus, Cattanach and Edwards believe the 11 to 14 day

period to reveal a real peak in sensitivity and attribute this to the utilization of treated spermatids in general. Bateman, on the other hand, believes this period to represent *late* spermatids only, and the rapid return to normal after fifteen days to indicate a low sensitivity of mid and early mouse spermatids. Furthermore, he does not consider the 11 to 14 day period ("late spermatids") to be more sensitive than the preceding one (epididymal sperm).

The relative sensitivity to dominant-lethal induction of spermatocytes is somewhat difficult to determine. In contrast to spermatids and spermatozoa, which are highly resistant to the direct killing or inactivating effects of radiation, spermatocytes are killed by higher doses. Thus, Oakberg and DiMinno (1960) find LD_{50}'s ranging from 205 to 837 r for the various meiotic prophase stages of the primary spermatocyte; in addition, a certain proportion of the surviving spermatocytes form grossly abnormal spermatozoa. Consequently, since there may be an increase in nonfertilized eggs, it cannot be assumed that a deficiency in implants represents embryonic death due to dominant lethals. Bateman, who calculates dominant-lethal frequency on the basis of implanted embryos only, has obtained results for mating-weeks 4 to 7 after irradiation with 200 r x-rays, an interval during which most of the sperm used is presumably derived from irradiated spermatocytes (although there may be some admixture from more advanced stages). In his first publication on the subject, however, Bateman (1956b) assumes that all spermatocytes are killed by 200 r and attributes these matings to spermatids and immature sperm. Later, following the criticism already mentioned, Bateman (1958a) makes the correct interpretation and finds that the rate of dominant lethals recoverable from irradiated spermatocytes is approximately equal to that found in testicular sperm. W. L. Russell et al. (1954) had already reported similar results for the two lowest neutron doses used, which showed an upswing in survival for the 24 to 31 day mating period (as compared to spermatids). The chemical TEM produces next to no dominant lethals in spermatocytes of both mouse and rat (Bateman, 1960). Bateman points out that, in view of the possibility of germinal selection of irradiated spermatocytes, dominant-lethal frequency, measured in terms of surviving embryos, may give an underestimate of actual chromosome breaks induced.

A similar explanation of selection had been invoked by earlier workers to reconcile the absence of dominant lethals from irradiated spermatogonia with the very high sensitivity to direct killing of these cell stages. It now appears, however, that at least a good portion of spermatogonial killing is not due to chromosome breakage, since the bulk of spermatogonial death occurs at the prophase of the first attempted mitotic division (Oakberg, 1955). It is also interesting, in this connection, that doses of TEM

which produce a high frequency of dominant lethals in spermatozoa and spermatids, cause very little, if any, killing of spermatogonia (Cattanach and Edwards, 1958; Bateman, 1960).

5. *Dominant lethals in females.* The ovary of the adult mouse, and probably of most other mammals as well, no longer contains oogonia. All germ-cell stages present are primary oocytes arrested in one of the prophase stages of the first meiotic division. In the mouse, the arrest occurs following diplotene, which is completed shortly after birth. The nucleus then assumes a pseudo-resting state, named dictyate, which persists until a few hours prior to ovulation, at which time the first meiotic prophase is again resumed with diakinesis. In other species, the arrest may occur in different stages of prophase. Completion of the first meiotic division occurs just prior to ovulation in most mammalian species (just after ovulation in a few), and the second meiotic division is immediately initiated without an intervening resting stage. At the time of sperm penetration, the egg is in metaphase of the second meiotic division.

It is thus obvious that, while in the male dominant-lethal induction can be studied in all stages of gametogenesis, such study in the adult mammalian female is restricted to the primary oocyte and the first meiotic division. A postmeiotic haploid stage (analogous to the spermatid and spermatozoon in the male) is never present by itself and occurs only after sperm entry, so that it is not possible to administer treatment to such a stage without also treating the fertilizing sperm. Because of the nature of the stages available in the adult female, the possibility exists that any treatment may produce, in addition to, or instead of, true dominant lethals, a number of other effects such as those already considered in the section on euploid heteroploidy (section I, A, 1), e.g., retention of polar bodies, movement of all chromosomes into a polar body, immediate cleavage, or disturbances in the vitelline surface that result in polyspermy or failure of sperm penetration. Hormonal effects may suppress ovulation completely. There is the further possibility, when females are treated, that death of embryos may be caused through disturbances in maternal physiology rather than abnormalities of chromosome constitution of the embryo. Consideration of these various complications will make it clear that dominant-lethal incidence in females cannot be measured by littersize reduction as it can in the case of irradiated spermatozoa. Partly because of this, no careful study of dominant-lethal induction had been undertaken in female mammals until the 1950's.

In these new experiments, a fresh, and hitherto unsuspected, complication was revealed: irradiation of female mice produces superovulation. The first indication of this effect was the finding of an increased number of corpora lutea of pregnancy in matings made in the first 5 days following

irradiation (L. B. Russell and Major, 1953). By counts of eggs present in the oviduct within a few hours after mating and by more detailed examination of 351 of these eggs for number of pronuclei and number of polar bodies, L. B. Russell then determined that the increase in number of corpora lutea of pregnancy was accompanied by a proportionate increase in the number of ovulated eggs fertilized normally (L. B. Russell and W. L. Russell, 1954, 1956; L. B. Russell and Spear, 1954). Irradiated females therefore have a higher *absolute* number of fertilized eggs, and it is thus possible to obtain normal litter sizes in spite of a fairly high incidence of embryonic death. (This again demonstrates dramatically the uselessness of littersize data in determining dominant-lethal frequencies in females.) Following a dose of 400 r., superovulation occurs from days 1½ to 14½ after irradiation; and, during the peak intervals, irradiated females average approximately half again as many copora lutea as do the controls. Following the two-week period of superovulation, the number of ovulated eggs remains normal for approximately 10 days, and then rapidly drops to zero, as expected from other work on the high sensitivity to killing of mouse oocytes (other than those contained in the most mature follicles).

With the demonstrations that the number of tubular eggs was as great as or greater than in controls and that the proportion of fertilized eggs was normal (as judged by the presence of two pronuclei), one of the criteria of dominant lethality was met, and many of the other possibilities enumerated above were eliminated. Further experiments proved that the postfertilization death was not due to disturbance of maternal physiology but was caused by chromosomal aberrations in the embryos. Examination of both fixed and living two-cell stages revealed the frequent occurrence of micronuclei and chromosome fragments (L. B. Russell and W. L. Russell, 1954). These are similar to the "Nebenkerne" or "subnuclei" found following irradiation of spermatozoa (section I, C, 1 and 2). Analysis of about 70 two-cell stages indicated that, for matings made 1½ or 2½ days following irradiation, 75 to 80 per cent of all eventual deaths were already represented by gross nuclear aberrations after the first cleavage (L. B. Russell and Major, 1956). It may, therefore, be concluded that the postfertilization death of embryos that occurs following preconception irradiation of the mother is the result of chromosome breaks induced in primary oocytes. Following 400 r., the majority of these deaths occur prior to implantation and the rest at or shortly after implantation (L. B. Russell and W. L. Russell, 1954), comparable to the situation with dominant lethals in males. The dominant-lethal frequency in matings made 1½ to 4½ days after irradiation of the oocyte is approximately the same as the frequency induced in spermatozoa by the same dose (L. B. Russell and Major, 1953).

Even the earlier experiments in females indicated that, in matings made

½ day after irradiation, dominant-lethal frequency was greater than in matings made 1½ to 4½ days after irradiation (L. B. Russell and Spear, 1954; L. B. Russell and W. L. Russell, 1954). These indications were followed up in experiments involving finer variations in the irradiation-to-fertilization interval. It was found that irradiation up to approximately 16 hours prior to ovulation yielded the same dominant-lethal frequency as that characteristic of the 1½ to 4½ day preovulation interval; but that a radical change in sensitivity occurred between 16 and 8.5 hours preovulation. Thus, a dose of 400 r, which allowed from 70 to 80 per cent survival of embryos (relative to control) if administered up to 16 hours preovulation, gave only 1.2 per cent survival when it was delivered 8.5 hours preovulation (L. B. Russell and Spear, 1955; L. B. Russell and W. L. Russell, 1956). Even a dose as low as 100 r at 8.5 hours preovulation allowed no more than 32 per cent survival (L. B. Russell, unpublished). Preliminary cytological analysis of this highly sensitive stage indicates that the majority of oocytes are in diakinesis or metaphase of the first meiotic division (L. B. Russell and W. L. Russell, 1956). It was felt necessary to carry out for this highly sensitive stage some of the same investigations that had been used in conjunction with the less sensitive intervals, since it was not inconceivable that the tremendous depletion in embryos could be due to some cause other than dominant-lethals. These tests revealed that even though only 0.1 embryos per female survived to two weeks gestation, there were, respectively, 9.7 fertilized eggs and 8.0 viable two-cell embryos per female (L. B. Russell, 1956). Therefore, as was the case with the less sensitive stages of irradiation, death occurs *post*fertilization and most, if not all, of it following the two-cell stage. About 75 per cent of the two-cell stages examined contained micronuclei or other chromosomal abnormalities; and these were, in general, more severe than was the case when irradiation was given at less sensitive stages (L. B. Russell and Major, 1956). It was concluded that at 8.5 hours preovulation, as at other stages, death of embryos resulting from chromosome breakage in oocytes had been induced; and that the greater severity of the effect was the result of a more sensitive nuclear state of the oocyte at the time of irradiation. The differential sensitivity of prophase and metaphase oocytes of the mouse to the induction of dominant-lethals is thus similar to that found in *Habrobracon* by Anna R. Whiting (1961).

For matings made 1½ to 4½ days postirradiation, dose curves (range 100 to 700 r in 100 r steps) indicate that at least some dominant-lethals in females are due to multiple-hit aberrations (L. B. Russell, unpublished). Hypoxia gives some protection against radiation-induced dominant-lethals in females (L. B. Russell and Major, 1953). This is in contrast to results with sperm (section I, C, 3). Hypoxia may also have some protective effect against the induction of superovulation (L. B. Russell and Major, 1953).

II. Duplications and Deficiencies

The terms duplication and deficiency are applied to addition and loss, respectively, of a *section* of chromosome, the total number of chromosomes remaining constant. There have been a number of studies of deficiencies in mice, but no report to date on pure duplications. The only duplication reported is accompanied by a deficiency in the same animals. This case will be discussed first.

A. *Duplications*

Animals heterozygous for a reciprocal translocation produce among their gametes some that are unbalanced for genetic content (section III, B, 2, Fig. 2). Among several possible types of these, the most frequent one is that in which one of the two chromosomes involved in the translocation is present intact (say, chromosome 1) and there is, in addition, a translocated chromosome (composed in part of 1 and in part of 2). When the mate contributes a normal gamete, containing the intact chromosomes 1 and 2, the resultant offspring then has—instead of two each of chromosomes 1 and 2—the following constitution: for 1, two whole chromosomes and a piece (i.e., a duplication); and for 2, one whole chromosome and a piece (i.e., a deficiency). Although such unbalanced offspring have been found to die in embryonic stages in the case of several autosomal translocations that have been studied (section III, B, 2), there is no *a priori* reason to assume lethality in every case. If the pieces exchanged between the two chromosomes involved in the translocation are very small, it is possible that a duplication-deficiency type could survive and, if there is some associated phenotypic expression, be transmitted like a dominant mutation.

In the case of a translocation between an autosome and the X-chromosome (section III, B, 5), Cattanach (1961b) has found one of the duplication-deficiency types to be viable, namely, the type that is deficient for a piece of the X and duplicated for a piece of the autosome. Since mouse females require only one X-chromosome for normal sexual development and almost normal viability and fertility, the X-deficiency may have no marked effect on females in the duplication-deficiency class (see discussion of this point in section III, B, 5). Such females may, therefore, reveal purely the effect of the autosomal duplication; and, in the case reported by Cattanach, they are fully viable and fertile. That this is not necessarily the usual situation is indicated by the fact that, in the case of 3 X-autosome translocations reported by L. B. Russell and Bangham (1959, 1960, 1961), duplication-deficiency offspring of all types die prenatally. Males of Cattanach's duplication-deficiency class are relatively inviable (only about 25 per cent survive to adulthood) and very small. This, however, is not surprising in

view of the fact that the deficiency for a piece of the X is not covered by a normal X chromosome, as is the case in the female. Males completely lacking an X, i.e., YO, have already been shown to be lethal (W. L. Russell et al., 1959) (section I, B, 2a).

If Cattanach's translocation is a simple reciprocal one, the duplicated piece of autosome should (according to the linkage map) be quite long. There are various lines of evidence to suggest that this is not so (section III, B, 5). Moreover, the break in the autosome is not far from where it was in two of Russell and Bangham's translocations; and the break in the X also approximately resembles one of Russell and Bangham's cases. Yet, in all of their X-autosome translocations, the duplication-deficiency offspring die prenatally. It is thus possible that Cattanach's translocation is not a simple reciprocal one and that the transposed piece of autosome (and, therefore, the duplication in the unbalanced offspring) is not much longer than the minimum 14 crossover units indicated by the genetic results.

B. Deficiencies

Studies on deficiencies in the mouse have concerned three regions: the region of *waltzer*, *v*, in linkage group X (one deficiency studied); the region of *dilute short-ear*, *d se*, in linkage group II (11 deficiencies studied); and the region of *belted*, *bt*, in linkage group VI (one deficiency reported). The deficiency involving *waltzer* is the classical case reported by Gates (1927) and Painter (1927). A cross of homozygous *waltzer* × homozygous wild-type, which should give only wild-type progeny, yielded one *waltzer* among several hundred wild-types. This exceptional animal, which could have been v/v as the result of a mutation to v in the wild-type parent, behaved on progeny tests as though she were, instead, $v/0$ (or, possibly, v/v^l, where v^l is a lethal allele recessive to v). While this could have been the result of loss of an entire chromosome, Painter showed that the normal number of chromosomes was present in spermatogonial metaphase, at the first maturation division, and in secondary spermatocytes. However, males of the constitution $v/0$ had an unequal pair of chromosomes in addition to the X-Y pair. The short chromosome presumably represented linkage group X deficient for a region including the *v*-locus.

Studies on deficiencies in the *d-se* region of linkage group II of the mouse (L. B. Russell and W. L. Russell, 1960) were the outcome of specific-locus mutation rate experiments. The loci for *d*, *dilute*, and *se*, *short-ear*, which are very closely linked (crossover frequency = 0.16 per cent) were included in these experiments in the hope that they would provide some answers concerning the relative frequencies of deletions and point mutations among induced and spontaneous genetic changes. The probability of simultaneous point mutation of both *d* and *se* is extremely low

(less than one in 100,000,000 even after high doses of radiation); consequently, the finding of an animal mutant for both *d* and *se* is presumptive evidence for deletion of a chromosome region including the *d* and *se* loci or even for loss of the entire chromosome. Over a period of several years, 26 *d se* double mutants have occurred from irradiation of various germ-cell stages as well as spontaneously. Of these, 22 occurred at Oak Ridge (L. B. Russell and W. L. Russell, 1960; Russell, unpublished) and 4 at Harwell (Searle, in discussion of L. B. Russell and W. L. Russell, 1960). Extensive genetic analysis of 18 of the Oak Ridge double mutants and of the stocks derived from them (L. B. Russell and W. L. Russell, 1960 and unpublished) leads to the following conclusions: (1) *d se* mutants recovered after irradiation of postspermatogonial stages and of oocytes carry deletions involving the *d* and *se* loci; (2) irradiation of spermatogonia yields such deletions only with extremely low frequency (only one *d se* double mutant—a deletion—was found in a population that contained 46 single mutants, *d* or *se*); (3) spontaneously occurring *d se* "mutants," instead of carrying deletions of *d* and *se*, are probably homozygous for the test-stock linkage group II as a result of nondisjunction in the test-stock parent combined with loss of that chromosome in the wild-type parent (section I, B, 1); (4) the deletions are recessive with respect to the coat color and ear phenotypes; but, in the case of several of them, the heterozygotes are smaller than normal; (5) several of the *d se* deletions are transmitted with less than normal frequency, and this may be the result of a semilethal effect of the deletion on gametes; (6) all *d se* deletions are prenatally lethal in the homozygous state, in all possible combinations with each other, and in combination with prenatally lethal *d*- or *se*-locus mutations.

A brief report has appeared of an x-ray-induced deficiency in linkage group VI of the mouse (Kidwell, Nash, Stadler and Gowen, 1961). This deficiency, which was induced in a postspermatogonial stage, includes the loci *bt* and *hl*, separated by seven crossover units, but not the locus *N*, which is approximately 14 crossover units away from *bt*. As was the case for some of the *d se* deficiencies, the *bt hl* deficiency has a dominant size effect, although it is recessive with respect to the specific phenotypic effects associated with the deleted loci.

III. Chromosomal Rearrangements

A. *Intrachromosomal*

1. Lack of good evidence on inversions in mammals. The information on inversions in experimental mammals is very meager indeed. Applying the findings from *Drosophila*, maize, and other organisms, inversions might be

expected to give one or more of the following results potentially detectable in breeding experiments: (a) crossover inhibition; (b) reduced number of living offspring from males in an inversion line; and (c) position effect. The first two of these three effects would be detectable in heterozygotes only. Inhibition of crossing-over has been found characteristic of certain alleles at the *t*-locus in the mouse, but no definite proof has yet been brought that this is due to inversions (section III, A, 2). Reduction in the number of living offspring—the result of the production of unbalanced gametes which form inviable embryos—can characterize either inversion or translocation heterozygotes. In the case of inversions, the expected litter-size reduction would be less severe and might affect the male parent only. However, wherever inherited partial sterility has been further analyzed, either genetically or cytologically, the phenomenon has been found to be the result of translocations rather than inversions (see section III, B). Position effects were until recently unknown in experimental mammals. Those that have been described since 1959 (section III, B, 5) have all been found to be associated with translocations, rather than inversions. In a specific search for inversions affecting linkage group III, none was found among more than 300 tested mice from irradiated fathers, though the same sample yielded many translocations (Carter and Phillips, unpublished data, cited in Carter, Lyon and Phillips, 1955).

Cytologically, inversions could be recognized by characteristic loops of the paired chromosomes in zygotene or pachytene of the first meiotic division. If the inverted piece of chromosome is long enough so that crossing-over within it occurs with measurable frequency, inversions can also be recognized by anaphase bridges and fragments in a certain proportion of meiotic divisions. Although several early reports of anaphase bridges in mammals were considered indicative of the presence of inversions, such evidence is now considered suspect in view of the frequent occurrence of pseudo-bridges and laggards (Jaffe, 1952). Only bridges accompanied by acentric fragments should be considered indicative of the presence of heterozygous inversion, and this condition has not been described except with very low frequency (not significantly different from control) in the case of *t*-locus heterozygotes (Jaffe, 1952). Slizynski (1952) mentions an unusually high frequency of anaphase bridges (with no information as to the presence of acentric fragments) in nine offspring of a single male. This suggested to him the presence of an inversion, and it was inferred "from a single pachytene figure that the chromosome involved is 19." No further evidence on this case has been published. Puttanna (1961) reports anaphase bridges and fragments and, on further analysis, the presence of "inversion loops" during zygotene and pachytene of Wistar rats. Since the rats used are de-

scribed as "inbred," it is not clear why the frequency of these various cytological findings, which characterize only inversion *heterozygotes* should be so frequent, unless the rate of occurrence of new inversions is very high.

2. *The t-locus of the mouse.* Some of the mutations at the *t*-locus in linkage group IX of the mouse inhibit crossing-over in adjacent regions and, for this reason, the locus has occasionally been suspected of being associated with an inversion or other type of chromosomal aberration. The total literature on the *t*-locus is voluminous and only that part which has a bearing on the problem of chromosome aberration will be reviewed here. Mutations at the *t*-locus are the dominant *T*, and a large number of recessive *t*'s (namely, t^0, t^1, t^2, ... etc.) to which the general term t^n may be applied. The phenotypic effects of the various compounds may be roughly summarized as follows: $T/+$ = short-tailed; T/T = lethal prenatally; T/t^n = tailless in almost all cases; $+/t^n$ = normal; t^n/t^n = either prenatally lethal, or viable and normal-tailed, though frequently male-sterile; t^n/t^x (where t^n and t^x are different *t*-locus mutations) = normal-tailed, though frequently male-sterile. Among the many unusual features of the *t*-locus is the complementary action of different *t*-alleles; i.e., t^n/t^x compounds are viable even in cases where both t^n/t^n and t^x/t^x are lethal. While this might suggest lack of true allelism, no recombination has ever been observed to occur either in t^n/t^x or in T/t^n (Dunn and Gluecksohn-Schoenheimer, 1950).

Since both *T*- and certain t^n-homozygotes are lethal, it is possible to maintain balanced-lethal lines by mating $T/t^n \times T/t^n$. In such lines, which breed true for taillessness, occasional normal-tailed exceptions occur; and these have, in almost all cases, turned out to be of genotype t^n/t^x, where t^x designates a newly arisen *t*-mutation. It should be noted that this method detects only t^x mutations which are complementary with t^n. Any t^x that renders the compound t^n/t^x lethal or tailless—if such a t^x should exist—will not be discovered. The frequency of *t*-mutations in balanced lethal stocks is about one in five hundred, whereas *t*-mutations are infrequent or absent as new occurrences in both $+/+$ and $T/+$ stocks (Dunn, 1956). One *t*-allele, t^{w18}, is known to have arisen from a $+/t$ heterozygote, $+/t^{w11}$, a wild female mouse (Bennett and Dunn, 1960).

An early working hypothesis concerning the nature of this mutable genetic entity was that an inversion had made unstable a locus, *t*, included in it, which then gave rise to new *t*-mutations. This hypothesis was, however, discarded when it was found that not all *t*-mutations acted as cross-over suppressors. Thus, the t^3 mutation, which had originated in a T/t^1 stock, did not suppress crossing-over in the region of *T-Fu*^{ki} (Dunn and Gluecksohn-Waelsch, 1953). The mutation t^{13} suppresses crossing-over in the *T-tf* interval in males only (Lyon and Phillips, 1959). The effect on crossing-

over has been most carefully studied for the mutation t^6 (Lyon and Phillips, 1959). It was found that the suppressing effect (which occurs in both sexes) acts over only a short distance: it is great but not complete over the interval *T-tf* (normal cross-over frequency = 8.3 per cent); it acts somewhat between *tf* and the translocation break *T138;* and it does not act at all on the interval between break *T138* and the *d*-locus in linkage group II which has been attached to linkage group IX as a result of the *T138* translocation. It had been shown earlier that t^0, of which t^6 is probably a repeat, suppresses crossing-over in the $T\text{-}Fu^{ki}$ interval (Dunn and Caspari, 1945), but since the *Fu*-locus lies in the interval between *T* and *tf*, no information is available on cross-over inhibition on the other side of the *t*-locus; t^6 does suppress crossing over between *T* and the translocation break *T190* (Lyon and Phillips, 1959), but the position of *T190* relative to *T* is not known.

If the *t*-"locus" is associated with an inversion at all, this inversion probably is quite small since the number of bridges and fragments in meiotic anaphase is low, probably not differing significantly from controls (Jaffe, 1952). Grüneberg (1952) suggests that newly arising *t*-factors, rather than being mutations in the ordinary sense, are the result of crossing-over in a region of chromosomal rearrangement, an event which "would give rise to new gene sequences which would contain duplications and/or deficiencies of chromosome segments."

This point of view has more recently been supported by Lyon and Phillips (1959) and Lyon (1960). One reason given is that the crossover frequency near the *t*-locus, in the presence of the crossover inhibitor t^6, is of the same order of magnitude as the frequency of *t*-mutation in balanced lethal T/t^n lines earlier determined by Dunn (1956). It might, however, be pointed out that one of the crossover determinations involved translocation break *T190* which may be at some distance from t^6 (and, as discussed above, the crossover inhibition of t^6 extends for short distances only). As a second piece of supporting evidence for the crossing-over theory of *t*-mutation, Lyon and Phillips note that, of four crossovers recovered from compounds of the crossover inhibitor t^6 with either *T* or +, one yielded a new *t* mutation, namely, t^{21}. This case, in which a $+\ t^{21}/+\ +$ animal was obtained from the cross of *T190* $t^6/+T \times +\ +/+\ +$ could, however, have been merely due to mutation, unassociated with crossing-over, of *T* to t^{21}. In another experiment (Lyon, 1960), of nine tested new *t* mutants obtained in a $T\ tf/t^6\ + \times T\ tf/t^6\ +$ balanced lethal line, four could conceivably have been a result of crossing-over combined with mutation, since they were of the type $t^n\ tf/T\ tf$ (where t^n is the new mutation). On the basis of the two experiments, Lyon favors the hypothesis that apparent mutation to new *t* alleles is in fact the result of crossing-over in an abnormal chromosome region. It should, however, be pointed out that

of the 10 tested new *t* mutations in the two experiments, 9 occurred in chromosomes that had originally carried the *T* allele. The alternative hypothesis, namely, that the presence of t^6 induces mutations from *T* to t^n in the homologous chromosome therefore seems attractive. A piece of evidence from *Drosophila* may also be cited in favor of the second hypothesis. Thompson (1960) has found that the presence of inversion in one chromosome increases the rate of mutation in the homologous chromosome and that these mutations tend to be clustered around the region of the inversion break. The effect may be due to asynapsis which could presumably be caused by other types of chromosomal aberrations also. Lyon (1960) found that whatever process was responsible for the high spontaneous mutability to *t*, its rate was little, if at all, affected by radiation.

Another unusual and very interesting feature of the *t*-locus is the abnormal transmission of some of its alleles. This has important implications in the fields of gene action, reproductive physiology, and population genetics. Since these fields are, however, outside the scope of this review, this particular property of the *t*-locus cannot be discussed here.

B. Translocations

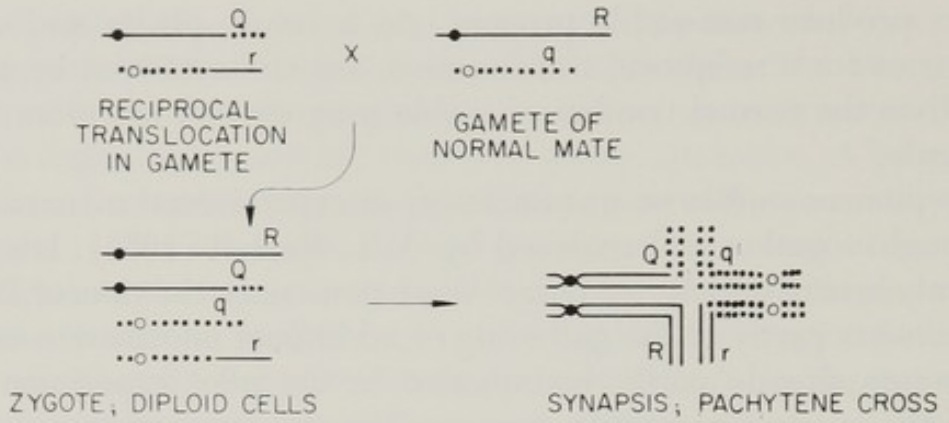
Rearrangement of genetic material involving at least two nonhomologous chromosomes is called translocation. Of several possible classes of translocations, the one encountered most frequently in organisms in general, and the only one to have been studied in experimental mammals, is the class of reciprocal translocation. This type comes about through the formation of one break in each of two non-homologous chromosomes and the subsequent re-attachment at the break point of the broken end of the other chromosome, i.e., a mutual exchange of material. Thus, chromosomes which might originally be designated as A B C D and T U V W become the reciprocally translocated chromosomes A B V W and T U C D. If the centromeres of the original chromosomes were in the A B and V W segments, respectively, then the first reciprocally translocated chromosome will have two centromeres (dicentric), and the second no centromeres (acentric). Both chromosomes will, therefore, be lost in subsequent cell divisions; and, if this type of translocation was induced in a haploid gamete, it will cause death after fertilization and, therefore, be classed with dominant lethals (section I, B, 2). If, however, the centromeres are in the A B and T U segments, the reciprocally translocated chromosomes will function normally in subsequent cell divisions. Syngamy with the normal-sequence chromosomes A B C D and T U V W provided by the mate, produces a zygote with a balanced genome (two doses each of A, B, C, D, T, U, V and W). Such a zygote forms a perfectly viable organism, but, as will be shown, a portion of its gametes will have unbalanced genomes and will,

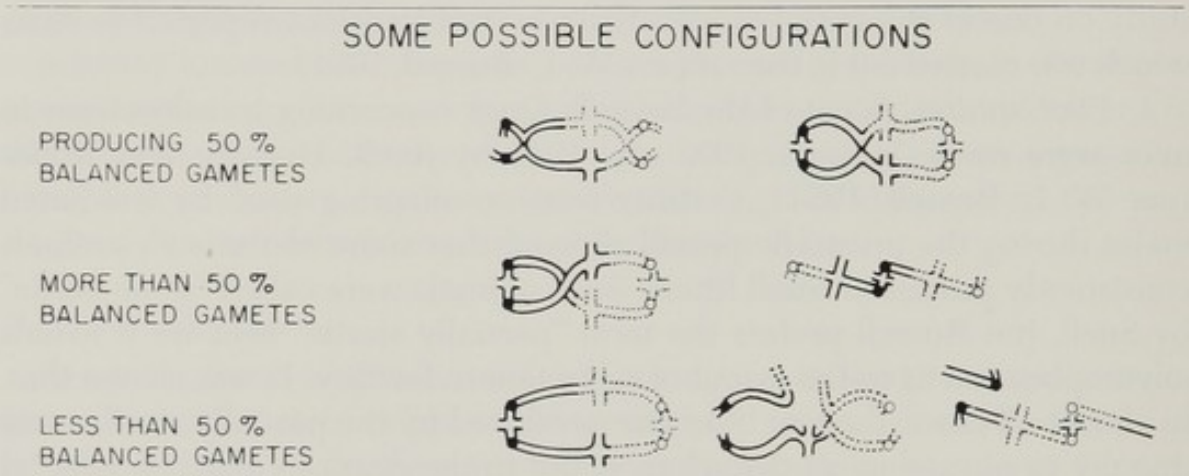
therefore, produce non-viable progeny. As a result, plants and animals heterozygous for a reciprocal translocation, are characterized by producing less than the normal number of viable progeny and are often termed "semi-sterile."

All the pioneer studies on translocations in experimental mammals have been brought together and analyzed by W. L. Russell (1954). His review will be only briefly summarized here. Work done since the time of Russell's review consists partly of the gathering of additional information on some of the points already partly investigated in the pilot experiments, and partly of the exploration of new avenues. The experimental mammal used in all translocation studies but one was the mouse; the one exception is a study on one of the very few spontaneous translocations reported to date, which was carried out in the rat (see W. L. Russell, 1954).

1. Pilot studies. Some of the basic findings concerning translocations in mice were made between 1933 and 1946 by Snell, Hertwig and Koller (see W. L. Russell, 1954). Fertility tests on offspring sired by irradiated males during the presterile period showed that some of these F_1 animals consistently produced small litters. Such animals were called "semi-sterile" by Snell, but Russell prefers the term "partially sterile" because it avoids any implication as to the extent of reduction in fertility. It was shown that, in almost all cases, the low litter size produced by the partially steriles was due not to physiological disturbance, but to the death *in utero* of some of their offspring. Partial sterility was inherited like a dominant in a quasi-Mendelian manner, i.e., approximately one-half of the viable offspring of partially sterile animals were also partially sterile. Snell showed that the results fitted the interpretation that the partially sterile animals were heterozygous for a reciprocal translocation. Later he brought elegant genetic proof of this hypothesis by demonstrating that, in a certain semi-sterile line, the two genes *a* and *b*, which would normally segregate independently, behaved as if they were linked (see also section III, B, 4b). Cytological confirmation of Snell's hypothesis came from the work of Koller, who showed that in three semi-sterile lines produced by him, one could find, in certain meiotic metaphases and anaphases, associations of four chromosomes in synapsis.

2. The types of gametes formed by translocation bearers. a. Partial Sterility. It has already been indicated that heritable partial sterility results from the segregation of unbalanced gametes from translocation heterozygotes. This process is shown diagrammatically in Figure 2. A large number of possible configurations can result from the pachytene cross (top portion of Figure 2) as a result of chiasma formation in one or more of the 6 regions of this cross (translocated segments, interstitial segments, short arms). The great majority of these configurations yield, upon disjunction





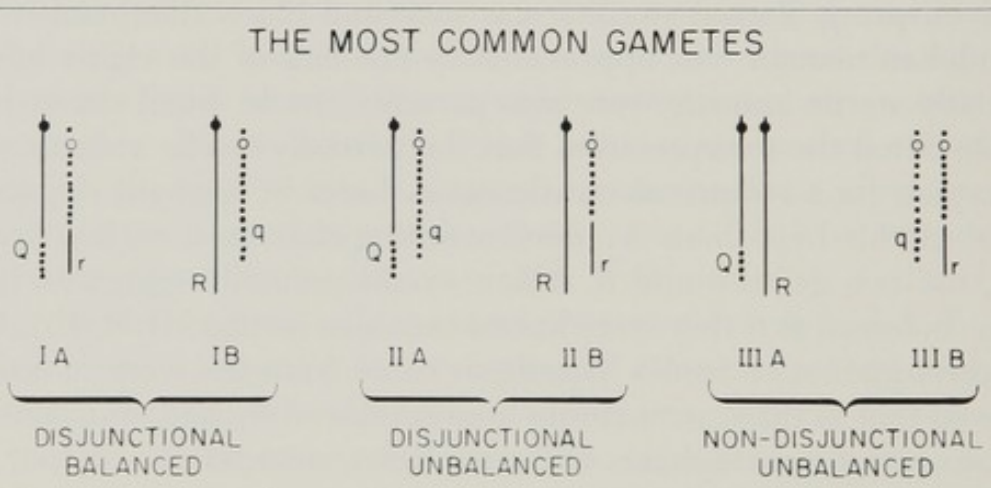


FIG. 2—Formation of translocation heterozygote and segregation in such an animal (see text, section III, B, 2). Only a few examples of numerous possible configurations are shown (modified from Slizynski, 1957b). The great majority would produce 50 per cent balanced gametes. The markers *Q*, *q* and *R*, *r* are used to illustrate text discussion of the use of translocations in cytogenetic investigations (section III, B, 4).

of centromeres, gametes of types I and II (bottom of Figure 2) in equal number, i.e., 50 per cent balanced gametes. Nondisjunction of both pairs of centromeres produces gametes of type III. If nondisjunction is rare, "fertility" will be approximately half of normal, provided that the special con-

figurations which give deviations from 50 per cent balanced gametes are not formed with unusual frequencies (see below).

It should be mentioned that a synaptic association of four chromosomes is not always formed. If the exchanged pieces are very small, affinity in the short distal regions is insufficient to lead to their pairing. The chromosomes will then associate predominantly as bivalents. One such translocation has been described by Cattanach (1961b) and one was mentioned by Auerbach and Slizynski (1956a). The fact that Slizynski, in another study (Slizynski, 1957a), found no evidence of figures-of-four at metaphase in 12 of 27 semi-sterile strains indicates that this situation may exist in a fair proportion of reciprocal translocations that cause semisterility in the mouse.

Koller (1944) had shown that several other types of unbalanced gametes, in addition to the types II A, B and III A, B shown in Figure 2, can also be formed by translocation heterozygotes. By tabulating various meta- and anaphase configurations observed and the proportion of unbalanced gametes expected from each one, he found that, in three translocation lines, the percentage of unbalanced gametes calculated from cytological analysis agreed well with reduction in "fertility" found in breeding experiments. More recently, Slizynski (1957b) has also found fairly good correspondence between cytologically calculated and actual "fertility" in the case of Snell's (1946) *T(5;8)a* and of 11 translocations analyzed by Carter et al. (1955, 1956). His cytological index is based on measurements of the 6 segments of the pachytene cross. Chiasma frequency is then computed for each segment in proportion to its length; and from this the relative frequencies of configurations with 6, 5, 4, etc., chiasmata are calculated. Of 64 possible disjunctional configurations, the great majority yield 50 per cent balanced gametes (types IA and B, Figure 2). Several, which do not, are also shown in Figure 2 and will be discussed below.

From Koller's analysis and other considerations, Snell (1946) concluded that it was theoretically possible for as many as 26 types of gametes to be formed by a translocation heterozygote. He showed, however, in a careful analysis of a genetically well-marked translocation, *T(5;8)a*, that unbalanced gametes of types other than II and III (Figure 2) are formed rarely, if ever. On crossing two translocation heterozygotes marked at three genetic loci, Snell found a class of offspring which could be formed only by the union of two unbalanced but complementary gametes (e.g., type IIIA with type IIIB; or IIA with IIB). By assigning gamete frequencies on the basis of this class and on the basis of the per cent "fertility" of heterozygotes (taken to represent the frequency of gametes of type I), Snell found a very good fit with observed segregation. He concluded that percentage fertility accurately measured the percentage of orthoploid gametes.

One curious effect found by Snell, and later also by Carter et al. (1955),

was that, within a given translocation line, the "fertility" of heterozygous females is lower than that of heterozygous males. Snell observed this to be true in eight out of nine crosses involving six translocations, with the difference being highly significant in one cross; and Carter et al. found a consistent difference in all of seven translocation lines where such a comparison could be made, although the total difference between the sexes, summed over all seven stocks, was not significant. Altogether, the range in average "fertility" of the translocation heterozygotes of various stocks was 41 to 69 per cent for males and 38 to 62 per cent for females in Snell's experiments; 38 to 59 per cent for males and 33 to 44 per cent for females in the experiments of Carter et al. Snell suggested that the proportion of orthoploid gametes produced by males was higher than that produced by females. A direct cytological check of this hypothesis would require improvements in methods for studying meiosis in female mammals. In the meantime, an alternative explanation that seems possible is that the translocation (as a result, perhaps, of some type of position-effect) slightly, but adversely, alters the fitness of animals. In the case of females, this could make them somewhat poorer mothers.

Differences between translocation lines in per cent "fertility" were, in the early experiments, attributed to chance. Later, however, Snell (1946) and Carter et al. (1955) found several translocation lines in which the percentage fertility was significantly different from 50 per cent, being lower in some and higher in others.

A "fertility" of *greater* than 50 per cent could be accounted for either on a segregational basis, or by the fact that one (or more) of the classes of unbalanced gametes produces viable progeny. On a segregational basis it could mean that, for some reason, alternate disjunction of centromeres is the most frequent type of segregation. Also, as Slizynski (1957b) has pointed out, certain configurations (Figure 2, center portion) could yield 100 per cent balanced gametes. However, these configurations would be formed with appreciable frequency only if at least one of the "short arms" is fairly long—a condition which probably does not exist in the mouse. To distinguish between the segregational and the viability explanations for a "fertility" of greater than half normal in a given translocation line, the frequency of translocation bearers among the viable progeny must be determined. If this frequency is about 50 per cent, it is evidence for the former explanation; if it is greater than 50 per cent, as determined by genetic markers, it is evidence for the latter. (Note that if the frequency of partial sterility is scored, rather than that of the genetic tag, viability of unbalanced type will result in less than 50 per cent partially sterile progeny.)

Using the genetically tagged translocation *T* (5; 8) *a*, the males of which show significantly more than 50 per cent fertility, Snell (1946) found that

very close to half of the viable progeny carried the translocation, indicating that, in this case, the greater-than-50 per cent fertility was probably due to preferential segregation of type-I gametes. On the other hand, in the case of a sex-linked translocation, to be described in greater detail below (section III, B, 5), Cattanach (1961b) found that one class of unbalanced gametes produced viable zygotes. This may, however, be a special case since the deficiency was for a piece of X-chromosome (a deficiency which can probably be tolerated without problem by females and, in this case, where it is apparently small, in some males also); and the duplication may be for a small piece of autosome. One translocation mentioned (but not further analyzed) by Auerbach and Slizynski (1956a) as probably involving the exchange of small segments gave littersizes considerably larger than 50 per cent of normal and may, therefore, also have yielded viable progeny from one class of unbalanced gametes.

"Fertility" of *less* than 50 per cent in a heterozygous translocation line could mean that the frequency of metaphase orientation leading to nondisjunction (gametes of type III) is high. Another type of nondisjunction, which can yield mono- and trisomics, would result from various types of associations-of-three plus a univalent (Fig. 2, center portion). Even in the absence of nondisjunction, a certain type of ring-of-four resulting from terminal chiasmata (Fig. 2) produces 100 per cent unbalanced gametes (Slizynski, 1957b). However, the conditions that would favor this configuration probably do not exist in the mouse, where centromeres are subterminal.

A somewhat different situation from that in which the fertility is less than 50 per cent—but more or less uniformly so within the line—is that of lines that frequently produce some completely sterile or almost sterile animals in addition to the partially steriles. W. L. Russell (1952) found such individuals in two out of eleven semisterile lines; and Carter et al. (1955) found them in one of eleven lines. In Carter's line *T6*, 20 of 28 males tested for fertility fell into the usual two categories—partially sterile and fertile—while the remaining 8, all known from the presence of the genetic markers to carry the translocation, were either completely sterile or almost so. One of Koller's lines contained a number of highly infertile animals in early generations which, however, disappeared in F_3. He suggested that the infertile animals were the result of additional aberrations or mutations which are lost by selection in later generations (Koller, 1944).

b. Complete Sterility. The sterility occasionally found in translocation lines must be distinguished from the sterility found with high frequency in the first-generation offspring sired during the presterile period by males treated with either x-rays or chemicals. A summary of the radiation work of several investigators led W. L. Russell (1954) to the conclusion that

this sterility occurred with a frequency of about 10 per cent in the sons of males exposed to 700 r, and that the frequency of completely sterile sons was approximately half that of semisteriles (presumed translocation heterozygotes). Following TEM injection into male mice, the number of completely sterile sons sired is as great as that of semisteriles (Cattanach, 1959).

One possible explanation that had been considered for the sterile F_1 individuals was that they were heterozygous for several translocations and that, therefore, the chance of their forming balanced gametes (and, thus, viable young) was low. Such animals would presumably have normal gametogenesis. However, scattered histological information on a few sterile sons of irradiated males indicated that most of these steriles did not complete spermatogenesis and that the interruption frequently occurred at the maturation divisions (W. L. Russell, 1954). The question might next be raised whether the interference with spermatogenesis could result from special types of translocations—either involving specific chromosomes or complex multiple rearrangements—which might lead to pairing difficulties; or whether it might be due to some cause other than translocation. Evidence will be cited for both of these possibilities; but, in general, it appears that the former may be a less common one.

L. B. Russell and Bangham (1959, 1960, 1961) have described three independent translocations, each between an autosome and the X-chromosome, which render the male heterozygote completely sterile as a result of degeneration of primary spermatocytes in pachytene. A fourth case, as yet unpublished, gives similar results. In an X-autosome translocation described by Cattanach (1961b), in which the exchanged piece of the X may be extremely small, the males are not completely sterile, although they are highly infertile. Thus, it appears possible that the bulk of X-autosome translocations may turn out to be male-sterile. On the other hand, this fact cannot have accounted for the high percentage of sterile sons of males treated with radiation or TEM, since any X-autosome translocations induced would have been transmitted to daughters only.

Scattered pieces of evidence give indications that multiple complex translocations may occasionally interfere with formation of normal spermatozoa. In animals heterozygous for two independently produced translocations, two associations-of-four are formed if the two translocations involve four separate chromosomes; one, more complicated, association-of-six, if one chromosome is involved twice (once in each translocation); and a very complex figure-of-four, if both translocations involve the same two chromosomes, each at two breakpoints (Slizynski, 1957a). Males representing 39 different combinations of the first type all had sperm; but of 16 combinations of the second type and 2 of the third type one each lacked

sperm heads in testis squashes. These two combinations were checked for fertility and found to be male-sterile, as expected, but female-fertile (Carter et al., 1956). Auerbach and Slizynski (1956a) also report the finding of a complex interchange in a completely sterile son of an irradiated male. It is, however, not known whether sterility was, in fact, associated with failure to form normal spermatozoa.

There is thus a suggestion that special types of translocations can lead to sterility. It is noteworthy that in both groups of cases—X-linked translocations and complex double heterozygotes—the males only are sterile. In the case of the X-linked translocations, there could, perhaps, be some upset in the timing of synapsis or subsequent events. The X-Y bivalent normally does not behave synchronously with the autosomal bivalents; and the translocated chromosome, in having affinity both to the Y and to an autosome, may, perhaps, upset various relations of meiosis. The reason why the sterility found for some complex double heterozygotes—if, indeed, the cases cited above were not due to chance—should afflict males only is not known. Carter et al. (1956) point out the parallelism with the t^n/t^x compounds, many of which are male sterile. The *t*-locus, as already discussed (section III, A, 2) is presumed to be associated with a rearrangement; and, in the case of t^n/t^x, each of the homologous chromosomes would carry a different rearrangement, as was the case in one of the male-sterile translocation heterozygotes discussed above.

While there is a suggestion that special types of translocations could lead to sterility, there is also evidence for the view that the great bulk of sterile sons of treated males do not carry translocations. Cattanach (1959) analyzed 16 such sons sired following TEM injection. Six had spermatogenesis arrested prior to meiotic metaphase and could, therefore, not be cytologically analyzed by his method. Among the remaining 10, however, he found only one to be a translocation heterozygote. The other nine gave no evidence of translocation at meiotic metaphase. Sterility, from unknown causes, was apparently associated with the frequent absence or abnormality of spermatozoa. Cattanach stresses the close parallelism in the frequencies of sterility and semi-sterility induced by TEM in sons of treated males and suggests that the mechanism responsible for the two effects may be similar—namely, breakage and reunion—but, in the case of sterility, resulting in deficiencies for genes required for some stage of spermatogenesis.

On the basis of both the x-ray and TEM evidence, it may be tentatively concluded that complete sterility in F_1 of treated males, while very probably due to chromosomal aberrations, only infrequently results from reciprocal translocations.

c. Fate of Unbalanced Gametes. The question of the manner in which

unbalanced gametes fail to produce viable progeny was already examined by the early investigators in this field (W. L. Russell, 1954). In general, it was found that zygotes formed from the unbalanced gametes produced embryos that died usually at or soon after the time of implantation; although in occasional lines death occurred much later, even up to shortly before birth, and was often due to failure of the neural groove to close. More recently, Carter et al. (1955), in a study of ten translocation lines, found that in all but two of them death occurred shortly after implantation. In the remaining two lines, about 10 to 14 per cent of the deficiency in living embryos was the result of either death prior to implantation or failure of fertilization.

Koller (1944) had suggested the possibility that severely unbalanced gametes might die prior to fertilization. If this were so in the case of sperm, normal gametes could take their place, with the result that the "fertility" could be greater than 50 per cent. In relatively crude experiments, Koller found no evidence of reduced sperm motility in translocation lines. If genetic imbalance of a spermatozoon, instead of affecting its chances of penetrating an oocyte, were instead to interfere with its ability to *complete* syngamy, the net result on "fertility" would be the same as if the mechanism were postfertilization (embryonic) death. However, it has been shown in at least one case (Snell, 1946) that it is the genotype of the zygote, rather than that of the gamete producing it that determines survival. Proof for this was the finding that two unbalanced but complementary gametes could form a viable zygote.

3. *The induction of translocations.* Spontaneous translocations in experimental mammals are apparently quite rare. One reported in the rat and two in the mouse have been reviewed by W. L. Russell (1954). Auerbach and Slizynski (1956a) found none in 248 unirradiated mice examined cytologically. Because of the rarity of spontaneous cases, all investigations on the properties of translocations (preceding sections) as well as investigations in which translocations are used as tools (subsequent section) have utilized induced translocations. Such investigations, therefore, frequently contain incidental information on induction of translocations. Other investigations were carried out specifically to study the effect of the agent used on the induction of translocations in various types of germ cells.

a. Methods of Induction. The bulk of the pilot studies of Snell and Hertwig had been carried out with acute x-rays. In a summary tabulation of their data, as well as some of his own, W. L. Russell (1954) found reasonably good agreement between the different results and concluded that the rate of induced translocations for the mouse was much higher than that for *Drosophila*. Thus, the yield from a dose of about 700 r. in the mouse was comparable to that from about 4,000 r. in *Drosophila*, being

between 25 and 30 per cent. Carter et al. (1955), who produced translocations for cytogenetic studies, found 8 cases of heritable semisterility among 45 sons sired during the early presterile period by males exposed to 700 r acute x-rays. Two "quasi-sterile" males may also have been translocation bearers. In any case, the frequency of induced translocation in that experiment was not dissimilar to that obtained by earlier workers. The induced translocation rate in mature sperm obtained with 400 to 600 r by Auerbach and Slizynski (1956a) is also in keeping with earlier results. This experiment will be discussed in more detail below (section III, B, 3b).

Three gaps in our knowledge of induced translocations already noted in W. L. Russell's review (1954) have not yet been filled. First, there have been no new data which would enable one to construct a good dose curve for translocation induction by acute x-rays. Second, no experiments on neutron induction of translocations have been carried out since the original one which indicated, but did not prove, that neutrons in mice (as is the case in other organisms) are more effective than x-rays in inducing translocations. Third, no conclusions can yet be drawn concerning the effect of dose rate on translocation induction: even though details of Charles' experiment have now been published (Charles, Tihen, Otis and Grobman, 1961), the reasons why these data are unsuitable for answering the question are still the same as discussed in W. L. Russell's review.

Two chemicals have been used in the study of induced translocations, namely, nitrogen mustard (Falconer et al., 1952) and TEM (Cattanach, 1957, 1959). The latter has been found much more effective than the former in this respect. Following nitrogen mustard treatment, 29 sons conceived in the presterile period were tested for fertility. Of these, one was completely sterile but showed no chromosome abnormalities or increase in abnormal sperm; a second was of moderately reduced fertility but was not further tested; and a third was semisterile and was found to carry two translocations, one between two autosomes and the other between the Y-chromosome and an autosome. This last case will be discussed below (section III, B, 5). Of 42 tested sons conceived in the poststerile period, one was completely sterile, with no normal germ-cell stages after diakinesis; one was semisterile but not analyzed; and two were of moderately reduced fertility, also not analyzed. In contrast to nitrogen mustard, TEM produces high frequencies of translocation at doses which are not prohibitively toxic to the treated males. Thus, among 21 F_1 offspring (10 males and 11 females) of early matings of males injected with either 0.8 or 0.64 mg./Kg. of TEM, 6 or 7 translocations were found (Cattanach, 1957). Following a dose of 0.2 mg./Kg., the percentage of translocations among 111 tested sons of early-period matings was 4.5 per cent (Cattanach, 1959).

One interesting finding is that, relative to semisteriles, TEM produces

many more steriles than is the case with x-rays. Thus, the higher doses of TEM were approximately equivalent to 700 r of acute x-rays with respect to translocation induction; but while 700 r of x-rays produces 10 per cent complete steriles in F_1, the TEM treatment gave 19 per cent. Again, the lower dose of TEM (Cattanach, 1959) produced approximately equal numbers of steriles and semisteriles; while, with x-rays, the frequency of steriles is less than half that of semisteriles. The nature of the complete sterility has already been discussed (section III, B, 2b).

b. Effect of Gametogenic Stage. It has already been shown (section I, C, 4) that induced dominant-lethal frequency is dependent on the stage in gametogenesis treated with the mutagen. Since translocations, like dominant lethals, are the result of chromosome breakage, it is not surprising that they should follow a similar relation to stage. Auerbach and Slizynski (1956a) produced translocations in an effort to determine the post-meiotic sensitivity pattern of the mouse for purposes of comparison with *Drosophila*. Translocations were scored cytologically, by checking all males that gave an average litter size of less than 9.0. The first four litters of irradiated males yielded 8 translocations among 60 tested sons; while litters subsequent to the fourth yielded 15 translocations among 51. The comparison between these two ratios gave a P value of slightly less than 0.05, and on this basis the authors concluded that in the mouse, as in *Drosophila*, spermatids are more sensitive to the induction of translocations than are spermatozoa. Bateman (1956a) criticized Auerbach and Slizynski for several procedures of classification and interpretation, all of which were effectively defended by these authors in their reply (Auerbach and Slizynski, 1956b). Among other things, they showed that Bateman's criticism of their identification of gametogenic stage irradiated was based on his faulty interpretation of the relative radiation sensitivities of the various gametogenic stages to lethal radiation effects. This was later admitted by Bateman himself (section I, C, 4). Auerbach and Slizynski, however, agree with Bateman that their data are relatively meager and should be regarded as only a pilot experiment.

Their conclusion on the high sensitivity of spermatids, based on x-ray results, was later confirmed with TEM by Cattanach (1959). In comparing offspring conceived 1 to 3 and 10 to 13 days, following injection of the male with 0.2 mg./Kg. of TEM, Cattanach found 4.5 and 14.0 per cent translocations, respectively; i.e., 3.3 times as many in the later period, which represents treated spermatids. Littersize reduction (dominant lethals) goes up in a similar fashion, and so does the frequency of completely sterile animals, which have already been discussed (section III, B, 2b). Cattanach's (1959) conclusion that the spermatid/spermatozoa ratio for the induction of translocations is higher with TEM treatment than

with x-rays (Auerbach and Slizynski, 1956a), must be considered doubtful in view of the relatively small volume of x-ray data.

The early experiments, particularly those of Hertwig, had shown that partially sterile animals were not found among the offspring of post-sterile period matings; and it is sometimes assumed that translocations, if induced in spermatogonia, will be eliminated as a result of germinal selection. However, W. L. Russell (1954) pointed out that elimination at meiosis seems unlikely in view of the fact that translocations, once induced, are easily transmitted through descendant generations. He concluded, therefore, that Hertwig's data indicated lack of occurrence of translocations in spermatogonia as a result of failure of the process of segmental interchange (since there is no reason for assuming failure of chromosome breakage). Griffen (1958) reports partially sterile offspring born up to ten months after irradiation and concludes that translocations were induced in spermatogonia "and even prespermatogonial stages" (it is not clear what the author means by these latter). Unfortunately, a line derived from only one of the partially sterile offspring was examined cytologically. The possibility is, therefore, not excluded that all, or almost all, of the partial sterility found in the experiment could have been due to the same translocation, namely, one that was already present in the stock. Although the data are not presented in a way which allows the calculation of percentages of partially steriles born at various intervals after irradiation, it would appear that the frequency for at least part of the interval during which irradiated spermatogonia are being utilized is so high that—were it a universal phenomenon—it should not have escaped detection in the experiments of other investigators. Incidental information obtained in the course of testing specific locus mutants induced in irradiated spermatogonia (Russell, unpublished) excludes a frequency of partial sterility of the magnitude indicated by Griffen's data.

Early data indicated that the frequency of translocations induced in females was probably lower than in males (W. L. Russell, 1954). More recently, L. B. Russell and Wickham (1957) have studied translocation induction in primary oocytes at various intervals prior to ovulation. Fertility tests were carried out on 320 males conceived 1½ to 34½ days after irradiation of their mothers with 400 r acute x-rays, as well as on 80 contemporary sons of nonirradiated mothers. Only one typical case of inherited partial sterility (i.e., 0.3 per cent) was found in the former group; an additional 1.3 per cent of this group of males had slightly but consistently reduced litter sizes, a condition which was probably heritable in one case and may have constituted a second example of translocation. Complete sterility also was found only among sons of irradiated females, the frequency being 1.9 per cent. Of the six sterile males, three had com-

pletely normal spermatogenesis, two had normal spermatogenesis in most tubules with only a few tubules being atrophic, and only one showed definite disturbances, with spermiogenesis stopping at various spermatid stages (Oakberg, personal communication). Another type of fertility disturbance found only in the irradiated group (0.9 per cent) was infrequency of litters resulting from a high percentage of the matings being infertile. On the other hand, infrequency of litters resulting from infrequent matings occurred with equal incidence in irradiated and control groups. Altogether, summing all categories of disturbed fertility, and including questionable as well as certain cases, the incidences were 8.4 and 2.5 per cent, respectively, in irradiated and control groups, a difference which, while suggestive, is not statistically significant. Certainly, the frequency of induced heritable partial sterility—which, even by including all doubtful cases, comes to only about 2 per cent—is exceedingly low in comparison with the frequencies induced in spermatozoa, where for a dose of 400 r Hertwig found 16.4 per cent, and for a dose of 500 r both Hertwig and W. L. Russell, independently, obtained 25 per cent partially sterile offspring (see W. L. Russell, 1954). This low frequency in females could be due partly to the circumstance that irradiation is prereductional, and that, therefore, a translocation resulting from chromosome breakage has only one chance in four of coming to be present in the egg in euploid condition; one resulting from chromatid breakage only one chance in sixteen. This is in contrast with induction in spermatozoa from which no loss due to segregation can occur. If the low induced translocation frequency in females is due only to the circumstance that irradiation is prereductional, then an equally low frequency should be found from irradiated primary spermatocytes. Unfortunately, no conclusive data on this point are available, although Auerbach's and Slizynski's (1956a) results gave some indication of a slight drop in translocation frequency in mice conceived near the end of the presterile period. An additional reason for low frequency of translocation-induction in females might be the peculiar dictyate state of the mouse oocyte nucleus, a state which persists from shortly after birth until a few hours prior to ovulation and which may not be conducive to exchange of genetic material, although it evidently does not prevent the induction of chromosome breakage (see dominant lethals, section I, C, 5).

4. The uses of translocations as tools in cytogenetic and other investigations. One of the most fertile fields of research on mammalian translocations in recent years has been their use in cytogenetic studies. The aim of most of these has been the establishment of independence of linkage group and/or correlation of the genetically established linkage groups with the cytological maps of pachytene chromosomes.

a. Cytological Procedures. The early cytological work of Koller (1944)

used meiotic metaphase and early anaphase to correlate cytological behavior with "fertility" of translocation heterozygotes (section III, B, 2a). Metaphase and late prophase (diakinesis and late diplotene) are still being used widely for cytological confirmation of the existence of heterozygous translocations inferred by breeding experiments, and/or to obtain some idea of the lengths of chromosomes involved and of the pieces exchanged. The procedure has been used by Slizynski (Falconer et al., 1952; Auerbach and Slizynski, 1956a; Slizynski, 1957a), Cattanach (1957, 1959, 1961b), and Jaffe (1952). An improved rapid technique has recently been reported by Welshons, Gibson, and Scandlyn (1962). The usual procedure for cytological search for translocations is a count of elements, the finding of twenty separate symmetrical bivalents (including a heteromorphic sex bivalent) in each of several division figures being taken as evidence for the probable absence of translocation (see, however, below); while the presence of only nineteen separate elements—one of these being a ring or chain of four—indicates that the animal is heterozygous for a translocation. It should be noted that translocations involving exchange of only small portions of chromosome can be missed by this method. Thus, Cattanach (1961b) in the study of a sex-linked translocation (section III, B, 5), regularly counted 20 bivalents; and Auerbach and Slizynski (1956a) report one male which showed the diagnostic reduction in elements in only one out of six metaphase plates.

For more detailed cytogenetic studies, mapping of chromomeres at pachytene has been carried out by Slizynski (Falconer et al., 1952; Slizynski, 1952, 1954, 1957 a and b) and by Griffen (1958). For other, special, purposes translocations have also been observed in diploid somatic cells (Ford, Hamerton, Barnes and Loutit, 1956; L. B. Russell and Bangham, 1961).

b. Genetic Localization of Breaks. The prerequisite for cytogenetic work with translocations is the genetic localization of at least one of the translocation break-points in a known linkage group. Pioneer work along these lines was done by Snell (1946) who found four linkage groups associated with three of his translocations. Subsequently, extensive work was done by Carter and co-workers (1955, 1956), who managed to tag eleven translocations with genes having visible phenotypes. Snell's procedure was to cross animals from known semisterile stocks to other stocks carrying a variety of genetic markers; to back-cross the offspring to the same marked stocks; and, finally, to test the progeny of this backcross for semi-sterility, with a view to detecting any association between semi-sterility and the given markers. By Carter's procedure, similar in basic principle, search for semi-sterility following irradiation was made in the progeny of a cross designed to be heterozygous for a large number of genes.

The general procedure will become clear from Figure 2. The pachytene cross shown is for an animal heterozygous both for the translocation and for genetic markers. In Snell's case, this would be an offspring of the cross between the translocation stock and the marker stock; in Carter's case, an offspring of the cross between the irradiated and nonirradiated marker stocks. The genes *R* and *r* may for the moment be ignored. The gene *q* on the standard chromosome has been contributed by the nonirradiated marker stock. If the translocation break is close to the *q*-locus, then the allele *Q* will segregate in almost all cases with the translocation. Assuming that gametes I A and I B are the only ones to produce viable progeny (as will be the case with most translocations), then, on back-crossing to the marker stock, which again contributes *q*, the semi-sterile progeny (derived from gamete type I A) will be of the *Q*/*q* genotype, while the fertile progeny (derived from gamete type I B) will be of the *q*/*q* genotype. The markers are, of course, picked in such a way that the *Q*/*q* and *q*/*q* genotypes are easily distinguishable phenotypically. If any crossing-over occurs between the translocation break and the *q*-locus, some of the progeny will be semi-sterile and *q*/*q;* or fertile and *Q*/*q*. The percentage of this crossover progeny in the total has been used to establish the distance between the translocation break and the *q*-locus on the linkage map. This procedure may have certain pitfalls since the presence of the translocation may affect frequency of crossing-over in that region of the chromosome (see below, in this section); but, in general, it will locate the break at at least an approximately correct distance on either side of the *q*-locus.

By measuring crossing-over between a translocation break and two or more loci on the same chromosome, the break can be located not only at a certain distance from a given gene, but also on a given side. By such procedures, Snell (1946) managed to locate the translocation break of his *T*(*5;8*)*a* translocation in linkage-group VIII on the opposite side of *m* from *b*, and the break of *T*(*1;?*)*c* in linkage-group I between *p* and *sh-1*. Carter and co-workers (1955, 1956) located 18 translocation breaks from 11 translocations according to linkage group, with 9 linkage groups being involved. Of the 18 breaks, 15 were located in more detail with respect to two or more genes in these linkage groups; and one of the three remaining ones was later located in this manner by Lyon and Phillips (1959).

As will be seen from Figure 2, if both chromosomes involved in a translocation are marked with genes fairly closely linked to the breaks (*Q* and *r*), not only will each gene show linkage with semi-sterility, but the two genes involved will show linkage with each other. Thus, the genes *Q* and *r* and the genes *R* and *q* will tend to segregate with each other (gametes I A and B), unless crossing-over occurs between the breakage point and the gene in question; and those combinations which would normally represent

random assortment, namely, *Q* with *R* and *q* with *r* will be contained in unbalanced gametes only (gametes III A and B). It may be noted that it was in this manner that Snell brought his original genetic proof of the translocation theory for heritable semi-sterility in mice.

The ultimate accuracy of mapping of translocation breaks depends on the extent to which the presence of a translocation interferes with crossing over. Snell (1946) found slight depressions in crossing-over in almost all marked chromosome segments in the vicinity of the breaks. Cattanach (1961b) found complete absence of crossing-over in a piece of linkage-group I translocated to the X-chromosome. His tentative explanation is that the translocated piece is so small that no appreciable pairing between it and the intact linkage-group I takes place and that there is, therefore, no opportunity for crossing-over. In contrast to these findings of reduced crossing-over is the report by Lyon and Phillips (1959) that the translocation *T138* enhances crossing-over in the *T-tf* interval, probably by altering chiasma localization in that region.

c. Tests for Independence of Linkage Groups. Using the tagged translocation stocks, a number of interesting investigations have been carried out. One of these is the test of independence of genetically established linkage groups. As Carter et al. (1955) point out, the lengths of most linkage groups known to date have not reached the 75 to 125 cM range estimated for the mouse; and it is, therefore, possible that two or more groups now considered separate may in reality represent different parts of a single group. The procedure used was to produce animals heterozygous for two different translocations, each of the translocations having at least one linkage group marked. Using twelve marked translocations (namely, Carter's 11 plus Snell's *T(5;8)a*), 57 of 66 possible combinations were cytologically examined by Slizynski (1957a). Three possible situations may be considered: (a) the two translocations may involve four different chromosomes, e.g., one may involve chromosomes 1 and 2, the other chromosomes 3 and 4 (designated *T(1;2)* and *T(3;4)*, respectively); (b) the two translocations may involve three chromosomes, one being *T(1;2)*, and the other, say, *T(2;3)*, with the break in the common chromosome 2 being in a different location for *T(1;2)* and *T(2;3)*; (c) the two translocations may involve only two chromosomes, both being *T(1;2)*, but the break-points in the two cases being in different locations. In situation (a), the metaphase plate will show 18 elements, two of them being large and asymmetric; in situation (b), there will also be 18 elements, but only one will be very large and asymmetric; and in situation (c), there will be 19 elements, one of them being of a complex nature. Slizynski's studies of the 57 double heterozygotes confirmed cytologically that linkage groups considered tentatively independent at the start of the experiment were, in fact, inde-

pendent, i.e., the cytological situations (a), (b) and (c) were found in accordance with expectations made on the basis of linkage groups known to be involved in the various double heterozygotes. In this manner, it could be established that linkage-groups I, II, III, V, VII, VIII, IX, XI, XIII and XIX of the mouse were all located in different chromosomes.

d. Correlation of Genetic and Cytological Maps. Another use made of genetically tagged translocations has been the correlation of the cytological with the genetic map. For this analysis, Slizynski (1957a) used the chromomeric patterns of pachytene chromosomes established by him earlier (Slizynski, 1949). The typical cross-figure found in translocation heterozygotes at pachytene, can be analyzed and the two chromosomes involved cytologically identified. If the two linkage groups involved in the translocation are also known, it can, in this manner, be established that either of the linkage groups can be associated with either of the identified chromosomes. The final step in matching comes from the analysis of a second translocation heterozygote in which one of the linkage groups involved is the same as one of the linkage groups in the first. Thus, it might be shown that a translocation of linkage-groups II and IX involves chromosomes 9 and 16, while a translocation of linkage-groups IX and XIII involves chromosomes 16 and 19. From this it can then be concluded that linkage-groups II, IX and XIII are, respectively, situated in chromosomes 9, 16 and 19. By this method, Slizynski localized seven linkage groups of the mouse, and partly localized two others. Using these correlations, Griffen (1958) concluded, on cytological grounds, that a translocation discovered by him, but not tested genetically, involved linkage-groups III and XI. The correlation of cytological and genetic maps has already found one important application. It had been concluded by Wright (1947) that linkage-group VII of the mouse was partially sex-linked. There were several reasons for making this conclusion doubtful, but the final evidence against it came from the finding that a translocation which involves the linkage group in question was between two autosomes, nos. 11 and 13 in the pachytene analysis. At diakinesis and metaphase, also, the translocation was shown to be independent of the XY-bivalent (Carter, Lyon and Phillips, 1954; Slizynshi, 1954).

A few cases are now known of translocations sufficiently unequal to be recognizable in somatic metaphase and involving known linkage groups (L. B. Russell and Bangham, 1961; Ford et al., 1956). These will be useful in correlating the genetic map with the mitotic karyotype.

One final cytogenetic analysis that has been carried out is the tentative localization of genes on the pachytene-chromosome map (Slizynski, 1957a). In general, this method can be applied to genes which have been genetically located between two translocation breaks. For example, in a given linkage group containing markers *a*, *b*, and *c*, if translocation-breaks

T1 and *T2* have been genetically located between *a* and *b* and between *b* and *c*, respectively, then it can be assumed that the *b* locus lies between *T1* and *T2*. The translocation breaks are then located cytologically from the cross-figure at pachytene; and the position of *b* is deduced accordingly. For example, if the break *T1* is within the pachytene-division 57 and the break *T2* in pachytene-division 59, then the *b* locus may be assumed to lie either in division 58, or in the distal or proximal ends, respectively, of divisions 57 or 59. Slizynski also drew a number of conclusions concerning the relation of certain loci to the centromeres. However, there are reasons to doubt the position of the centromere as mapped by Slizynski at pachytene. Carter et al. (1956) point out that once the centromeres have been firmly located at this stage, it will be possible to establish proximal and distal ends of linkage groups for cases where there are two or more cytologically separable and genetically located breaks in the same chromosome.

e. Other Uses of Translocations. While the main uses of translocations as tools have been in the measurement of induced chromosome breakage (section III, B, 3) and cytogenetic studies (section III, B, 4 a–d), translocations have also been utilized in a few other miscellaneous studies. Thus, Ford et al. (1956) used the unequal translocation *T6* as a cytological cell marker to show that the protective effect of injected spleen against lethal radiation doses was due to cellular repopulation by the injected material. Another translocation was used by Lyon (1954) as a means of reducing littersize in a study made of the effect of littersize on gene penetrance. Undoubtedly, with the greater availability of translocation lines and the improved cytological methods developed in recent years, the miscellaneous uses of translocations will increase greatly in the future.

5. *Position effect; sex-linked translocations.* Since translocations involve rearrangements of portions of chromosomes, they obviously entail changes from the original order of genes. It is a well known phenomenon in *Drosophila* that the action of specific genes may be influenced by their position, and this has been referred to as position-effect. One of the main types of these is the variegated, or V-type, position effect, whereby the action of a rearranged gene is made uncertain. Such a gene fails to function in a certain proportion of somatic cells, leading, if the effect is a visible one, to a variegated, or mottled, phenotype.

For two reasons it had until recently been assumed that position effects in experimental mammals were rare or absent. First, before 1959 there had been no report of V-type position effects for any of the translocations studied in the mouse, even though, in the case of some of the tagged translocations, the breaks had been close to genes with easily visible effects; and even though a considerable number of translocations have now been produced in animals heterozygous for seven visible markers. (W. L. Rus-

sell, unpublished). A second reason for the tentative conclusion that position effects were rare or nonexistent in the mouse was the fact that the bulk of translocation homozygotes that had been produced were viable. Thus, Hertwig reported two or three; Snell, two (one of which gave a suggestion of reduced viability); and Carter and co-workers (1955, 1956) reported six viable and only one lethal type of translocation homozygote. All of the viable homozygotes were fully fertile and all of their progeny semi-sterile. Since position effects can inactivate genes concerned with cell survival and, since, in *Drosophila*, position effects are generally enhanced by homozygosis, the viability of translocation homozygotes in the mouse appeared to argue that position effect was less important than it is in *Drosophila* (W. L. Russell, 1954).

In 1959, L. B. Russell and Bangham reported 2 cases of V-type position effect in the mouse. Both resulted from sex-linked translocations. Since then, the number has risen to 6: an additional one reported by L. B. Russell and Bangham (1961); one reported by Cattanach (1961b); and two found at this laboratory but not yet described in detail (L. B. Russell, Bangham and Saylors, 1962). All 5 of these position-effect cases involve X-autosome translocations. It thus appears possible that only the X has the power to produce such effects in the mouse; and it is even conceivable that this power is restricted to a specific region of the X (L. B. Russell and Bangham, 1961; L. B. Russell et al., 1962). It may be that there is very little heterochromatin on the autosomes, while the X is strongly heterochromatic (L. B. Russell, 1961).

Russell and Bangham's findings (L. B. Russell and Bangham, 1959, 1960, 1961; L. B. Russell, 1961) will be summarized first and then compared with the case of Cattanach's (1961a and b), which shows basic similarities but also some important differences. Russell and Bangham have described variegation at three loci, namely *b* (=*brown*) in linkage-group VIII, *c* (= *albino*) in linkage-group I, and *p* (= *pink-eyed*) also in linkage-group I. The results of the basic cross, which are essentially similar in all cases, are outlined in Figure 3 (where the symbol ® denotes Russell and Bangham's findings). They may be summarized as follows: (a) when the translocated chromosome carries the wild-type allele, and the standard chromosome the recessive marker (line 1 of Fig. 3), the animal is variegated for the marker if female, but wild-type if male (i.e., in males, the wild-type allele behaves fully dominant, as if it were in its normal position); (b) while female translocation bearers show the typical partial sterility, male translocation bearers are completely sterile, as a result of degeneration of primary spermatocytes in pachytene (line 1 of Fig. 3); (c) crossing-over between the variegating locus and break point has been observed and produces the types shown in the right-hand portion of the diagram; (d) there is no evidence for the survival of offspring formed from unbalanced

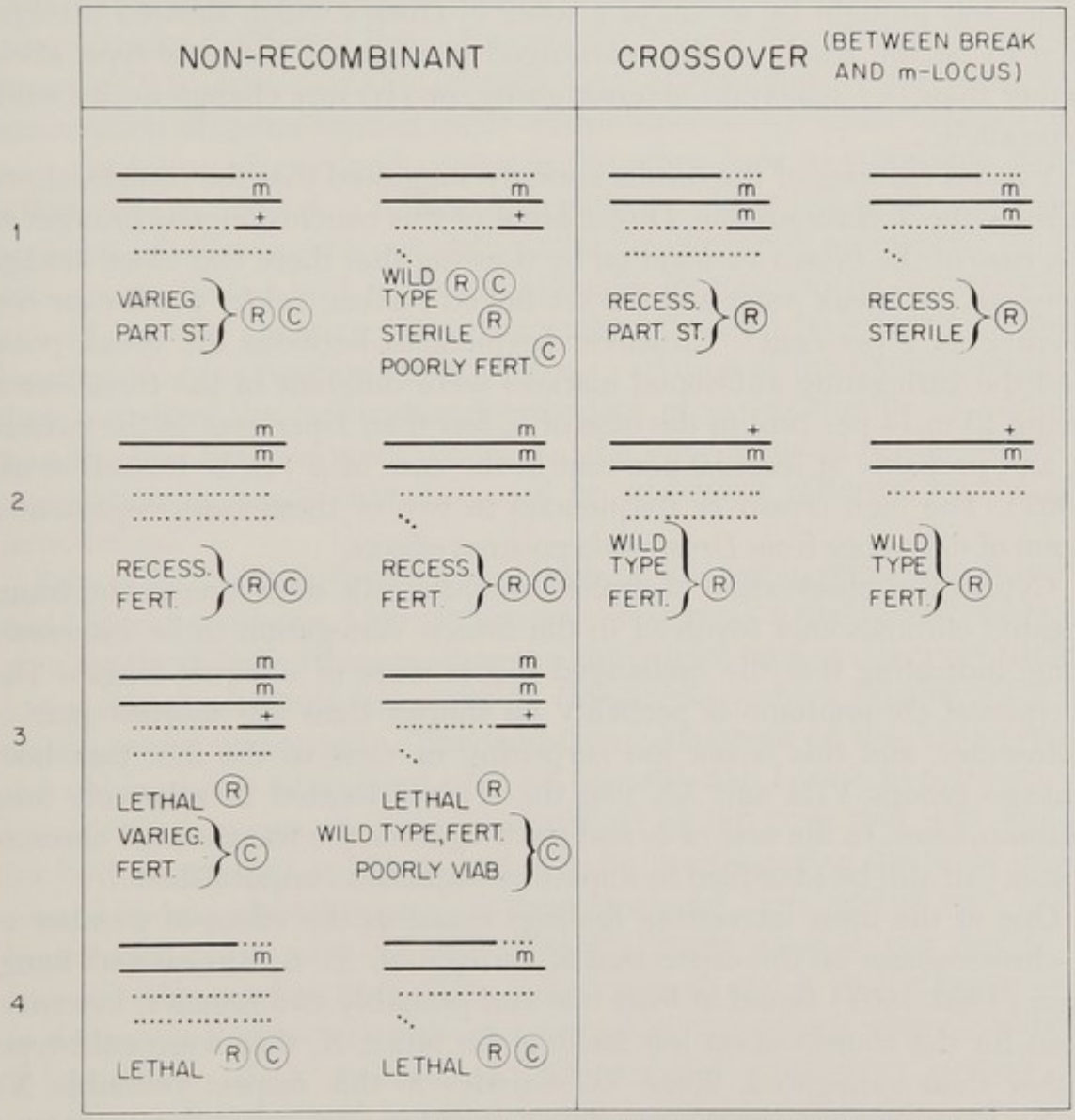


FIG. 3—Presumed chromosomal basis for variegated-type position effect in the mouse (see text, section III, B, 5). The position effect is thought to result from X-autosome translocation. The wild-type allele, when transposed to the X, does not act in all cells of the body, thus allowing the recessive marker (on the intact autosome) to express itself in scattered regions of the body (= variegation). This effect occurs only when two X's are present.

..... = X chromosome.

˙ ˙ . = Y chromosome.

——— = autosome.

m = recessive marker.

Ⓡ = results of L. B. Russell and Bangham on three independent radiation-induced X-autosome translocations (Russell and Bangham, 1950, 1960, 1961; L. B. Russell, 1961).

Ⓒ = results of Cattanach on a TEM-induced translocation (Cattanach, 1961b).

gametes (lines 3 and 4 of Fig. 3), the littersize of the variegated females being no greater than half of normal, and the percentage of translocation-bearing animals being no higher than 50 per cent of all viable animals. In the case of the *brown* variegation, the classic proof of V-type position effect was brought by means of a series of crosses which showed variegation to be the result of the rearranged position of the wild-type allele rather than (a) structural heterozygosity, or (b) any change in the wild-type allele.

Various features of the results strongly suggested that the translocations involve the X-chromosome. Direct proof of this conclusion was brought in the case of the *brown* variegation by showing that there was close linkage between the break point and the sex-linked marker *Tabby* (crossover frequency = 7 per cent). Crossover frequencies between the break point and the variegating autosomal markers were different in the three cases, being 13 to 14 per cent in the case of *b*, less than 1 per cent in the case of *c*, and probably at least 10 per cent in the case of *p* (L. B. Russell et al., 1962). The high crossover frequencies in two of these cases represent a point of difference from *Drosophila* position effects.

Cytological observation of diploid somatic cells show one of the translocated chromosomes involved in the *brown* variegation to be extremely long, indicating that the exchanged pieces were of unequal length. The reciprocal chromosome is probably no shorter than the shortest pair of autosomes, and this is not too surprising in view of the fact that both linkage groups VIII and XX (on the X) are located in relatively long chromosomes. In the case of *c*- and *p*-variegation, the translocated chromosomes can also be identified in somatic metaphases (unpublished).

One of the most interesting findings concerns the effect of number of X-chromosomes on the expression of variegation. L. B. Russell and Bangham (1960, 1961) found at least one and probably two females, heterozygous for the translocation but lacking the intact X, that were wild-type, rather than variegated. Since XO females in this respect resemble XY males heterozygous for the translocation, while XX translocation heterozygotes were invariably variegated, these authors suggested that, in the mouse, two X-chromosomes are required to produce variegation. Cattanach (1961b) also found an XO wild-type translocation heterozygote which occurred spontaneously, and, following this, deliberately produced an animal of this genotype and found it also to be wild-type. In addition, Cattanach (1961a and b) had also found two translocation heterozygous males which were XXY and were variegated. This finding greatly strengthened the hypothesis.

By relating these findings to certain cytological observations in other mammals, L. B. Russell (1961) developed the hypothesis that, in mam-

mals, genic balance requires the action of one X in a manner which precludes realization of its heterochromatic potentialities, so that only *additional* X's present assume the properties characteristic of heterochromatin. Lyon (1961) has developed a hypothesis which starts with Russell's basic assumption—namely, that only one X is euchromatically active—but then proceeds along different lines of emphasis. Thus, while Russell states that the other X is instrumental in causing the position-effect (variable somatic inactivation of genes located on or translocated to X), Lyon believes that no gene action of any kind occurs in the other X or in autosomal pieces attached to it. According to Lyon's hypothesis, either one or the other X is active at random up to a certain point in development, accounting for the variegation caused both by genes normally located on the X (sex-linked genes), and by genes secondarily attached to the X as a result of translocation (V-type position effect). Lyon's variation of the basic assumption does not affect the explanation for the lack of variegation in XY and XO translocation heterozygotes. However, the interpretation of the causes of variegation in two-X animals would be somewhat different by the two hypotheses.

Two of Cattanach's findings are of special interest in relation to Russell's and Lyon's hypotheses. One of them is the observation that females *homozygous* for the X-linked transposition are either wild-type or only very slightly variegated, in contrast to results in *Drosophila* (where homozygotes are usually *more* variegated). The wild-type phenotype of the homozygotes can obviously be interpreted both on Russell's and Lyon's hypotheses. Alternatively, however, it could be explained on the basis that it is unlikely for both rearranged wild-type genes to be inactivated in the same cell. A second finding of Cattanach's, on the other hand, is in conflict with Lyon's hypothesis, but in keeping with Russell's. Variegation in the case of his translocation involves both of the linked genes *c* and *p*, with *c* being closer to the break. Preliminary indications are that, in analogy with *Drosophila*, a "spreading effect" is obtained: light-colored patches in animals that carry recessives for both *c* and *p* on the standard chromosome, are either of the *c* phenotype or of the *c p* phenotype on a wild-type background. If this indication can be further substantiated, it would disprove Lyon's hypothesis, which allows only for wild-type or *c p* areas, but not for *c* areas. It would, however, be in keeping with Russell's hypothesis, according to which the position effect is similar to that in *Drosophila*, provided a second X is present.

In the remaining discussion, Cattanach's case of V-type position effect, which has already been mentioned in connection with a variety of subjects, will be examined mainly from the point of view of the dissimilarities that it presents in comparison with the cases of Russell and Bangham. As al-

ready noted, there is similarity in the basic facts that the V-type position effect is due to an X-autosome translocation and that two X-chromosomes are required to produce variegation. The differences can probably all be explained in terms of *one* basic one: most of the results indicate that the chromosome portions involved in Cattanach's translocation are quite small. Fig. 3, where the symbol © denotes the behavior of Cattanach's translocation, and the symbol ® that of Russell and Bangham's three translocations, gives the comparison at a glance. The features in which Cattanach's case differs from the others may be summarized as follows.

(a) One type of unbalanced gamete produces viable progeny (line 3 of Fig. 3), females and males having 100 per cent and about 25 per cent normal viability, respectively. The viable unbalanced animals have a duplication for that part of the autosome which carries the markers, and probably a deficiency for a portion of the X-chromosome. Such a deficiency can apparently be perfectly well tolerated in females, where even the XO genotype is viable. (It seems that the presence of whole numbers of X's is not required for genic balance in mammals, as it is in *Drosophila;* and this, again, supports the assumption that only one X is active.) The X deficiency is apparently small enough to be at least sometimes tolerated in males also. It is, incidentally, the poor viability and small size of these males that leads Cattanach to conclude tentatively that the translocation is a reciprocal one: If a piece of X were *not* translocated to the autosome, the unbalanced translocation males (line 3) would not be deficient for X and should, by analogy with the females, be of equal viability with balanced translocation males (line 1). (An alternative, but unlikely, explanation is that the autosomal duplication has a much more deleterious effect on males than on females.) Balanced and unbalanced translocation females (lines 1 and 3, respectively, of Fig. 3) can be phenotypically distinguished by the use of the proper markers at the *c*-locus, where multiple alleles are available. (b) Balanced heterozygous males (line 1 of Fig. 3), in the case of Cattanach's translocation, are not completely sterile, although their fertility is very poor. Even those that produce no litters at all apparently do complete spermiogenesis. (c) No crossing-over occurs between the variegating loci and the break point. This has been explained in terms of lack of pairing between the transposed piece and the standard autosome (due to the smallness of the transposed pieces), for which there is cytological confirmation (section 111, B, 4a).

While all these lines of evidence point to the presence of a translocation involving very small pieces of the autosome and the X, it appears to the present writer from a study of the linkage groups involved that one would have to postulate a considerable length of the genetic map of linkage-group I to be involved in the translocation if it is a simple reciprocal one.

For this reason, other types of translocations between linkage-group I and the X should perhaps be considered.

Mention should be made of a Y-linked translocation briefly reported by Falconer et al. (1952). This translocation had two unusual features: it did not cause partial sterility; and it was no longer present in a grandson of the original male, even though such a translocation is expected to be passed in a straight male line except for possible cases of crossing-over between the break point and the male-determining region of the Y. The former feature suggests that unbalanced gametes may have produced viable zygotes, some of which may well have lacked a portion of the Y. Such a situation, as well as the possible crossovers mentioned above, would make this translocation a most valuable tool for establishing how much of the Y chromosome is male-determining. For this reason, it is to be hoped that more work will be forthcoming, either on this or on other Y-linked translocations.

References

Auerbach, C. 1957. Sensitivity of immature mouse sperm to the mutagenic effects of X-rays. Nature 179: 725.

——, and B. M. Slizynski. 1956a. Sensitivity of the mouse testis to the mutagenic action of X-rays. Nature 177: 376–377.

——, and ——. 1956b. Reply to A. J. Bateman's letter on "Sensitivity of the mouse testis to the mutagenic action of X-rays." Nature 177: 934–935.

Austin, C. R. 1960. Anomalies of fertilization leading to triploidy. J. Cell. Comp. Physiol. (Suppl. 1) 56: 1–15.

——, and A. W. H. Braden. 1953. An investigation of polyspermy in the rat and rabbit. Australia J. Biol. Sc. 6: 674–692.

——, and ——. 1954a. Induction and inhibition of the second polar division in the rat egg and subsequent fertilization. Australia J. Biol. Sc. 7: 195–210.

——, and ——. 1954b. Anomalies in rat, mouse, and rabbit eggs. Australia J. Biol. Sc. 7: 537–542.

Bateman, A. J. 1956a. Sensitivity of the mouse testis to the mutagenic action of X-rays. Nature 177: 934.

——. 1956b. Sensitivity of immature mouse sperm to the mutagenic effects of X-rays. Nature 178: 1278–1280.

——. 1957. Sensitivity of immature mouse sperm to the mutagenic effects of X-rays. Nature 179: 727.

——. 1958a. Mutagenic sensitivity of maturing germ cells in the male mouse. Heredity 12: 213–232.

——. 1958b. The two classes dominant lethal in the mouse. Proc. X Intern. Congr. Genetics 2: 14.

——. 1958c. The partition of dominant lethals in the mouse between unimplanted eggs and deciduomata. Heredity 12: 467–475.

——. 1960. The induction of dominant lethal mutations in rats and mice with triethylenemelamine (TEM). Genet. Res. 1: 381–392.

Beatty, R. A. 1957. Parthenogenesis and polyploidy in mammalian development. Monogr. in Experimental Biology, Cambridge 7: Univ. Press.

——, and M. Fischberg. 1952. Heteroploidy in mammals. III. Induction of tetraploidy in pre-implantation mouse eggs. J. Genet. 50: 471–479.

Bennett, D., and L. C. Dunn. 1960. A lethal mutant (t^{W18}) in the house mouse showing partial duplications. J. Exper. Zool. 143: 203–219.

Bock, M., and H. Jackson. 1957. The action of triethylenemelamine on the fertility of male rats. Brit. J. Pharmacol. 12: 1–7.

Braden, A. W. H. 1957. Variation between strains in the incidence of various abnormalities of egg maturation and fertilization in the mouse. J. Genet. 55: 476–486.

——. 1958a. Variation between strains of mice in phenomena associated with sperm penetration and fertilization. J. Genet. 56: 1–11.

——. 1958b. Strain differences in the incidence of polyspermia in rats after delayed mating. Fertil. & Steril. 9: 243–246.

——, and C. R. Austin. 1954. Fertilization of the mouse egg and the effect of delayed coitus and of hot-shock treatment. Australia J. Biol. Sc. 7: 552–565.

Carter, T. C., M. F. Lyon, and R. J. S. Phillips. 1954. Partial sex linkage in the mouse. Nature 174: 309.

——, ——, and ——. 1955. Gene-tagged translocations in eleven stocks of mice. J. Genet. 53: 154–166.

——, ——, and ——. 1956. Further genetic studies of eleven translocations in the mouse. J. Genet. 54: 462–473.

Cattanach, B. M. 1957. The induction of translocations in male mice by triethylenemelamine. Nature 180: 1364–1365.

——. 1959. The sensitivity of the mouse testis to the mutagenic action of triethylenemelamine. Ztschr. Vererb. 90: 1–6.

——. 1961a. XXY mice. Genet. Res. 2: 156–158.

——. 1961b. A chemically-induced variegated-type position effect in the mouse. Ztschr. Vererb. 92: 165–182.

——, and R. G. Edwards. 1958. The effects of triethylenemelamine on the fertility of male mice. Proc. Roy. Soc. Edinb. B, 67: 54–64.

Chang, M. C. 1950. Cleavage of unfertilized ova in immature ferrets. Anat. Rec. 108: 31–44.

Charles, D. R., J. A. Tihen, E. M. Otis, and A. B. Grobman. 1961. Genetic effects of chronic X-irradiation exposure in mice. Genetics 46: 5–8; and U. of Rochester Atomic Energy Project Report UR-565.

Dunn, L. C. 1956. Analysis of a complex gene in the house mouse. Cold Spring Harbor Symp. Quant. Biol. 21: 187–195.

——, and E. Caspari. 1945. A case of neighboring loci with similar effects. Genetics 30: 543–568.

——, and S. Gluecksohn-Schoenheimer. 1950. Repeated mutations in one area of a mouse chromosome. Proc. Nat. Acad. Sc. U.S. 36: 233–237.

——, and S. Gluecksohn-Waelsch. 1953. The failure of a t-allele (t^a) to suppress crossing over in the mouse. Genetics 38: 512–517.

Edwards, R. G. 1957a. The experimental induction of gynogenesis in the mouse. I. Irradiation of the sperm by X-rays. Proc. Roy. Soc. London S.B. 146: 469–487.

——. 1957b. The experimental induction of gynogenesis in the mouse. II. Ultra-violet irradiation of the sperm. Proc. Roy. Soc. London S.B. 146: 488–504.

——. 1958a. Colchicine-induced heteroploidy in the mouse. I. The induction of triploidy by treatment of the gametes. J. Exper. Zool. 137: 317–348.

——. 1958b. Colchicine-induced heteroploidy in the mouse. II. The induction of tetraploidy and other types of heteroploidy. J. Exper. Zool. 137: 349–361.

——. 1958c. The experimental induction of gynogenesis in the mouse. III. Treatment of sperm with trypaflavine, toluidine blue or nitrogen mustard. Proc. Roy. Soc. London S.B. 149: 117–129.

——. 1958d. The number of cells and cleavages in haploid, diploid, polyploid and other heteroploid mouse embryos at 3½ days gestation. J. Exper. Zool. 138: 189–207.

——. 1961. Induced heteroploidy in mice: effect of deacetylmethylcolchicine on eggs at fertilization. Exper. Cell Res. 24: 615–617.

——, and J. L. Sirlin. 1959. Identification of C^{14}-labelled male chromatin at fertilization in colchicine-treated mouse eggs. J. Exper. Zool. 140: 19–27.

Falconer, D. S., B. M. Slizynski, and C. Auerbach. 1952. Genetical effects of nitrogen mustard in the house mouse. J. Genet. 51: 81–88.

Ferguson-Smith, M. A. 1961. Chromosomes and human disease. Progr. in Med. Genet. 1: 292–335.

Fischberg, M., and R. A. Beatty. 1951. Spontaneous heteroploidy in mouse embryos up to mid-term. J. Exper. Zool. 118: 321–335.

——, and ——. 1952. Heteroploidy in mammals. II. Induction of triploidy in pre-implantation mouse eggs. J. Genet. 50: 455–470.

Ford, C. E., J. L. Hamerton, D. W. H. Barnes, and J. F. Loutit. 1956. Cytological identification of radiation-chimaeras. Nature 177: 452–454.

Gates, W. H. 1927. A case of non-disjunction in the mouse. Genetics 12: 295–306.

Griffen, A. B. 1958. Occurrence of chromosomal aberrations in prespermatocytic cells of irradiated male mice. Proc. Nat. Acad. Sc. U.S. 44: 691–694.

——. 1960. Mammalian pachytene chromosome mapping and somatic chromosome identification. J. Cell. Comp. Physiol. 56, Suppl. 1: 113–121.

Grüneberg, H. 1952. The Genetics of the Mouse, ed. 2. The Hague: Martinus Nijhoff.

Haldane, J. B. S. 1959. Parthenogenese. Naturw. Rundschau 12: 453–458.

Hancock, J. L. 1959. Polyspermy of pig ova. Animal Production 1: 103–106.

Hollander, W. F., and J. W. Gowen. 1955. Estimation of the induction rate for dominant lethal effects in mouse sperms by X-rays. Genetics 40: 575.

Jaffe, J. 1952. Cytological observations concerning inversion and translocation in the house mouse. Am. Nat. 86: 101–104.

Jude, A. C., and A. G. Searle. 1957. A fertile tortoiseshell tomcat. Nature 179: 1087–1088.

Kidwell, J. F., D. G. Nash, J. Stadler, and J. W. Gowen. 1961. An X-ray induced deficiency in linkage group VI of the mouse. Am. Zool. 1: 365.

Kindred, B. M. 1961. Abnormal inheritance of the sex-linked Tabby gene. Australia J. Biol. Sc. 14: 415–418.

Koller, P. S. 1944. Segmental interchange in mice. Genetics 29: 247–263.

Komai, T., and T. Ishihara. 1956. On the origin of the male tortoise shell cat. J. Heredity 47: 287–291.

Lea, D. E. 1947. Effects of radiation on germ cells: dominant lethals and hereditary partial sterility. Brit. J. Radiol. (Suppl. 1) 120–141.

Lyon, M. F. 1954. Stage of action of the litter-size effect on absence of otoliths in mice. Z. indukt. Abstamm. u. Vererbungslehre 86: 289–292.

——. 1960. Effect of X-rays on the mutation of t-alleles in the mouse. Heredity 14: 247–252.

——. 1961. Gene action in the X-chromosome of the mouse (Mus musculus L.) Nature 190: 372–373.

——, R. J. S. Phillips. 1959. Crossing-over in mice heterozygous for t-alleles. Heredity 13: 23–32.

McLaren, A. 1960. New evidence of unbalanced sex-chromosome constitutions in the mouse. Genet. Res. 1: 253–261.

Oakberg, E. F. 1955. Sensitivity and time of degeneration of spermatogenic cells irradiated in various stages of maturation in the mouse. Radiation Res. 2: 369–391.

——. 1956a. A description of spermiogenesis in the mouse and its use in analysis of the cycle of the seminiferous epithelium and germ cell renewal. Am. J. Anat. 99: 391–414.

——. 1956b. Duration of spermatogenesis in the mouse and timing of stages of the cycle of the seminiferous epithelium. Am. J. Anat. 99: 507–516.

——. 1957. Duration of spermatogenesis in the mouse. Nature 180: 1137–1139; also p. 1497.

——, and R. L. DiMinno. 1960. X-ray sensitivity of primary spermatocytes of the mouse. Intern. J. Radiation Biol. 2: 196–209.

Odor, D. L., and R. J. Blandau. 1956. Incidence of polyspermy in normal and delayed matings in rats of the Wistar strain. Fertil. & Steril. 7: 456–467.

Ohno, S., W. D. Kaplan, and R. Kinosita. 1959. Do XY- and O-sperm occur in Mus musculus? Exper. Cell Research 18: 382–384.

Painter, Th. S. 1927. The chromosome constitution of Gates' 'non-disjunction' (v-o) mice. Genetics 12: 379–393.

Pikó, L. 1958. Étude de la polyspermie chez le rat. Compt. rend. Soc. Biol. Paris 152: 1356–1357.

——, and O. Bomsel-Helmreich. 1960. Triploid rat embryos and other chromosomal deviants after colchicine treatment and polyspermy. Nature 186: 737–739.

Puttanna, C. R. 1961. The role of paracentric inversions in populations with reference to *Rattus norvegicus*. Excerpta Medica (Intern. Cong. Series No. 32), p. E9.

Russell, L. B. 1956. Dominant lethals induced at a highly sensitive stage in mouse oogenesis. Anat. Rec. 125: 647–648.

——. 1960. Blotchy. Mouse News Letter 23: 58.

——. 1961. Genetics of mammalian sex chromosomes. Science 133: 1795–1803.

——, and J. W. Bangham. 1959. Variegated-type position effects in the mouse. Genetics 44: 532.

——, and ——. 1960. Further analysis of variegated-type position effects from X-autosome translocations in the mouse. Genetics 45: 1008–1009.

——, and ——. 1961. Variegated-type position effects in the mouse. Genetics 46: 509–525.

——, ——, and C. L. Saylors. 1962. Delimitation of chromosomal regions involved in V-type position effects from X-autosome translocations in the mouse. Genetics(in press).

——, and E. H. Y. Chu. 1961. An XXY male in the mouse. Proc. Nat. Acad. Sc. U.S. 47: 571–575.

——, and M. H. Major. 1953. Dominant lethals in mouse oocytes induced by X-rays in air and in 5% oxygen. Genetics 38: 687–688.

——, and ——. 1956. Fertilization and the first cleavage following dominant lethal induction in female mice. USAEC Report ORNL-2060, pp. 27–28.

——, and W. L. Russell. 1954. Pathways of radiation effects in the mother and embryo. Cold Spring Harbor Symp. Quant. Biol. 19: 50–59.

——, and ——. 1956. The sensitivity of different stages in oogenesis to the radiation induction of dominant lethals and other changes in the mouse. In Mitchell, J. S., Holmes, B. E., and Smith, C. L., Eds.: Progress in Radiobiology, Edinburgh: Oliver and Boyd Ltd., pp. 187–192.

——, and ——. 1960. Genetic analysis of induced deletions and of spontaneous nondisjunction involving chromosome 2 of the mouse. J. Cell. Comp. Physiol. (Suppl. 1) 56: 169–188.

——, ——, M. H. Major, and C. M. Coffman. 1956. Dominant lethals induced in mouse sperm by 14-Mev neutrons from a deuterium-tritium reaction. USAEC Report ORNL-2155, pp. 26–27.

——, and C. L. Saylors. 1960. Factors causing a high frequency of mice having the XO sex-chromosome constitution. Science 131: 1321–1322.

——, and ——. 1961. Spontaneous and induced abnormal sex-chromosome number in the mouse. Genetics 46: 894.

——, and ——. 1962a. Induction of paternal sex chromosome losses by irradiation of mouse spermatozoa. Genetics 47: 7–10.

——, and ——. 1962b. Chromosome loss and nondisjunction in mouse spermatocytes. USAEC Report ORNL-3267, pp. 57–59.

——, and R. J. Spear. 1954. X-ray-induced dominant lethals in mouse oocytes and their relation to irradiation-to-ovulation interval. Genetics 39: 991.

——, and ——. 1955. Relation between dominant lethal incidence and stage in oogenesis irradiated. Radiation Res. 3: 342–343.

——, and L. Wickham. 1957. The incidence of disturbed fertility among male mice conceived at various intervals after irradiation of the mother. Genetics 42: 392.

Russell, W. L. 1951. X-ray-induced mutations in mice. Cold Spring Harbor Symp. Quant. Biol. 16: 327–336.

——. 1952. Mammalian Radiation Genetics. In Nickson, J. J., Ed.: Symposium on Radiobiology. New York: John Wiley & Sons, pp. 427–440.

——. 1954. Genetic Effects of Radiation in Mammals. In Hollaender, A., Ed.: Radiation Biology. New York: McGraw-Hill, pp. 825–859, vol. 1.

——, J. C. Kile, Jr., and L. B. Russell. 1951. Failure of hypoxia to protect against the radiation-induction of dominant lethals in mice. Genetics 36: 574.

——, L. B. Russell, and J. S. Gower. 1959. Exceptional inheritance of a sex-linked gene in the mouse explained on the basis that the X/O sex-chromosome constitution is female. Proc. Nat. Acad. Sc. U.S. 45: 554–560.

——, ——, ——, and C. W. Sheppard. 1953. Neutron-induced dominant lethals in the mouse. Genetics 38: 688.

——, ——, and E. M. Kelly. 1958. Radiation dose rate and mutation frequency. Science 128: 1546–1550.

——, ——, and ——. 1960. Dependence of mutation rate on radiation intensity. Intern. J. Radiat. Biol., Suppl., pp. 311–320.

——, ——, and A. W. Kimball. 1954. The relative effectiveness of neutrons from a nuclear detonation and from a cyclotron in inducing dominant lethals in the mouse. Am. Naturalist 88: 269–286.

——, ——, and E. F. Oakberg. 1958. Radiation Genetics of Mammals. In Claus, W. D., Ed.: Radiation Biology and Medicine. Reading, Mass.: Addison-Wesley Publishing Co., Inc., pp. 189–205.

Sirlin, J. L., and R. G. Edwards. 1957. Sensitivity of immature mouse sperm to the mutagenic effects of X-rays. Nature 179: 725–727.

Slizynski, B. M. 1949. A preliminary pachytene chromosome map of the house mouse. J. Genet. 49: 242–245.

——. 1952. Pachytene analysis of Snell's T(5;8)*a* translocation in the mouse. J. Genet. 50: 507–510.

——. 1954. Partial sex linkage in the mouse. Nature, London 174: 309–310.

——. 1957a. Cytological analysis of translocations in the mouse. J. Genet. 55: 122–130.
——. 1957b. Chromosomal mechanism in translocation infertility. Proc. Roy. Phys. Soc. Edinburgh 26: 49–60.
Snell, G. D. 1946. An analysis of translocations in the mouse. Genetics 31: 157–180.
Steinberger, E., W. O. Nelson, A. Boccabella, and W. J. Dixon. 1959. A radiomimetic effect of TEM on reproduction in the male rat. Endocrinology 65: 40–50.
Thibault, C. 1959. Analyse de la fécondation de l'oeuf de la truie après accouplement ou insémination artificielle. Colloquium on Reproduction and Artificial Insemination of the Pig. Institut National de la Recherche Agronomique, Paris, pp. 165–188.
Thompson, P. E. 1960. Effects of some autosomal inversions on lethal mutation in Drosophila melanogaster. Genetics 45: 1567–1580.
Thuline, H. C., and D. E. Norby. 1961. Spontaneous occurrence of chromosome abnormality in cats. Science 134: 554–555.
Welshons, W. J., B. H. Gibson, and B. J. Scandlyn. 1962. Slide processing for the examination of male mammalian meiotic chromosomes. Stain Technology 37: 1–6.
——, and L. B. Russell. 1959. The Y-chromosome as the bearer of male determining factors in the mouse. Proc. Nat. Acad. Sc. U.S. 45: 560–566.
Whiting, Anna R. 1961. Genetics of Habrobracon. In Caspari, E. W., and Thoday, J. M., Eds.: Advances in Genetics 10: 295–348.
Wright, M. E. 1947. Two sex linkages in the house mouse, with unusual recombination values. Heredity 1: 349–354.

Chapter 8

Mammalian Genetics in Medicine

Salome Gluecksohn-Waelsch
Albert Einstein College of Medicine,
New York, New York

I. Introduction

The surge of interest in human and medical genetics in recent years stems to a large degree from the remarkable contributions to basic genetic knowledge by research in these fields themselves. It is hardly necessary in this series of reviews to point to the developments in the area of hereditary molecular diseases the analysis of which, since Pauling's first discoveries, is largely responsible for many of our recent concepts about the fine structure of the gene and its mode of action. The stimulation which problems of medicine thus provided for basic genetics has in turn engendered interest in human and medical genetics to a degree which can hardly be satisfied by basic geneticists. Reasons for this failure are manifold; among them is the fact that the transfer of knowledge from one type of organism to another encounters great difficulties in spite of the apparently universal nature of the genetic coding mechanism. Anyone who has ever been confronted with this problem is aware of the impossibility of fitting complex genetic problems in higher organisms into some of the present models of bacterial gene action. Nevertheless, there is no question that the analysis of problems of gene action will also in the future derive great benefit from continued mutual exchange of methods and concepts between studies of the genetics of human molecular diseases and of microorganisms.

It seems hardly necessary to enumerate the contributions which microbial genetics and other specialities of basic genetics such as statistical and biochemical genetics have made and will continue to make to knowledge

The author's own studies discussed here were supported by grants from the National Institutes of Health, Public Health Service (B-931) and from the American Cancer Society.

in medical genetics. It might, however, be of some value to justify here the inclusion of a chapter on mammalian genetics in a volume of Progress in Medical Genetics.

It is an interesting phenomenon that medical people interested in genetics expect from the field of microbial genetics the solution of their medical genetics problems. This approach is based on the belief, confirmed to a very large degree, that the principles of heredity apply throughout nature in the same way, and that the principle of genetic coding appears to be universally valid. Thus, findings in microbial organisms can be assumed to apply to other organisms which resist direct analysis because of their complex nature. There seems to be much reason for agreeing with this as far as the basic mechanisms of gene action are concerned. However, as soon as one begins to proceed from this level to further steps of gene effects, the reasoning by analogy encounters difficulties. Not only haploidy against diploidy, but also the unicellular against the multicellular state, which in higher organisms involves interaction of cells, tissues, and biochemical processes, have to be taken into consideration when transferring conclusions from microorganisms to higher ones. When attacking the problem of how genes produce their effects in higher organisms and particularly in man, it is at least debatable whether the microbial organism presents the ideal model. Certainly the prejudice in its favor finds its justification in the area of hemoglobin genetics, for example. However, there are a great many, if not the majority of problems in human genetics for which better models may be provided by other mammalian organisms whose development, biochemistry, metabolism, physiology, and pathology show a great many similarities to those of man.

The problem of gene expression in man can hardly be studied in microbial models. A closely related experimental mammal with the same type of interaction of cells and tissues during development and the same interrelationships of developmental processes may lend itself much better for the analysis of gene effects in man.

There exists only a limited number of ways in which mammalian organisms are able to react to various traumata, be they gene or environmentally induced (Dunn, 1939–1940). This restriction of possibilities is the consequence of the limited degree to which the very specialized developmental and metabolic processes of higher vertebrates are able to deviate from their normal course without leading to immediate death of the organism. Identical abnormalities of the nervous system for example, may be produced in mammals by a number of hereditary as well as environmental factors (Gluecksohn-Waelsch, 1955). It is well known that the congenital malformation of the brain known as pseudencephaly or exencephaly may be observed in mice as the result of the effect of different genetic factors,

or as the result of X-irradiation or injection of trypan blue into the pregnant female. This phenomenon of similarity of response to different stimuli demonstrated in experimental laboratory mammals is of course equally apparent in man where identically appearing pathological entities may arise on the basis of different etiologies.

It is the purpose of this review to bring to the attention of those interested in medical genetics the vast amount of material of great potential value to them buried in the literature on mammalian genetics usually not considered by the medical scientist. Those approaches and results in the field of animal, specifically mammalian genetics will be singled out which either have contributed to the illumination of problems of medical genetics, or which show promise of being able to help progress in medical genetics. Obviously, therefore, this chapter does not attempt to represent an exhaustive literature review of mammalian genetics. The choice of topics has been determined by a number of criteria. Problems in genetics of theoretical as well as of practical nature have been chosen for consideration; contributions from the field of mammalian genetics have been selected which in the writer's opinion advance the solution of these problems. It is of course impossible to avoid a certain bias in such a choice. Most of the work to be discussed has been done on the mouse (Mus musculus) and the author's primary interest in different aspects of the physiology of the gene played a decisive role in the choice of topics.

II. The Application of Concepts Derived from Experimental Genetics

In the discussion of the material to be covered, we shall begin with a consideration of theoretical genetic problems which play a role in medical genetics and to the solution of which experimental approaches in mammalian genetics have made contributions.

Concepts of considerable importance in medical genetics such as penetrance and expressivity, complex genetic loci, pleiotropism, stem from experimental genetics where they were developed as the result of genetic observations. Further analysis and elucidation of such concepts were frequently based on work with experimental material.

a. Penetrance and Expressivity

Two concepts of physiological genetics which are called upon frequently to explain complex genetic situations in man are those of penetrance and expressivity. Whereas the specific conditions affecting penetrance and expressivity of human genes frequently resist analysis, it is possible to undertake such an analysis in the case of experimental animals. The incompletely

dominant Sd-mutation in mice is an example of a study of penetrance and expressivity; this mutation affects kidney development, resulting in absence or abnormality of one or both kidneys (Gluecksohn-Schoenheimer, 1943, 1945).

The behavior of this gene on different genetic backgrounds was studied, and the existence of modifiers which affect its penetrance and expressivity was demonstrated (Dunn and Gluecksohn-Schoenheimer, 1945). Outcrosses to different strains resulted in a change from high penetrance of the gene where 94 per cent of all heterozygotes showed the kidney defect to low penetrance where only a small percentage of heterozygotes had abnormal kidneys. In addition to incomplete penetrance, the Sd-gene showed in heterozygotes variable expressivity on all backgrounds tested, from only a slight decrease in size of one kidney to absence of both kidneys. Furthermore, the degree of expressivity varied on different backgrounds. A shift occurred in the distribution of the kidney defects from a higher percentage of severe effects on some backgrounds to a higher percentage of slight effects on other backgrounds.

Both penetrance and expressivity of genes are under the influence of other genes and the environment. Genes affecting these two phenomena are known as modifying genes. They can be identified and their effects studied more easily in organisms other than man.

b. Complex Genetic Loci

The problem of complex genetic loci has received much attention in modern genetics (Pontecorvo, 1958). Observations of recombination and complementation between so-called alleles at one locus have resulted in the breaking up of the gene as the ultimate unit of heredity, and our knowledge of the fine structure of the gene has increased greatly. Not only in microorganisms and in Drosophila, but also in mammals have such complex genetic loci been discovered. It would seem that their analysis might have considerable bearing on problems of medical genetics since similar complex loci appear to exist in man also.

The T-locus in the mouse seems to represent a suitable model for analysis. Dunn (1954, 1956) has discussed this complex locus and the theoretical implications of its structure and action. Different alleles at this locus seem to interfere with processes of early growth and differentiation raising the problem of spatial and functional gene relationships (Gluecksohn-Waelsch, 1954). It is obvious that the experimental analysis of complex loci in the mouse, such as the T-locus or the H-2 locus (Snell, 1958) may contribute greatly to our understanding of complex loci in man, such as the Rh or the MN locus.

In connection with the problem of the action of genes in relation to their

spatial organization, attention is called to reports by Paigen (1961) and by Paigen and Noell (1961). These authors studied two closely linked genes in the mouse, one affecting the enzyme glucuronidase and the other responsible for a hereditary degeneration of the visual cells of the retina. Similar time patterns of expression were shown by these two genes. The enzyme ceases to appear in the liver after the 17th day of life in homozygous mutants. The degeneration of visual cells starts around 10 days and by 3 weeks no visual cells remain. Failure of the enzyme is restricted to the liver cells of mutants, whereas the spleen, for example, maintains normal enzyme production. In the eye, only the visual cells degenerate, but other cells of the same developmental origin, e.g., pigment epithelium, bipolar cells and ganglion cells, remain normal.

There seems to be some similarity in the time patterns of expression of these two functionally different, linked genes, both of which show normal function up to a certain time at which functional failure occurs. The authors offer a highly speculative interpretation of their results in the light of recent speculations on the genetic control of differentiation. They wonder if particular regions of chromosomes may be specialized to control the timing of expression of genes located within these regions; in this way a nuclear control of differentiation may be provided.

c. The Problem of Pleiotropic Gene Effects in Higher Organisms.

One of the most intriguing problems of physiological genetics is that of the pleiotropic effects of genes. The problem has to be approached at different levels between primary gene action and phenotype. This is particularly necessary in the case of an organism as complex in structure, function and metabolism as the mammal where multiplicity of effects as observed in the phenotype by no means necessarily indicates multiplicity of effects on the level of primary gene action.

Pleiotropy has been the object of much argumentation. The concept of "pedigree of causes" by Grüneberg (1943) although a useful working hypothesis, has led to attempts to line up different effects of a gene observed in the organism in sequential order, sometimes to the extent of forcing them into such order. Subsequently causal relations have been constructed between different effects simply on the basis of correlation between them in space and time.

In view of recent progress in the analysis of the fine structure of the gene and the problem of gene action at the primary level, it now seems futile and rather primitive to present elaborate arguments for the "unity of gene action" based on studies at the phenotypic level and on the construction of "pedigrees of causes," arguments which have resulted in the formulation of rigid categories of different types of pleiotropism by Grüneberg

(1943). The time has come for considerations of pleiotropism to proceed to a more sophisticated level. An example of this is the model for primary pleiotropic gene action proposed by Monod (Jacob and Monod, 1961) on the basis of his concept of the "operon" consisting of structural genes on the one hand and operator or regulator genes on the other hand. The interaction of operator or regulator genes with several closely linked structural genes may simulate a pleiotropic effect.

Although argumentation of the "unity of gene action" based on phenotypic observations at a complex level of organization should have no place in modern genetics, the author feels obliged to make reference to it since it unfortunately still has because of its simplicity, a great appeal to the medical investigator interested in genetics.

In spite of reservations regarding the usefulness of further debate, the thoughtful discussion of pleiotropism by Hadorn (1961) should offer a good background for the analysis of pleiotropic patterns of gene effects on the phenotypic level in man.

Hadorn's concepts of pleiotropism have been made use of productively in the analysis of an interesting human hereditary disease (multiple adenomatosis), which results from a dominant gene (Wermer, 1954). In this disease, adenomata of different organs appear throughout the organism, and it was not possible to construct a "pedigree of causes" in the sense that the abnormality of one organ might cause that of the others. Despite the fact that some of the organs are affected at earlier times than others, a causal relation between the earlier and later effects has been excluded. Wermer concludes (personal communication) that we are dealing here with a type of mosaic pleiotropism (Hadorn) where the gene expresses itself independently and differently in different cell systems.

III. Gene Effects in Mammalian Development

a. Interaction of Developmental Systems

Pleiotropic effects of genes in mammals as well as in man have been utilized to investigate and perhaps reveal possible relationships between diverse developmental or biochemical processes.

In this connection studies of two mutations in the mouse should be mentioned. In the first (Auerbach, 1954), a dominant mutation (Splotch) with recessive lethal effects was investigated with the help of embryological methods and those of transplantation. Abnormalities of the nervous system, the neural crest and the pigment developing system were found in the homozygotes, and it was concluded that a disturbance of the dorsal part of the neural tube and of the neural crest might underlie the abnormal syndrome. In the discussion of these findings, Auerbach places particular em-

phasis on the role which such mutations may play in furnishing information about normal inductive relationships in mammalian development and the role of genetic factors in such processes of induction. For more details of this discussion the reader is referred to the original paper (Auerbach, 1954).

The second paper (Bennett, 1956) reports a similar approach to the study of a mutation in the mouse with pleiotropic effects where germ cell development, hematopoiesis and the pigment system are affected simultaneously. Again, this analysis stresses the importance of such studies for the revelation of new developmental relationships between different cell systems in mammals, and the role genes play in these developmental events.

The two papers just mentioned are examples of studies which aim at the analysis of the developmental steps by which a hereditary congenital malformation arises while at the same time revealing the role of genes in normal mammalian development (Gluecksohn-Waelsch, 1961).

b. Congenital Malformations

Congenital defects of different organ systems show remarkable resemblances in man and mouse. As stated earlier, the similarities of developmental processes in these two mammals are directly responsible for similarities of deviations from the normal course of development whenever these are caused by any traumata, genetic or environmental. Thus, studies such as the two just reviewed, will no doubt contribute to knowledge of the etiology of congenital defects in man. Furthermore, they help considerably towards the evaluation of the role of genetic factors in normal and abnormal developmental processes in mammals. The significance of this approach to studies of congenital malformations has been amply stressed in the first volume of this series (Fraser, 1961) and does not require further elaboration.

c. Early Embryonic Lethals

Among the genes with the earliest embryonic effects, before and immediately after implantation in the uterus, is the remarkable series of alleles at the complex T-locus in Chromosome IX in the mouse mentioned before; these alleles have been shown to interfere with processes of early differentiation and inductive interaction in development. At least six of these mutations result in death of the homozygote in early stages of embryonic differentiation. One of them (t^1) leads to failure of further development at a time between fertilization and implantation of the blastocyst in the uterine mucosa. Another allele in the same series (t^{12}) is the earliest mammalian embryonic lethal to have been analyzed in detail. In these t^{12}/t^{12}

embryos the concentration of RNA seems to be abnormally low and the shape of the nucleoli abnormal at a stage of about 30 cells, approximately 80 hours after fertilization. Still another lethal allele (t^0) interferes in homozygous condition with the differentiation of mesoderm and the organization of the embryonic material beyond the blastocyst stage. Two more lethal alleles (t^1 and t^9) lead when homozygous to failure of differentiation of mesoderm into somites, to abnormal formation of the allantois and to disturbances of the pattern of neural fold formation with overgrowth and asymmetry of head folds and neural folds. The dominant mutation *T* itself for which the locus was named, causes in homozygous condition a failure of development of the posterior region of the embryo. Consequently, no structures are found caudal to the forelimb buds in a 10 day embryo; since the missing region includes the allantois, umbilical connection with the maternal placenta fails to be established and the embryo dies at a stage when it becomes dependent upon the mother for its oxygen supply, nutrition, and waste product elimination. It is interesting that two additional mutations also affecting early processes of differentiation are located close to the T-locus in Chromosome IX of the mouse. In one of these homozygotes (*Ki/Ki*) more than one embryonic axis develops resulting in inviable monsters with duplications of various organ systems. The other one (*Fu/Fu*) shows overgrowth and partial duplications of the differentiating nervous system.

All of the mutations just described show effects similar to those produced with various techniques by experimental embryologists in lower vertebrates in the causal analysis of developmental mechanisms. Much of our knowledge of early mammalian differentiation is derived from these studies of gene produced lesions in the mouse embryo. For detailed descriptions and discussions of these interesting genes the reader is referred to several earlier publications (Dunn, 1956; Gluecksohn-Waelsch, 1954; Lyon and Phillipps, 1959).

At this point, it might be worthwhile to direct attention to the possible significance of this research for problems in medicine. Studies of pregnancy wastage in man should take into account the existence of well analyzed mutations in a mammal with definite patterns of inheritance and with effects on implantation, placenta formation, and early differentiation, i.e., on processes which in principle are the same in man as in the mouse.

d. Thymus and Lymphoid Differentiation

Interesting implications for medical problems are contained in a report which deals with the role of genetic factors in lymphoid differentiation of the mouse thymus (Auerbach, 1961). Using methods of transplantation of embryonic thymus rudiments into the anterior eye chamber

of adult males Auerbach (1960) could show that lymphoid differentiation in the rudiments occurred only when donor and host were immunologically compatible. In contrast to this, embryonic rudiments of other organs, e.g., lung, grew and differentiated normally in all combinations of host and donor. Consequently, lymphoid differentiation took place in combinations of embryonic thymus rudiments from parents as donors with F_1 as hosts, or of rudiments from F_1 as donors with F_1 as hosts. It failed to occur in the case of thymus in combinations of genetically incompatible strains, or F_1 as donors with parents as hosts. These results indicate that lymphoid differentiation of the thymus may be subject to genetically determined control mechanisms.

The thymus has been reported to play a role in mouse leukemia, and mice of the AKR strain show a high incidence, those of the C3H strain a low incidence of leukemia. A comparison was therefore made between in vitro morphogenesis of thymus rudiments from the high leukemic strain (AKR) and the low leukemic strain (C3H); also, thymus primordia from hybrid matings of C3H and AKR, and of C3H and Bagg albino C were included in this comparison (Auerbach, 1961). Growth and differentiation in organ tissue culture of thymus rudiments from the high leukemic strain and from the F_1 between the high and the low leukemic strains, were considerably faster than of any of the other rudiments: four days after explantation the "high leukemic" rudiments showed lymphoid differentiation whereas it took 7 to 8 days for the rudiments of the low leukemic strain and those of the F_1 between two low leukemic strains, C3H and Bagg albino C.

There is no doubt that studies of this kind in experimental mammals attempting to analyze genetic and developmental mechanisms of lymphoid differentiation show great promise of increasing our understanding of the role of lymphoid systems in problems of immunity and of leukemia in man.

IV. Transplantation Genetics of Mammals

a. Historical

Transplantation immunity and its genetic basis have come to play a most significant role in several areas of medicine and particularly, of course, in surgery and in cancer research. Knowledge in the field of transplantation genetics stems largely from studies in experimental mammalian genetics tracing back for over 50 years. A number of excellent reviews have been written on the exciting subjects of transplantation genetics and tolerance, and the reader is referred to them for extensive coverage (Owen, 1959, 1960; St. Whitelock, 1958). In the present review, we shall attempt only the briefest summary from the very beginning to the most recent results in this field.

A clearly reproducible and uniform difference in susceptibility to tumor transplants was shown first by Leo Loeb (1901, 1902) who obtained good growth of tumors from Japanese waltzing mice transplanted to animals of the same strain. On the other hand, these tumors were rejected by animals of a different unrelated strain. The possible genetic basis of susceptibility and resistance to tumor transplants was subjected to experimental test in 1909 by Tyzzer in a series of carefully controlled experiments which confirmed the genetic nature of these phenomena. However, the exact mode of the genetic transmission remained obscure until Little in 1914 demonstrated that the peculiar ratios of transplant susceptible to transplant resistant animals could be explained if one assumed a large number of dominant factors to be responsible for susceptibility and resistance to transplantation. This interpretation was put to experimental test by Little and Tyzzer who published their results in 1916. They crossed partly inbred mice with each other and tested by transplantation successive hybrid generations for resistance to tumor. The ratios obtained conformed to expectation if the simultaneous presence of 14 to 15 dominant genes was assumed to be responsible for susceptibility to transplanted tumors in these particular mouse strains (Little, 1941). Subsequently, the same type of genetic transmission could be demonstrated to underlie transplantation reactions to tissues other than tumors.

Studies in mammalian genetics, specifically of the mouse, the development of isogenic-resistant lines, and the identification of a large number of histocompatibility genes, which determine the compatibility between transplanted tissue and host (Snell, 1957), all of these factors are responsible for the tremendous development of the field of transplantation genetics and its application to medical problems. It is important to point out that the specific genetic methods required for the analysis of histocompatibility genes have thus far been used primarily in the mouse, although Billingham and Silvers have begun to extend this work towards other species, e.g., rats and hamsters (Billingham, Sawchuck and Silvers, 1960; Billingham and Silvers, 1961).

In mice there exist a number of histocompatibility loci: H-1, H-2, H-3, each of them with a considerable number of alleles. The H-2 locus appears to be the most interesting of the histocompatibility loci in the mouse and has been studied most extensively. It has been reported to have as many as 18 alleles. According to Snell (1958) it is a matter of definition whether the H-2 locus be considered a single compound locus or a group of closely linked loci. The complexity of the H-2 locus has been discussed fully by Hoecker (1956): it controls an antigenic situation similar in its complexity to that of the Rh locus in man. H-2 determines not only transplantation resistance or susceptibility but also agglutinability or nonagglutinability of

erythrocytes by antisera from mice which have rejected transplants, as shown by Gorer (1937). This makes the H-2 factor a blood group gene in the mouse as well. Typing is done with the help of histogenetic and serologic methods. The first method determines susceptibility and resistance to transplants and the second the agglutinability or nonagglutinability of erythrocytes by antisera. The genetic structure of the H-2 locus, its mode of action and antigenic components might well present a model for similar genetic loci in man.

c. Antigenic Differences Between Males and Females

It was shown some time ago that in an inbred strain of mice a high proportion of females rejected tissues transplanted to them from male donors although tissues were accepted between all other combinations of sexes. This phenomenon was interpreted as being due to the presence of a histocompatibility locus in the Y-chromosome of the mouse (Snell, 1956). Recently, support for this interpretation has been provided by the demonstration that female mice may be made tolerant of male isografts (Billingham and Silvers, 1959). It was shown that the Y factor is a transplantation antigen comparable to other antigens responsible for homograft rejection and the H-Y locus is considered to be a "fully qualified member of the histocompatibility group" (Billingham and Silvers, 1959). The Y antigen seems to be identical in all male mice of different strains, and the H-Y locus seems to lack alleles. Thus far no crossing-over could be demonstrated. It has been argued, however, that this antigenic difference between male and female may be a secondary sexual characteristic not related to the Y-chromosome (Owen, 1959).

It is impossible in the framework of the present review to discuss in detail all the significant contributions from the field of experimental mammalian genetics towards the understanding of the genetic basis of transplantation immunity in man, or to do more than mention work on the genetics of tolerance or of graft versus host reaction. For summaries of much current work in this field the reader is referred to several reviews (Huestis, Anderson and Motulsky, 1956; Owen, 1959; Snell, 1957).

V. Sex Chromosomes

a. Sex Determination in Mammals

The interpretation of the chromosomal basis of sex-determination has undergone considerable revision in recent years; it presents perhaps one of the best illustrations for the statement that it is not possible to reason by analogy when analyzing basic mechanisms of gene effects in different organisms.

In the past, it had been assumed that in man and other mammals sex was determined, as in Drosophila, by a balance of female-tendency genes, more numerous or more effective in the X-chromosome, and male-tendency genes more effective in the autosomes. In Drosophila, Bridges showed that this genic balance depended on the proportion of X-chromosomes to sets of autosomes: the females have two X-chromosomes and two sets of autosomes, with a ratio of one between them, and the male one X-chromosome and two sets of autosomes with a ratio of 1/2. Deviations from this sex balance ratio result in deviations from normal sex differentiation in Drosophila. During the last few years evidence has been accumulating which indicates that a different mechanism of chromosomal sex determination operates in mammals.

In the mouse (W. L. Russell, L. B. Russell and Gower, 1959; Welshons and Russell, 1959) genetic and cytological evidence suggested that XO females were normal and fertile. This phenomenon was demonstrated in the course of the analysis of a sex-linked gene which in certain females, presumably heterozygous, produced a phenotypic effect similar to that of the same gene in males. Transplantation of ovaries from such exceptional females which as a result of the effect of the sex-linked gene die before the reproductive age, to normal ovariectomized females and subsequent matings of these hosts to normal males gave sons, *all* of whom expressed the presence of the sex-linked gene, and daughters of two kinds, those transmitting and those not transmitting the sex-linked gene. The latter were presumed to be XO females which thus appeared to be fertile.

Proof for this conclusion came from work with other X-chromosome markers where exceptional females showed the genetic behavior concomitant with the hypothesis that they were hemizygous for the X-chromosome. These exceptional females were then shown cytologically to carry only 39 chromosomes, instead of the normal number of 40.

The fact that a fertile female could have an XO chromosome constitution was not compatible with the balance theory of sex determination; the Y-chromosome in the mouse was therefore considered to be male-determining. The simultaneous demonstration (Ford, Jones, Polani, de Almeida and Briggs, 1959) of a woman with Turner's syndrome and the chromosomal constitution XO clearly showed a similarity of the chromosomal sex determining mechanisms in mouse and man and a common difference from that in Drosophila.

The production of XO females in mice was ascribed to nondisjunction of sister chromatids of one of the X-chromosomes during the first cleavage division of the mouse egg, presumably with subsequent loss of the XXX blastomere. The reason for this was the failure to find any abnormalities in

over 2,000 figures of male meiosis in Mus musculus which might have led to the formation of O-sperm (Ohno, Kaplan and Kinosita, 1959).

Subsequently a male mouse presumed to be XXY on the basis of X-chromosome genetic markers was described (McLaren, 1960). This finding was confirmed by the report of another male mouse whose XXY constitution was proven by X-chromosome markers as well as cytologically (Russell and Chu, 1961). Simultaneously, the existence of XXY mice with a paternal X-chromosome proved the occurrence of nondisjunction in male meiosis (Cattanach, 1961). The frequency of meiotic nondisjunction seems to be smaller than that of chromosome loss occurring during cleavage. The age of the male is suspected as a possible factor influencing the frequency of nondisjunction, with younger males showing a higher frequency.

Several conclusions may be drawn from this work on chromosomal sex determination in mice in relation to human conditions:

1. XO individuals are females and, at least in mice, fully fertile; the existence of Turner's syndrome in XO human individuals with infertility seems to indicate that in contrast to the mouse one X chromosome is not enough for normal female differentiation in humans.

2. The Y chromosome is male determining and XXY mice are males (Klinefelter in humans).

3. Loss of X leading to XO occurs most frequently after fertilization during first cleavage.

4. Nondisjunction leading to XY sperm occurs less frequently and is the result of first-meiotic nondisjunction with apparently a higher frequency in young males, in contrast to Mongolism where nondisjunction occurs more frequently in older females.

b. Gene Action in the X-Chromosome

An interesting interpretation of gene action in the X-chromosome of the mouse has been advanced recently. The so-called sex chromatin had been assumed to be composed of one heteropycnotic X-chromosome in the mouse (Ohno and Hauschka, 1960). Genetic evidence seems to indicate that only one X-chromosome is genetically active in any one cell, and it is suggested that the heteropycnotic X-chromosome has been inactivated genetically (Lyon, 1961). Consequently in the case of X-linked genes in the mouse with localized action, the phenotype should appear mosaic which actually is the case in all sex-linked mutants in the mouse affecting coat color. An intermediate and variable phenotype results if the phenotype is not due to localized gene action. Or a normal phenotype may develop if, for example, the presence of any normal cells is enough to insure normalcy; or there may be incomplete penetrance if the proportion of normal and mutant

cells varies. The inactivation of one X-chromosome is assumed to occur in early embryogenesis.

In a report to the Second International Conference of Human Genetics in Rome, Lyon (1961) applied this concept to the interpretation of the phenotypes in several X-linked conditions in man, e.g., hemophilia, color-blindness, sex-linked muscular dystrophy.

Lyon's intriguing hypothesis was also used for an interpretation of observations in humans with glucose-6-phosphate-dehydrogenase deficiency, a hereditary abnormality determined by an X-linked gene. Beutler, Yeh and Fairbanks (1962) present data which they interpret as showing that women heterozygous for this X-linked gene have a mixed red blood cell population consisting of cells deficient in glucose-6-phosphate dehydrogenase and of normal cells, corresponding to cells with an active mutant X-chromosome and those with an active normal X-chromosome.

VI. Genetics of Mammalian Reproduction

a. The Effect of Genes on Phenotype of Gametes

The problem of the role of genes in determining morphology and function of gametes was discussed and reviewed a few years ago (Braden, 1960). There has been much argumentation about the effects of genes on gametic phenotype, particularly about the problem whether gametic phenotype is subject to gene action in the haploid phase. This latter question is still being debated although of course there is no doubt about the effect of the genotype as a whole on gamete development. In individuals heterozygous for a gene affecting gamete morphology, 2 types of gametes should be found if there is gene action in the haploid phase in contrast to the existence of only one type in the case of homozygotes. Attempts to demonstrate such morphological dimorphism in homozygous males have been unsuccessful thus far.

However, there exist some data in the literature which suggest a functional dimorphism of spermatozoa from heterozygous males.

1. Separation of X and Y sperm in the electrical field. A sexual dimorphism of rabbit spermatozoa is indicated by the studies of Gordon (1957) who followed up work of several Russian authors. Gordon observed microscopically a separation into about equal amounts, of sperm from the ejaculates of rabbits into those which at a certain pH migrated to the anode and those which migrated towards the cathode. This migration was reversed when the current was reversed. Subsequently anode-migrating and cathode-migrating spermatozoa were used for artificial insemination and the sex ratios produced were studied using blind tests and nonblind tests.

Statistical evaluation of the sex ratios obtained seems to show that anode and cathode spermatozoa represent two different populations, one bearing an X-chromosome, the other a Y-chromosome. The separation of X and Y sperm in an electrical field is probably accounted for by different isoelectric points of the two types, although this is not generally accepted. If X and Y sperm do indeed have different isoelectric points this may be due to different surface proteins, but here again other possibilities of interpretation exist. For a more detailed discussion the reader is referred to the paper by Gordon (1957).

The report unfortunately leaves several questions unanswered. First of all, the statistical significance of the results varied between the nonblind test and the blind test groups. Secondly, the significance decreased as more data accumulated. Furthermore, there has been no follow up in later publications and no report of a confirmation of these results by other workers. In view of the extreme importance of this work for a possible control of sex ratio in mammals, one would like to see these experiments continued and extended.

2. *Sperm of blood group A or B.* Another study of dimorphism of gametes was done in man. Spermatozoa in ejaculates of individuals of the AB blood type were reported to be either A or B as shown with serological methods (Gullbring, 1957). However, Owen (1960) reports the failure to find any red cell antigenic specificities in the sperm of segregating males of cattle. On the basis of these findings Owen doubts that there is as yet good reason to assume cellular antigen dimorphism of sperm from males heterozygous for cell antigen controlling genes.

3. *The effect of T-locus mutants on sperm phenotype.* A report indicating differences between sperms carrying different alleles deals with a study of the peculiar effects of alleles at the complex T-locus in the mouse on male fertility and gene transmission ratios (Braden, 1960; and Gluecksohn-Waelsch, 1958). It had been reported many years ago (Dunn and Gluecksohn-Schoenheimer, 1939, 1943) that males heterozygous for certain of the recessive t-alleles do not transmit sperm carrying *t* and sperm carrying the other allele in equal proportions, but that there is transmitted a highly significant excess of *t*-sperm. On the other hand, males heterozygous for two of such alleles—each of them transmitted in excess when alone—are either completely sterile or show severe reduction of fertility. A recent study (Braden and Gluecksohn-Waelsch, 1958) shows clearly that the sterility of males heterozygous for 2 *t*-alleles cannot be accounted for by the presence of morphologically abnormal sperm or sperm with reduced motility and viability. The cause of the sterility of these males seems to lie rather in some disturbance of the normal functioning of these sperm in the female genital tract rendering them unable to traverse the uterotubal junction.

It is interesting that the ability of sperm to traverse the uterotubal junction may be under genetic control, exerted in this case in the premeiotic phase.

In this connection attention is directed towards a discussion remark at the Symposium on Mammalian Genetics and Reproduction, where Curt Stern (1960) pointed out a possibility which would make it difficult to decide between pre- and postmeiotic gene effects. The existence of intercellular bridges connecting the four spermatids with each other, demonstrated by Fawcett (1957), may permit an exchange of gene products across these bridges. Such exchange could lead to a result indistinguishable from that of gene interaction in the premeiotic phase. Stern therefore suggests subdivision of the postmeiotic phase into two stages: the early spermatid stage when there is communication between genetically different spermatids, and the sperm stage when the genes are presumably inactive.

While the sterility effect of *t*-alleles has thus found a possible physiological basis, there exists as yet no explanation for the fact that males heterozygous for one of the *t*-alleles transmit an excess of sperm carrying the particular allele. However, in view of the demonstration of a physiological abnormality of sperm from sterile males carrying two different *t*-alleles, it is conceivable that the disturbance of the segregation or better transmission ratio in males heterozygous for one *t*-allele may be due also to a genetically controlled physiological and functional difference between the two types of sperm expressed in the haploid phase. Support for such an assumption comes from the demonstration (Braden, 1960) that the segregation ratio of heterozygous males is affected by the time interval between ovulation in the females and copulation. This result indicates the existence of a physiological property of the sperm which is under the control of the *t*-alleles in the haploid phase and also subject to effects of the environment of the female genital tract. One might suspect a disturbance of this physiological mechanism to be responsible for the deviating ratios of gene transmission in males heterozygous for one of the *t*-alleles.

4. Genetic transformation of sperm. Attempts have been made to transform genetically labelled rabbit spermatozoa with DNA extracted from a different genotype, but these were unsuccessful (Beatty and Billett, 1961).

VII. Genetic Pathology of Mammals

A number of hereditary disease entities have been described in the mouse which show close resemblance to conditions in man. These diseases include those of the blood (e.g., macrocytic anemias), of the nervous system and of the musculature. One of the most interesting of such experimental diseases is:

a. Muscular Dystrophy

The existence of an autosomal recessive mutation in the mouse causing a syndrome resembling closely human muscular dystrophy was reported in 1955 (Michelson, Russell and Harman, 1955). The lesions found in mice homozygous for this recessive mutation (dy) showed striking similarity to those described in human muscular dystrophy. Progressive ataxia, atrophy and paralysis are typical symptoms of the dystrophic mice which may be recognized at the age of two or three weeks by their characteristic behavior. The affected animal has subnormal weight throughout its life span which varies between 1 and 6 months. Mice with muscular dystrophy show a drastic reduction of fertility and hardly ever reproduce. In order to facilitate the production of large numbers of mice with muscular dystrophy, ovaries from dystrophic females were transplanted to compatible normal female hosts (Stevens, Russell and Southard, 1957) using the necessary genetic color markers. Matings of these normal females, carrying ovaries from donors homozygous for muscular dystrophy, to heterozygous or normal males produced offspring either homozygous or heterozygous for the dystrophy gene.

The enzyme phosphoglucomutase has a markedly lower activity in biopsy muscle samples of human patients with progressive muscular dystrophy than in samples from normal individuals. Studies in mice (Hazzard and Leonard, 1959) reveal a similar significant decrease in mutase activity in the muscles of dystrophic mice, and measurements at different ages indicate a progressive decline in enzyme activity with increasing age and severity of muscular dystrophy. The similarity of the biochemical defect in man and mouse muscular dystrophy is striking.

Further metabolic studies of mice with hereditary muscular dystrophy revealed that kidney transamidinase activity in these abnormal mice was higher than in normal mice maintained on the same basic semisynthetic ration (Coleman, 1961). Addition of creatine to the diet resulted in a more severe depression of the enzyme activity in dystrophic mice than in normals. This enzyme depression may be reversed by the addition of glycine to the diet. Dystrophic mice were more sensitive than normal mice to such dietary changes. It seems that kidney transamidinase exerts a control over creatine synthesis in mouse hereditary muscular dystrophy which is in contrast to findings in dystrophic rabbits. Many of the additional metabolic and biochemical abnormalities reported in mouse muscular dystrophy are considered to be the results rather than the causes of the disease itself (E. S. Russell, 1961).

The fine structure of muscle elements of dystrophic mice was studied (Ross, Pappas and Harman, 1960) with the help of the electronmicroscope.

A loss of the contractile elements was observed within the afflicted cell. The process seems to originate at random along the myofibrils and to progress slowly with more and more myofibrils disappearing as the dystrophy increases in severity. The endoplasmic reticulum undergoes swelling and vacuolization in the dystrophic cell. No alterations were observed in the nuclei of muscle cells or the sarcolemma.

Penetrance and expressivity of the gene for muscular dystrophy were studied in outcrosses to 13 different mouse strains (Loosli, Russell, Silvers and Southard, 1960). Whereas clinical expressions of the dystrophy, e.g., weakness of the hindlegs, remained uniform, time of clinical onset varied in different groups. Genetic modifiers seem to affect the penetrance of mouse muscular dystrophy, without necessarily affecting its expressivity.

The clinical aspects of mouse muscular dystrophy resemble most closely those of the sex-linked Duchenne type of the human disease. It is interesting to note that the Duchenne type of muscular dystrophy in man is usually due to a sex-linked recessive gene whereas the mode of inheritance of the mouse dystrophy which clinically resembles most closely that of the Duchenne type, is that of a recessive autosomal gene. However, there exist in man also examples of muscular dystrophy clinically identical with the Duchenne type but due to an autosomal recessive gene.

The existence of a muscular dystrophy with a simple genetic basis in an experimental mammal provides the possibility of studies of the metabolism, physiology and pathology of this myopathy to a degree not possible in human patients.

b. Neurological Diseases

Studies in the field of mammalian genetics have devoted much effort to the investigation of hereditary neurological diseases. Obviously, such material may reveal much important information on mechanisms of hereditary transmission, neuroembryology and neuropathology of related diseases in man. At the same time possible metabolic errors with neurological effects may be discovered in this way.

1. Tremor and convulsions. Hereditary tremor and convulsions in rabbits have been studied and described extensively by Nachtsheim with particular attention to the relation of these conditions to similar ones in man. The results of these studies have been summarized relatively recently in a review (Nachtsheim, 1958) to which the reader is referred for detailed information.

2. *Reeler.* Since the publication of the last review of abnormalities of the nervous system in mice (Huecksohn-Waelsch, 1959) further results were obtained on the neuropathology and neurochemistry of "*reeler*," a reces-

sive mutation in the mouse. Animals homozygous for this autosomal recessive gene show lack of muscular coordination, balancing difficulties and tremors (Falconer, 1951). Studies of enzyme concentrations with standard manometric methods revealed a doubling of cholinesterase activity in the cerebellum of animals homozygous for the reeler gene (Hamburgh, 1958). Histological investigations of brains from reeler homozygotes obtained 18 to 21 days after birth revealed severe alterations of the organization of the cerebellum. "The typical appearance of the folia is missing. The arrangement of Purkinje cells which normally surround the granular layer is severely disturbed. The granular layer is much reduced and the area of white matter contains large numbers of cells which resemble Purkinje cells" (Hamburgh, 1960).

In this connection it might be of interest to mention another neurological mutation in the mouse, "agitans," which resembles "reeler" symptomatically and in which abnormalities of Purkinje cells were also reported (Martinez and Sirlin, 1955).

3. *Lurcher.* Several new mutations with neurological manifestations were described in the last few years: "*Lurcher*" is a dominant or semi-dominant mutation with neuromuscular effects (Phillips, 1958). Heterozygotes resemble homozygous "reelers" (cf. above) and show characteristic swaying of the hind quarters and unsteadiness of gait. At the same time, jerky movements are observed. The neurological manifestations progress with age. Heterozygotes are not deaf and show no disturbance of their labyrinth mechanism as shown by several tests, e.g., swimming. Homozygotes die soon after birth. Their behavior and pathology have not been described.

4. *Zig-zag.* Another neurological abnormality in the mouse is "*zig-zag*" whose genetic transmission is not quite clear but which seems to be due to the action of a number of genes, i.e., polygenes (Lyon, 1960). Animals with the defect show a zig-zag motion of the head and occasionally walk in circles; they hear normally. There was a gradation from severely affected animals who circled repeatedly to almost normal animals whose head motions were slight and who did not circle. Reduction or absence of the horizontal canals of the inner ear was found in abnormal animals.

5. *Dreher.* The "*dreher*" mutant of the mouse, a recessive autosomal gene with neurological effects (Falconer and Sierts-Roth, 1951), such as circling in the homozygotes, was shown to have, in addition, effects on pigmentation and on the expression of a skeletal hereditary abnormality (Lyon, 1961). One could fit the pleiotropic effects of this gene only with the greatest difficulty into a pedigree of causes. Their relationships to each other remain obscure.

6. *Dilute lethal.* Hereditary demyelinating diseases play an important

role in human neuropathology, and multiple sclerosis, for example, has been and is the object of many investigations on the genetic, metabolic and biochemical level. A recessive autosomal gene (dilute-lethal) has been reported in the mouse which in homozygous condition is responsible for demyelination in the central nervous system (Kelton, 1961).

Animals homozygous for d^l begin myelination at the same time as normal controls. By 11 days of age the somato- and vestibulo-cerebellar systems show widespread degeneration. Subsequently demyelination progresses in severity. "No degeneration was seen in olfactory lobes; in the cerebrum the putamen and tecto-spinal tract, and in the medulla the pyramidal tract and its decussation contained Marchi granules. The dorsal, lateral and ventral funiculi of the spinal cord anterior to the fourth cervical vertebra were demyelinated." (Kelton, 1961.)

7. *Premature senescence.* The genetic basis of presenile dementias in man is well established for some diseases, e.g., Huntington's Chorea, and Pick's and Alzheimer's diseases. Nothing is known about the mechanisms by which the abnormal genes produce their effects nor about a metabolic error underlying or accompanying these diseases. Suggestions for further approaches to the study of these diseases may be expected if experimental mammals were found with similar syndromes of abnormalities on a genetic basis. In this connection the description of hereditary premature senescence in rabbits appears to be interesting.

Pearce and Brown (1960a and b) described an acute and a chronic form of such a disease in the rabbit. The chronic form is more frequent. Degeneration of coat and skin, lesions of eyes and feet, general deterioration are described. The disease follows a progressive course with symptoms of muscle wasting, fat reduction, emaciation, all leading to early death.

In the acute form, the same symptoms are found to be more severe. The rate of progression of systemic degeneration is greater, and the animals die even earlier. Prior to death, deteriorative changes of the coat produce a prematurely senile appearance of the animals.

The authors planned a systematic study of the pedigree records and the breeding results in order to obtain information about the mode of inheritance of the condition. However, no report of such an analysis has appeared, so that we do not know whether the condition is determined by a single gene or by multiple factors. Since both authors are no longer alive, one would hope that the material is still available so that the work may be continued by other laboratories.

8. *Audiogenic seizures in mice.* It is well known that certain human individuals are subject to epileptic attacks which may be provoked by specific afferent stimuli. In addition to "photogenic epilepsy" which seems to run in families, musicogenic seizures are known. It may, therefore, be of

some interest to direct attention to a large body of investigations concerned with the genetic basis of susceptibility to audiogenic seizures in mice (Ginsburg, Miller and Zamis, 1950). Resistance and susceptibility to such seizures are determined by many genes, i.e., polygenes. Mice of the DBA/2 strain are most susceptible, whereas other strains, e.g., C57Bl/10, A/Jax, C3H are resistant (Hamburgh and Vicari, 1960).

It is interesting that the thyroid activity of the seizure susceptible DBA strain is twice as high as that of all other inbred strains tested (Chai, 1958).

c. Blood Dyscrasias

1. Macrocytic anemia. Studies of a hereditary, macrocytic, normochromic anemia produced by the series of W genes in the mouse have proven to be a rich source of information about the mode of genetic transmission in such diseases, the role of genes in controlling differences in the functioning of blood-forming tissue, and about various aspects of the genetics of transplantation of blood forming tissue between normal and genetically anemic mice [see E. S. Russell (1954) for review]. Such information should prove to be of great value when applied to similar blood abnormalities in man.

Erythrocyte numbers are reduced in the newborn from 4.8×10^6/ml. in normals to about 0.8×10^6/ml. in animals homozygous for one of the alleles, which die soon after birth, and to 2.2×10^6/ml. in viable animals homozygous for a less extreme allele. Sixteen day old embryos show an erythrocyte level of 0.5×10^6/ml. in the severely anemic genotype, and 1.3×10^6/ml. in the less extreme genotype, compared with 2.6×10^6/ml. in the normal embryo. Maturation of erythrocytes is arrested in the anemic mice prior to formation of mature erythroblasts. It is assumed that genes of the W-series block some stage of normal erythropoiesis, thus causing the development of macrocytic anemia.

Hemoglobin abnormalities were excluded as causal factors in this anemia. Studies with radioactive tracers showed normal formation of the globin portion of the hemoglobin molecule in anemic genotypes. On the other hand, similar studies in viable anemic mice revealed a significant reduction in the rate of protoporphyrin formation.

Experiments have been reported (E. S. Russell, 1960; E. S. Russell, Bernstein, Lawson and Smith, 1959) which show that the anemia of genetically anemic viable adults may be cured by transplantation of cells of normal embryonic liver. The normal blood picture obtained by such implantations was maintained for more than 500 days, i.e., throughout the normal life span of the mouse. Success, though in a smaller proportion of mice, was achieved even when anemic juvenile hosts, homozygous for the most extreme W-allele, received normal fetal liver cells by injection. There

seems to be competition in the injected anemic host between the large amount of its own defective cells, subject to maturation arrest, and the small amount of implanted normal erythropoietic tissue. As a result of this competition, the effect of the normal implant increases continuously in magnitude as the donor cells overcome the larger number of host cells, because of the higher proliferative capacity and the more rapid maturation rate of the normal erythropoietic tissue.

It seems hardly necessary to stress here the significance of this experimental material, present in the form of genetically anemic mice, for studies of the mechanisms of hereditary anemias in man and of the possible therapy of such anemias by implantation of normal erythropoietic tissue of the proper genetic constitution.

2. *Spherocytosis in Peromyscus.* An investigation of pale and jaundiced newborn mice in a colony of the deer mouse (Peromyscus) revealed the presence of spherocytosis, poikylocytosis and polychromasia in the blood smears of such anemic mice (Motulsky, Huestis and Anderson, 1956; Huestis et al., 1956). The anemia disappeared by the tenth day of life as the result of bone marrow erythroid hyperplasia. Consequently reticulocyte counts in the peripheral blood were elevated from 1.65 per cent in normal animals to 14.9 per cent in spherocytic animals. The spleens of the affected animals were enlarged.

Red cell survival was studied with the help of Chromium 51 and was shown to be shorter in spherocytic animals than in normals. Such spherocytes from abnormal animals also had a shortened life time when transfused into normal recipients. In fact, their lifetime in the normal host was even shorter than in the spherocytic host. Survival time of red cells was normal after splenectomy of the spherocytic mice. Apparently both normal and enlarged spleens destroy spherocytes, whereas normal red cells show normal survival time even in the presence of an enlarged spleen.

Hereditary spherocytosis in the deer mouse is due to an autosomal recessive gene. The expressivity of the gene varies, some animals being considerably more jaundiced than others. There are indications for the presence of genetic modifiers influencing the severity of the disease.

The resemblance of the mouse disease to human hereditary spherocytosis is indeed striking in its clinical and hematological features. Genetically, the human condition seems to be inherited as a dominant. The experimental material gives great promise for further studies of hereditary hemolytic disease.

3. *The Pelger anomaly.* An interesting hereditary abnormality of the granulocytes for which the rabbit supplies an experimental model, is the so-called Pelger anomaly inherited in man and rabbit as an incomplete

dominant. Heterozygotes have granulocytes with bilobed nuclei, described as rod-like, dumbbell or peanut-shaped. In homozygotes, the nuclei of the granulocytes do not segment at all. In addition, homozygotes have symptoms of chondrodystrophy and most of them die as early embryos. Those which survive to birth die soon thereafter.

For a more detailed description of this interesting anomaly the reader is referred to Nachtsheim's review (1958) where also a complete list of references to the original publications may be found. Nachtsheim reports, incidentally, the case of a little girl from a consanguinous marriage both of whose parents had the Pelger anomaly. This little girl had a blood picture closely resembling that of rabbits homozygous for this incompletely dominant gene.

d. Hereditary Deafness

Pathological changes associated with hereditary deafness in the mouse have been studied (Kocher, 1960) in a number of mouse mutants including those of the waltzer-shaker group in which deafness had been reported previously (Gluecksohn-Waelsch, 1959). These observations resemble to a large extent those described by Altman (1950) in hereditary human deafness. The detailed descriptions of the genetics, pathology and development of hereditary deafness in the mouse are thus of great potential value for further studies in man.

e. Hereditary Cataract of the Lens

The investigation of a hereditary cataract in mice (Paget and Baumgartner-Gamauf, 1961) showed this to be a dominant trait with a persisting hyaloid artery playing a possible causal role in the development of the cataract. This analysis is of potential medical interest in view of the close resemblance of the mouse cataract to congenital cataract in man.

An independent occurrence of cataract has been observed in our own mouse colony. Its genetics, pathology and development are being studied, and preliminary results indicate that they are different from those in the dominant cataract mentioned above; it is too soon to compare this new cataract with similar conditions in man. In view of the existence of many different forms of human cataract it would be interesting if the recent form of mouse cataract were different from the one studied previously (Paget and Baumgartner-Gamauf, 1961).

f. Vitamin A Utilization

An interesting study (Mauer, 1961) deals with the effects of vitamin A feeding to mice with mutations affecting skin and hair development. Cu-

taneous lesions have been described in vitamin A deficient humans and vitamin A has been employed in the treatment of rare familial diseases with skin and hair abnormalities. Such treatment in humans was based on the hypothesis that the skin defect in these diseases is correlated with a hereditary metabolic defect in the utilization of vitamin A by skin cells.

The administration of large doses of vitamin A to mice with hereditary skin and hair abnormalities resulted in modification of the hyperkeratotic process. However, systemic toxicity with liver dysfunction and cyrrhotic changes were produced by high doses of vitamin A. Mutants of one type were more sensitive to the excessive doses than those of another mutant type.

Mice with hereditary hyperkeratosis and hypotrichosis are assumed to suffer from an inborn lack or dysfunction of the ability to utilize vitamin A. This defect expresses itself primarily in the skin cells. The systemic effects of excess doses of vitamin A may possibly be related to a general inability of the mutants to utilize the vitamin.

g. Hyperglycemia and Diabetes

A recessive mutation in mice is responsible for the simultaneous occurrence of obesity and of hyperglycemia. Studies of blood serum and of pancreatic extracts have revealed increased insulin-like activity (Wrenshall, Andrus and Mayer, 1955; Christophe, Dagenais and Mayer, 1959; Mayer and Silides, 1958).

In this connection, attention is called to a report of hereditary diabetes in the hamster (Meier and Yerganian, 1959).

In view of the many questions still surrounding the problem of the genetic basis of diabetes in man, the existence of hereditary diabetes in experimental mammals assumes considerable significance.

h. Hereditary Jaundice

A mutation in the mouse causing neonatal jaundice (Stevens, Mackensen and Bernstein, 1959) should yield valuable material for studies of comparative biochemical genetics.

In man a form of hereditary hemolytic jaundice has been studied and found to be due to a gene-controlled failure of conjugation of bilirubin with glucuronic acid (Crigler and Najjar, 1952). This failure is the result of a deficiency of the enzyme glucuronyl transferase.

The same gene-controlled failure has been found in rats where, as a result of a recessive mutation, congenital jaundice was observed (Axelrod, Schmid and Hammaker, 1957). It is interesting that in this case a decrease of enzyme activity rather than complete absence was demonstrated in the mutants.

i. Osteoarthritis in Mice

Reference is made here to a report of osteoarthritis in mice which seems to be determined by polygenes (Sokoloff, Yamamoto, Crittenden and Joy, 1961).

j. Other Diseases

There exist a great many additional reports of specific genetic pathologies in mammals which resemble conditions in man. It is not possible within the framework of this review to refer to them all and reference is made once more to the excellent and comprehensive review of hereditary diseases of rodents by Nachtsheim (1958).

VIII. Cancer Susceptibility of Mice

A discussion of the inheritance of cancer susceptibility in experimental animals is outside the scope of this review. The reader is referred to the collection of papers in the volume "Genetics and Cancer" (1959) where most of the recent work in this field is discussed fully and where reference is made to the fundamental contributions of Heston, Law and others.

Since many genes are involved in the determination of cancer susceptibility and since environmental factors come into play significantly, ordinary methods of Mendelian genetics are not adequate for such studies, and methods of quantitative genetics must be applied. In this connection, attention is called to an exemplary study by Falconer and Bloom (1961) who applied such methods to the analysis of the relative role of heredity, environment and chance, in a study of the susceptibility of mice to the action of urethane which induces pulmonary tumors. In view of the multifactorial genetic basis of tumor susceptibility Bloom (1961) questions the validity of studies which attempt to determine linkage of tumor susceptibility and other genetic traits in mice as though single genes were responsible for such susceptibility.

IX. Genetics of Experimental "Allergic" Encephalomyelitis

Studies of experimental "allergic" encephalomyelitis revealed an interesting difference between mouse strains in susceptibility to the injection of brain tissue with adjuvants. Susceptible strains react with encephalomyelitis to such injections, whereas resistant strains are unaffected.

The resistance has been shown to be controlled by two pairs of genetic factors R_1 and r_1 and R_2 r_2 (Lee, Olitsky, Schneider and Zinder, 1954). The presence of either R_1 or R_2 in single dose confers resistance upon the carrier.

Backcrosses of F_1 between resistant ($R_1R_1R_2R_2$) and susceptible individuals ($r_1r_1r_2r_2$) to their resistant parents give all resistant offspring whereas backcrosses of such F_1 to susceptible parents give 25 per cent susceptible offspring. In the F_2, a ratio of 15 resistant to 1 susceptible is obtained. This is indeed a remarkable case of genetic control of resistance and susceptibility. The resistant strain (BRVR) is resistant also to Salmonella and the virus of St. Louis encephalitis, whereas the susceptible strain (BSVS) is susceptible to both of these infectious agents.

The genetically controlled resistance and susceptibility to experimental allergic encephalomyelitis are nutritionally dependent: folic acid, vitamin B_{12} and biotin have been found to be necessary nutritional components to maintain susceptibility (Schneider, 1959). The dependence of susceptibility rather than resistance on vitamins in the diet seems noteworthy and one looks forward to further investigations of the mechanism underlying this phenomenon.

There exist numerous examples in the literature of mammalian genetics which demonstrate the genetic basis of resistance or susceptibility to agents in addition to carcinogenic or encephalitogenic ones. This is true, for example, for the susceptibility to cortisone which produces cleft palate in the young of treated mothers in a high percentage in some strains and a low percentage in others (Fraser, 1961). The literature of experimental genetics is full of examples which point towards the genetic control of many varieties of resistance and susceptibility. Excellent summaries of such work have been prepared by Gowen (1962) and by Hutt (1958). This should be kept in mind in observations of individual variability among patients, as for example, in respect to drug reaction.

X. Biochemical Variations in Mammals

Biochemical genetics of mammalian organisms other than man with particular emphasis on multiple hemoglobins was discussed and reviewed about 2 years ago (Gluecksohn-Waelsch, 1960). Only some recent additions in this field will be reported here, particularly those which seem to have bearing on problems of medical genetics.

a. Multiple Hemoglobins in Mice

Two types of hemoglobin which may be separated by filter paper or starch electrophoresis have been reported to exist in different strains of mice (Gluecksohn-Waelsch, Ranney and Sisken, 1957; Ranney and Gluecksohn-Waelsch, 1955). Further studies failed to demonstrate additional hemoglobin types (E. S. Russell and Gerald, 1958). Genetic tests agree with the hypothesis that these two hemoglobins are controlled by a pair

of alleles with co-dominance: Hb^1 determines the presence of hemoglobin type I, and Hb^2 that of hemoglobin type II. F_1 hybrids show the presence of both type I and type II hemoglobins. A number of mouse strains are homozygous for Hb^1, others for Hb^2, and some strains, not highly inbred, segregate for the two types of hemoglobin.

Whereas hemoglobin type I in the mouse appears to be homogeneous, several components with different electrophoretic mobilities are found in type II mouse hemoglobin, indicating the existence of several molecular species and a genuine heterogeneity of this hemoglobin (Ranney, Smith and Gluecksohn-Waelsch, 1960). To account for this heterogeneity the following hypothesis has been proposed (Gluecksohn-Waelsch, 1960):

It is assumed that there exists in the mouse a gene locus which controls the synthesis of one of the polypeptide chains of the hemoglobin molecule. Mutations at this locus are responsible for the differences between hemoglobins type I and type II. This locus is complex and consists of several neighboring sites which might have arisen by duplication. In the case of type I hemoglobin, these sites are identical in their function resulting in the production of a homogeneous hemoglobin of a single molecular species. A mutation at one of these originally identical sites may lead to the production of a slightly different hemoglobin molecule in addition to the normal one produced at the neighboring unchanged site. Eventually, mutation may lead to the differentiation of 3 or 4 originally identical neighboring sites and consequently the production of 3 or 4 different hemoglobin molecules, all of them contained in the heterogeneous type II hemoglobin.

One may expect these different hemoglobin molecules to show slight differences in physiological properties, as e.g. oxygen-binding capacity. The existence of different molecular species of hemoglobin with different physiological properties might confer an adaptive advantage upon the carrier since the organism would have available a variety of hemoglobins to meet various specific requirements. This hypothesis would receive support from the demonstration of molecules with different polypeptide chains in the heterogeneous type II hemoglobin.

The existence of the A_2 component in normal human hemoglobin with δ-chains instead of β-chains is suggestive of similarly duplicated hemoglobin genes contained in a complex locus in man since the genes for δ- and β-chains appear to be linked and it has not been possible to separate A_2 genetically from the main component of human hemoglobin (Ingram and Stretton, 1961).

The study of multiple hemoglobins in the mouse is being continued in our laboratory. Current experiments are directed toward the analysis of the basis of the hemoglobin polymorphism in mouse populations. Experiments are under way to test the possible correlation of a particular type of mouse

hemoglobin with resistance to Plasmodium berghii. Interest in such studies stems from the reports on the distribution of malaria and different hemoglobins in human populations and the role of malaria in the maintenance of balanced polymorphism in the case of sickel-cell hemoglobin.

b. Phenylananine Hydroxylase in Dilute Mice.

Mice with a hereditary pigment deficiency were chosen for studies of metabolic abnormalities possibly associated with coat color dilutions (Coleman, 1960). Animals homozygous for the *d* (dilute) recessive gene were subjected to phenylalanine tolerance tests. These revealed limitation of tyrosine production in dilute homozygotes.

Further studies of conversion of phenylalanine to tyrosine in vitro with liver slices showed only 50 per cent of normal activity of phenylalanine hydroxylase in the liver of mice homozygous for *d*. Subsequently it could be shown that this was due to the presence of an inhibitor in the mutant liver homogenate. Thus, the dilute gene does not seem to control directly the enzyme phenylalanine hydroxylase but rather is responsible for the presence of an inhibitor or a competing enzyme. There is some indication for multiple gene action underlying the decrease of enzyme activity in these mutants.

This situation is different from that in human phenylketonuria where Jervis (1953) could show actual absence of the enzyme from the liver of individuals homozygous for the gene for phenylketonuria.

c. Transferrins (β-Globulins)

In connection with the interesting studies on polymorphism of human β-globulins (Smithies and Connell, 1959), attention is directed here to reports of polymorphism of β-globulins in mice. Ashton and Braden (1961) found three serum β-globulin types in mice determined by a pair of alleles β^A and β^B. Each allele controls three protein zones as shown by starch gel electrophoresis. An effect of parental β-globulin type on fertility has been shown in cattle (Ashton and Braden, 1961), but a similar effect on fertility in mice has not been studied as yet. The study by Ashton and Braden does not attempt to identify the β-globulins further by their iron binding property. Such identification of β-globulins as transferrins was accomplished in the case of another study (Cohen and Shreffler, 1961) which demonstrates the existence of a pair of co-dominant alleles Trf^a and Trf^b responsible for three phenotypes Trf-a, Trf-ab, Trf-b, in mice.

d. Serum Factor Variation

A possible association of a heritable serum variant with the H-2 locus has been reported (Shreffler and Owen, 1961). Five inbred lines have one type of serum factor and all carry the $H\text{-}2^K$ allele, and 8 inbred lines with

the other serum type all carry other *H-2* alleles. In 88 of 91 progeny tested, the two traits segregated together.

Preliminary studies which have tried to identify the serum antigen further suggest that the difference between the 2 serum types is quantitative. The component has the mobility of an α-globulin, probably of high molecular weight.

In the choice of topics for discussion in this chapter, a major omission will be noted, namely that of mammalian radiation genetics. The reader is referred to several excellent reviews, cited by Crow (1961) in the first volume of this series, for information and references to the literature and to the review by Russell in this volume. Because of its pertinence to problems of medical genetics today, this subject requires a discussion surpassing the framework of the present review.

XI. Conclusions and Prospects

At this point, one would like to stop and give some thought to future prospects of contributions by mammalian genetics to problems of human genetics.

In addition to the type of work discussed in the preceding pages, much of which will be continued with further valuable contributions, there seems to be great promise in studies of mammalian somatic cell genetics of the kind discussed in the Gatlinburg Symposium on "Genetic Approaches to Somatic Cell Variation" (1958). The work of Puck, who transferred methods from genetic and biochemical studies of microorganisms to the study of genetic and biochemical relations in somatic mammalian cells, is well known. In these studies, normal cells from different mammals as well as those from malignant tissues were used. There seems no limit to the problems of somatic cell variation which can be studied with these and similar methods as discussed at this symposium: genetic causes of cell variation in mammals, somatic antigen variation in erythrocytes (Atwood), antibody formation (Coons), cell population dynamics (Braun) and nucleo-cytoplasmic interaction in cell differentiation (Ephrussi and Stern).

Attention is called also to a recent extremely interesting paper by Sorieul and Ephrussi (1961) who demonstrated the occurrence of hybridization of somatic cells in vitro. They used two cells each derived from a single cell of mouse adipose tissue which differ from each other in terms of their karyotypes. In mixed cultures of these two cell types, it was possible to demonstrate up to 10 per cent hybrid cells characterized by chromosome counts which were equal to the sum of the individual chromosome numbers of the two cell types; identification of hybrid cells was accomplished also with the help of chromosome markers and configurations. The hybrid cells are assumed to be the result of fusion, demonstrating a kind of zygote for-

mation of somatic cells. This method and observation appear most promising for an approach to the various problems of somatic cell genetics mentioned above.

At this point I would like to make an addition to Joshua Lederberg's in his "appeal on behalf of the inbred mouse for sophisticated studies of somatic cell genetics." Lederberg (1958) in the summary comment to the symposium gives full credit to the "anthropocentric glamour" of using human tissues, and the standardization of HeLa cells. However, he warns of the unpredictable genetic constitution of the HeLa cells and the lack of a defined compatible host for retransplantation and he predicts failure to reach a definite conclusion with the use of human material.

The present chapter should add to this by calling attention to the existence of genetically well defined material in the form of mutant strains of mice, which should prove extremely useful in studies of somatic cell variation by making accessible to experimental approaches a variety of genetically caused deviations from the normal pattern of differentiation.

The solution of problems of the genetics of somatic cell variation will no doubt benefit greatly from a synthesis of concepts, methods and results of mammalian genetics with those derived from microbial studies and applied to mammalian cells.

Another area of experimentation where genetically defined material obtained from mouse mutants should prove useful and productive is that concerned with cell dissociation and cell aggregation (Moscona, 1959). These studies have contributed greatly to our understanding of the pattern of organization of embryonic tissues and have given us an insight into the role such developmental patterns may play in differentiation and morphogenesis.

For example, the mechanisms which lead to kidney agenesis might be studied by applying methods of cell dissociation and aggregation to the genetically abnormal kidney rudiments of Sd-mutants of mice. Similarly, changes leading to abnormal skeletal development and to chondrodystrophy in the mouse (Gluecksohn-Waelsch, Hagedorn and Sisken, 1956; Sisken and Gluecksohn-Waelsch, 1959) could be analyzed with such methods. In view of the existence of similar conditions, e.g., kidney agenesis and chondrodystrophy, in man such work would have important bearing on medical problems.

This review has attempted to deal with those contributions of mammalian genetics which appeared most pertinent and promising for the field of medical genetics. If it has succeeded in demonstrating to the reader the immediate pertinence of the many interesting contributions of mammalian genetics to problems of medical genetics, the author will feel that she has accomplished her purpose.

References

Altmann, F. 1950. Histologic picture of inherited nerve deafness in man and animals. Arch. Otolaryng. 51: 852–890.

Ashton, G. C., and A. W. H. Braden. 1961. Serum β-globulin polymorphism in mice. Australia J. Biol. Sc. 14: 248–253.

Auerbach, R. 1954. Analysis of the developmental effects of a lethal mutation in the house mouse. J. Exper. Zool. 127: 305–330.

——. 1960. Morphogenetic interactions in the development of the mouse thymus gland. Devel. Biol. 2: 271–284.

——. 1961. Genetic control of thymus lymphoid differentiation. Proc. Nat. Acad. Sc. 47: 1175–1181.

——. 1961. In press. Tissue culture in the analysis of developmental processes in mammals. Rutgers Symposium on Tissue Culture.

Axelrod, J., R. Schmid, and L. Hammaker. 1957. A biochemical lesion in congenital, non-obstructive, non-hemolytic jaundice. Nature 180: 1426–1427.

Beatty, R. A., and F. S. Billett. 1961. Genetics of Gametes. VI. Attempted genetic transformation in the rabbit. Proc. Roy. Soc. Edin. B 68: 83–90.

Bennett, D. 1956. Developmental analysis of a mutation with pleiotropic effects in the mouse. J. Morphol. 98: 199–234.

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. Acad. Sc. 48: 9–16.

Billingham, R. E., G. H. Sawchuck, and W. K. Silvers. 1960. Studies on the histocompatibility genes of the Syrian hamster. Proc. Nat. Acad. Sc. 46: 1079.

——, and W. K. Silvers. 1959. Studies on tolerance of the Y chromosome antigen in mice. J. Immunol. 85: 14–26.

——, and——. 1961. Transplantation of Tissues and Cells. Philadelphia. Wistar Institute.

Bloom, J. L. 1961. Pulmonary tumours and Group VII in the house mouse. Nature 191: 1124–1125.

Braden, A. W. H. 1960. Genetic influences on the morphology and function of the gametes. J. Cell. Comp. Physiol. 56: 17–29.

——, and S. Gluecksohn-Waelsch. 1958. Further studies of the effect of the T-locus in the house mouse on male fertility. J. Exper. Zool. 138: 431–452.

Cattanach, B. M. 1961. XXY Mice. Genet. Res. 2: 156–158.

Chai, C. K. 1958. Endocrine variation. Thyroid function in inbred and F_1 hybrid mice. J. Hered. 49: 143–148.

Christophe, J., Y. M. Dagenais, and J. Mayer. 1959. Increased circulating insulin-like activity in obese hyperglycemic mice. Nature 184: 61.

Cohen, B. L., and D. C. Shreffler. 1961. A revised nomenclature for the mouse transferrin locus. Genet. Res. 2: 306–308.

Coleman, D. L. 1960. Phenylalanine hydroxylase activity in dilute and non-dilute strains of mice. Arch. Biochem. 91: 300–306.

——. 1961. Effects of dietary creatine and glycine on transamidinase activity in dystrophic mice. Arch. Biochem. 94: 183–186.

Crigler, J. F., and V. A. Najjar. 1952. Congenital familial nonhemolytic jaundice with kernicterus. Pediatrics 10: 169–180.

Crow, J. F. 1961. Mutation in man. In Steinberg, A. G., Ed.: Progress in Medical Genetics. New York: Grune & Stratton, vol. 1, pp. 1–26.

Dunn, L. C. 1939–40. Heredity and development of early abnormalities in vertebrates. The Harvey Lectures, 35: 135–165.

——. 1954. The study of complex loci. Proc. IX Internat. Cong. Genet., Caryologia, (suppl.) 155–166.
——. 1956. Analysis of a complex gene in the house mouse. Cold Spring Harbor Symp. on Quant. Biol. 21: 187–196.
——, and S. Gluecksohn-Schoenheimer. 1939. The inheritance of taillessness (anury) in the house mouse. II. Taillessness in a second balanced lethal line. Genetics 24: 587–609.
——, and ——. 1943. Tests for recombination amongst three lethal mutations in the house mouse. Genetics 28: 29–40.
——, and ——. 1945. Dominance modification and physiological effects of genes. Proc. Nat. Acad. Sc. 31: 82–84.
Falconer, D. S. 1951. Two new mutants, "Trembler" and "Reeler" with neurological actions in the house mouse. J. Genet. 50: 192–201.
——, and U. Sierts-Roth. 1951. Dreher, Ein Neues Gen der Tanzmausgruppe bei der Hausmaus. Zeit. indukt. Abstammungs-und Vererb. 84: 71–73.
——, and J. L. Bloom. 1961. Inheritance of susceptibility to induced plumonary tumours in mice. Nature 191: 1070–1071.
Fawcett, D. W. 1957. Changes in the fine structure of the cytoplasmic organelles during differentiation. Developmental Cytology. New York: Ronald Press Co.
Ford, D. 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.
Fraser, F. C. 1961. Genetics and congenital malformations. In Steinberg, A. G., Ed.: Progress in Medical Genetics. New York: Grune and Stratton, vol. 1, pp. 38–80.
Genetics and Cancer. 1959. Austin: University of Texas Press.
Ginsburg, B. E., D. S. Miller, and M. J. Zamis. 1950. On the mode of inheritance of susceptibility to sound-induced seizures in the house mouse. Genetics 35: 109.
Gluecksohn-Schoenheimer, S. 1943. The morphological manifestations of a dominant mutation in mice affecting tail and urogenital system. Genetics 28: 341–348.
——. 1945. Dominance modification and physiological effects of genes. Proc. Nat. Acad. Sc. 31: 82–84.
Gluecksohn-Waelsch, S. 1954. Some genetic aspects of development. Cold Spring Harbor Symp. on Quant. Biol. 19: 41–49.
——. 1955. Genetic factors and the development of the nervous system. Biochemistry of the Developing Nervous System. New York: Academic Press, pp. 375–396.
——. 1959. Abnormalities of the nervous system of the mouse. Biology of Myelin. New York: Hoeber-Harper, pp. 108–121.
——. 1960. The inheritance of hemoglobin types and other biochemical traits in mammals. J. Cell. Comp. Physiol. 56: 89–101.
——. 1961. Developmental genetics of mammals. Am. J. Human. Genet. 13: 113–121.
——, D. Hagedorn, and B. F. Sisken. 1956. Genetics and morphology of a recessive mutation in the house mouse affecting head and limb skeleton. J. Morphol. 99: 465–479.
——, H. M. Ranney, and B. F. Sisken. 1957. The hereditary transmission of hemoglobin differences in mice. J. Clin. Invest. 36: 753–756.
Gordon, M. J. 1957. Control of sex ratio in rabbits by electrophoresis of spermatozoa. Proc. Nat. Acad. Sc. 43: 913–918.
Gorer, P. A. 1937. The genetic and antigenic basis of tumour transplantation. J. Path. & Bact. 44: 691–697.
Gowen, J. W. 1962. Transactions of the Second Conference on Genetics, Josiah Macy Jr. Foundation.

Grüneberg, H. 1943. Congenital hydrocephalus in the mouse, a case of spurious pleiotropism. J. Genet. 45: 1–21.

Gullbring, B. 1957. Investigation on the occurrence of blood group antigens in spermatozoa from man, and serological demonstration of the segregation of characters. Acta med. scandinav. 159: 169–172.

Hadorn, E. 1961. Developmental genetics and lethal factors. London: Methuen & Co., Ltd.

Hamburgh, M. 1958. The distribution of acetylcholinesterase in the mouse brain. Anat. Rec. 130: 311.

——. 1960. Observations on the neuropathology of "Reeler," a neurological mutation in mice. Experientia 16: 460.

——, and E. Vicari. 1960. A study of some physiological mechanisms underlying susceptibility to audiogenic seizures in mice. J. Neuropath. & Exper. Neurol. 19: 461–472.

Hazzard, W. R., and S. L. Leonard. 1959. Phosphoglucomutase activity in hereditary muscular dystrophy in mice. Proc. Soc. Exper. Biol. & Med. 102: 720–722.

Heustis, R. R., R. S. Anderson, and A. G. Motulsky. 1956. Hereditary spherocytosis in Peromyscus. J. Hered. 47: 225–228.

Hoecker, G. 1956. Genetic mechanisms in tissue transplantation in the mouse. Cold Spring Harbor Symp. on Quant. Biol. 21: 355–362.

Hutt, F. B. 1958. Genetic Resistance to Disease in Domestic Animals. Comstock Publishing Associates, Cornell University Press.

Ingram, V. M., and A.O.W. Stretton. 1961. Human Haemoglobin A_2: Chemistry, Genetics and Evolution. Nature: 190: 1079–1085.

Jacob, F., and J. Monod. 1961. On the regulation of gene activity. Cold Spring Harbor Symposia of Quantitative Biology. 26: 193–211.

Jervis, G. A. 1953. Phenylpyruvic oligophrenia deficiency of phenylalanine-oxidizing system. Proc. Soc. Exper. Biol. & Med. 82: 514–515.

Kelton, D. E. 1961. Myelin degeneration in the central nervous system of dilute-lethal mice. Records of the Genetics Society of America. 30: 84–85.

Kocher, W. 1960. Untersuchungen zur Genetik und Pathologie der Entwicklung spät einsetzender hereditärer Taubheit bei der Maus. Archiv. Ohren usw. Heilk. u. Z. Hals usw. Heilk. 177: 108–145.

Lee, J. M., P. K. Olitsky, H. A. Schneider, and N. D. Zinder. 1954. Role of heredity in experimental disseminated encephalomyelitis in mice. Proc. Soc. Exper. Biol. & Med. 85: 430–432.

Little, C. C. 1914. A possible Mendelian explanation for a type of inheritance apparently non-Mendelian in nature. Science 40: 904–906.

——. 1941. Biology of the laboratory mouse. Philadelphia: Blakiston Co.

——, and E. E. Tyzzer. 1916. Further studies on inheritance of susceptibility to a transplantable tumor of Japanese waltzing mice. J. Med. Res. 33: 393–425.

Loeb, L. 1901. Transplantation of tumor in rats. J. Med. Res. 6: 28–38.

——. 1902. Transplantation of benign tumors in the rat. J. Med. Res. 7: 44–82.

Loosli, R., E. S. Russell, W. K. Silvers, and J. L. Southard. 1960. Variability of incidence and clinical manifestation of mouse hereditary muscular dystrophy on heterogeneous backgrounds. Genetics 46: 347–355.

Lyon, M. F., and R. J. S. Phillips. 1959. Crossing over in mice heterozygous for t-alleles. Heredity 13: 23–32.

——. 1960. Zigzag: a genetic defect of the horizontal canals in the mouse. Genet. Res. 1: 189–195.

——. 1961. Linkage relations and some pleiotropic effects of the dreher mutant of the house mouse. Genet. Res. 2: 92–95.

——. 1962. Sex chromatin and gene action in the mammalian X-chromosome. Am. J. Human Genet. 14: 135.

——. 1961. Gene action in the X-chromosome of the mouse. (Mus musculus L.) Nature 190: 372–373.

Martinez, A., and J. I. Sirlin. 1955. Neurohistology of the Agitans mouse. J. Comp. Neurol. 103: 131–138.

Mauer, I. 1961. The effect of vitamin A in hyperkeratotic mouse mutants. J. Exp. Zool. 146: 181–207.

McLaren, A. 1960. New evidence of unbalanced sex-chromosome constitutions in the mouse. Genet. Res. 1: 256–261.

Mayer, J., and Silides, O. J. 1958. Fat metabolism in experimental obesities. VIII. Blood total lipids and ketones in four kinds of obese mice. Experientia 14: 96.

Meier, H., and G. A. Yerganian. 1959. Spontaneous hereditary diabetes mellitus in Chinese hamster. 1. Pathological findings. Proc. Soc. Exper. Biol. & Med. 100: 810–815.

Michelson, A. M., E. S. Russell, and P. J. Harman. 1955. Dystrophia Muscularis: A Hereditary Primary Myopathy in the House Mouse. Proc. Nat. Acad. Sc. 41: 1079–1084.

Moscona, A. A. 1959. Patterns and mechanisms of tissue reconstruction from dissociated cells. Developing Cell Systems. New York: Ronald Press.

Motulsky, A. G., R. R. Huestis, and R. Anderson. 1956. Hereditary spherocytosis in mouse and man. Acta. Genet. Stat. Med. 6: 240–245.

Nachtsheim, H. 1958. Erbpathologie der Nagetiere Pathologie der Laboratoriumstiere: 309–452. Berlin: Springer-Verlag.

Ohno, S., and T. S. Hauschka. 1960. Allocycly of the X-chromosome in tumors and normal tissues. Cancer Res. 20: 541–545.

——, W. D. Kaplan, and R. Kinosita. 1959. On the end-to-end association of the X and Y chromosomes of Mus musculus. Exper. Cell. Res. 18: 382–384.

Owen, R. D. 1959. Genetic aspects of tissue transplantation and tolerance. J. Med. Ed. 34: 366–384.

——. 1960. Current status of mammalian immunogenetics. J. Cell. Comp. Physiol. 56: 73–87.

Paget, O. E., and M. Baumgartner-Gamauf. 1961. Histologische Untersuchungen an einer dominant erblichen Form einer Cataract bei der Hausmaus. Zool. Anz. 166: 55–69.

Paigen, K. 1961. The genetic control of enzyme activity during differentiation. Proc. Nat. Acad. Sc. 47: 1641–1649.

——, and W. K. Noell. 1961. Two linked genes showing a similar timing of expression in mice. Nature 190: 148–150.

Pearce, L., and W. H. Brown. 1960a. Hereditary premature senescence of the rabbit. I. Chronic Form; General Features. J. Exper. Med. 111: 485–503.

——, and ——. 1960b. Hereditary premature senescence of the rabbit. II. Acute Form: General Features. J. Exper. Med. 111: 505–516.

Phillips, R. J. S. 1958. "Lurcher," a new gene in linkage group XI of the house mouse. J. Genet. 57: 35–42.

Pontecorvo, G. 1958. Trends in genetic analysis. New York: Col. Univ. Press.

Ranney, H. M., and S. Gluecksohn-Waelsch. 1955. Filter-paper electrophoresis of mouse haemoglobin: Preliminary note. Ann. Human Genet. 19: 269–272.

——, G. M. Smith, and S. Gluecksohn-Waelsch. 1960. Haemoglobin differences in inbred strains of mice. Nature 188: 212–214.

Ross, M. H., G. D. Pappas, and P. J. Harman. 1960. Alterations in muscle fine structure in hereditary muscular dystrophy of mice. Lab. Invest. 9: 388–403.

Russell, E. S. 1954. Review of the pleiotropic effects of W- series genes on growth and differentiation. Aspects of Synthesis and Order in Growth. New Jersey: Princeton U. Press, pp. 113–126.

——. 1960. Genetic aspects of implantation of blood-forming tissue. Fed. Proc. 19: 573–578.

——. 1961. Genetic studies of muscular dystrophy in the house mouse. Second International Conference of Human Genetics, Rome, 1961.

——, S. E. Bernstein, F. A. Lawson, and L. J. Smith. 1959. Long-continued function of normal blood-forming tissue transplanted into genetically anemic hosts. J. Nat. Cancer Inst. 23: 557–566.

——, and P. S. Gerald. 1958. Inherited electrophoretic hemoglobin patterns among 20 inbred strains of mice. Science 128: 1569–1570.

Russell, L. B., and E. H. Y. Chu. 1961. An XXY male in the mouse. Proc. Nat. Acad. Sc. 47: 571–575.

Russell, W. L., L. B. Russell, and J. S. Gower. 1959. Exceptional inheritance of a sex-linked gene in the mouse explained on the basis that the X/O sex-chromosome constitution is female. Proc. Nat. Acad. Sc. 45: 554.

St. Whitelock, O. v (Ed.) 1958. Immunogenetics of tissue transplantation. Third Tissue Homotransplantation Conference 73: 590–686.

Schneider, H. A. 1959. Genetic and nutritional aspects of experimental "allergic" encephalomyelitis. "Allergic" Encephalomyelitis. Springfield, Ill.: Charles C. Thomas.

Shreffler, D. C. and R. D. Owen. 1961. Association between a heritable serum variant and the histocompatibility-2 locus in the mouse. Records of the Genetics Society of America. 30: 108.

Sisken, B. F. and S. Gluecksohn-Waelsch. 1959. A developmental study of the mutation "Phocomelia" in the mouse. J. Exper. Zool. 142: 623–641.

Smithies, O. and G. E. Connell. 1959. Biochemical aspects of the inherited variations in human serum haptoglobins and transferrins. Biochemistry of Human Genetics, CIBA Foundation Symposium, pp. 178–193.

Snell, G. D. 1956. A comment on Eichwald and Silmser's communication. Communication. Transpl. Bull. 3: 29–31.

——. 1957. The genetics of transplantation. Ann. New York Acad. Sc. 69: 555–560.

——. 1958. Histocompatibility genes of the mouse. II. Production and analysis of isogenetic resistant lines. J. Nat. Cancer Inst. 21: 843–877.

Sokoloff, L., R. S. Yamamoto, L. B. Crittenden, and G. E. Joy, Jr. 1961. Genetics of degenerative joint diseases in mice. Arth. & Rheumat. 4: 440.

Sorieul, S. and B. Ephrussi. 1961. Karyological demonstration of hybridization of mammalian cells in vitro. Nature 190: 653–654.

Stern, C. 1960. Genetic influences on the morphology and function of the gametes. J. Cell. Comp. Physiol. 56: 27.

Stevens, L. C., J. A. Mackensen, and S. E. Bernstein. 1959. A mutation causing neonatal jaundice in the house mouse. J. Hered. 50: 35–39.

——, E. S. Russell, and J. L. Southard. 1957. Evidence on inheritance of muscular dystrophy in an inbred strain of mice using ovarian transplantation. Proc. Soc. Exper. Biol. & Med. 95: 161–164.

Symposium on Genetic Approaches to Somatic Cell Variation. 1958. J. Cell. Comp. Physiol. (suppl. 1.) 52.

Tyzzer, E. E. 1909. A study of inheritance in mice with reference to their susceptibility to transplantable tumors. J. Med. Res. 21: 519.

Welshons, W. J., and L. B. Russell. 1959. The Y-chromosome as the bearer of male determining factors in the mouse. Proc. Nat. Acad. Sc. 45: 560.

Wermer, P. 1954. Genetic aspects of adenomatosis of endocrine glands. Am. J. Med. 25: 363–371.

Wrenshall, G. A., S. B. Andrus, and J. Mayer. 1955. High levels of pancreatic insulin co-existent with hyperplasia and degranulation of beta cells in mice with hereditary obese-hyperglycemic syndrome. Endocrinology 56: 335.

Chapter 9

Progress in Ophthalmic Genetics

J. François
University of Ghent,
Ghent, Belgium

I. Varieties of Inherited Ocular Diseases

Some ocular diseases such as albinism and pigmentary retinitis are always and exclusively genetic in origin, whereas others such as wounds from trauma or operation are exclusively external in origin. In addition, there is a series of intermediate diseases in which both hereditary and external factors are important to a varying extent.

It is not surprising, therefore, that it becomes difficult sometimes to differentiate between an essentially hereditary disease and an essentially exogenous disease—in other words—to distinguish between a phenotype and a phenocopy.

It is for this reason that the hereditary character of many diseases of the eye has been ascertained only after long delays or is still in doubt.

Furthermore, just as a hereditary factor can influence an exogenous disease, so also can an external factor modify the clinical picture of a hereditary disease.

It was believed for a long time that an inherited disease is not only congenital, but also static, in that the clinical picture could not be modified or evolved. We now know that this idea is incorrect. In fact, a congenital abnormality which is itself the outcome of a prenatal evolution can become further modified during life. Also, and this happens frequently, a hereditary disease can manifest itself some time after birth or even quite late in life and develop for a period of years. Examples are pigmentary retinopathy, senile degeneration of the macula, xeroderma pigmentosum, etc.

All of these considerations show that it is instructive to classify inherited diseases into the following main clinical groups (Sorsby, 1951).

Congenital Defects

Abiotrophies. The term abiotrophy is applied to progressive, partial, or complete destruction of a tissue which has begun to develop normally. This is not brought about by an inflammation or trauma but by the hereditary causation of premature tissue death.

Examples in ophthalmology include chorioretinal peripheral degenerations (such as pigmentary retinopathy), central degenerations (such as degeneration of the macula), corneal dystrophies, some cataracts, some glaucomas, Leber's optic atrophy, and other diseases.

Phakomatoses or neuroectodermal dysplasias. Van der Hoeve studied the relations between the following four syndromes which affect the nervous system, the eye, and the skin: Recklinghausen's neurofibromatosis, von Hippel-Lindau's cerebroretinal angiomatosis, Sturge-Weber-Dimitri's neurocutaneous angiomatosis, and Bourneville's cerebral tuberous sclerosis. Van de Hoeve groups these under the term phakomatoses.

Malignant tumors.

Endocrine disturbances.

Metabolic disturbances.

Functional abnormalities. In patients with purely functional abnormalities known to be congenital, such as color blindness and essential hemeralopia, morphological examination of the eye supplies no information.

Syndromes. Syndromes are often hereditary, sometimes congenital, sometimes abiotrophic, and sometimes mixed. An example of the first is macular coloboma and apical dystrophy of the hands and feet (Sorsby's syndrome). An example of the second group is the generalized elastorrhexis with angioid streaks of the retina (Groenblad-Strandberg-Touraine syndrome). As an example of mixed syndromes, one can mention that of Bardet-Biedl in which the polydactylia is congenital and the pigmentary retinopathy abiotrophic.

II. Patterns of Inheritance of Ocular Diseases

Hereditary ocular diseases and defects are transmitted in various ways. A summary of the modes of inheritance of the common ocular disorders has recently been compiled in tabular form (François, 1961).

III. Carriers of Genes and their Detection

The term carrier of a gene is applied to an individual who can transmit a hereditary disease without himself being manifestly affected by this disease (this does not, of course, apply to cases of true dominance). The

carrier is sometimes apparently normal, but he might show minor abnormalities which permit his identification. This section is devoted to the examination of such minor abnormalities, microsymptoms of attenuated forms of the disease.

It is in fact very important to be able to recognize the carriers of pathologic genes: first, from a scientific point of view, because it might help in understanding the mechanism of gene action by comparison of the minor manifestations in the carrier with the major manifestations in the phenotypically affected individual, and second, from a practical point of view, because the discovery of the carrier might permit the working out of a method of transmission and a precise genetic prognosis.

We can distinguish three groups of carriers of genes: (1) individuals heterozygous for an incompletely recessive gene, (2) individuals carrying a dominant gene of delayed expression, and (3) individuals carrying a gene which is dominant but incompletely penetrant.

Individuals Heterozygous for an Incompletely Recessive Gene

A. *Intermediate sex-linked inheritance.* It is now known that some sex-linked genes are neither strictly dominant nor recessive but give rise to intermediate phenotypes in heterozygous women. Actually, we know at least eight diseases of the eye which follow this form of inheritance.

Choroideremia. In men (Fig. 1), a progressive loss of visual function begins at an early age (reduction of the central vision and of the visual field, hemeralopia), and the choroid and retina atrophy completely. In heterozygous women (Fig. 2), there is no functional sign whatsoever, but only certain abnormalities in the interior of the eye, namely, irregular peripheral pigmentation, sometimes depigmented retinal zones, and peripapillary atrophy (Goedbloed, 1942; McCulloch, 1948; Sorsby et al., 1952; personal observation).

Sex-linked chorioretinal degeneration or pigmentary retinopathy. This is a rare form of pigmentary degeneration of the retina, characterized in man by typical pigmentary retinopathy (Fig. 3) and progressive choroidal sclerosis leading to complete blindness.

In heterozygous or carrier women (Fig. 4), one finds a tapetoretinal reflex—brilliant, scintillating, golden hued, patchy, and distributed around the macula or over the whole retina but more pronounced in the macular and perimacular regions and gradually diminishing toward the periphery. This refiex is perhaps due to the irregular density of the amina vitrea, or more probably it is localized in the retina itself. It is never accompanied by any visual complaint (Falls and Cotterman, 1948; personal observation).

It appears to us that there is no fundamental difference between choroideremia and sex-linked pigmentary retinopathy, as we have seen the evolu-

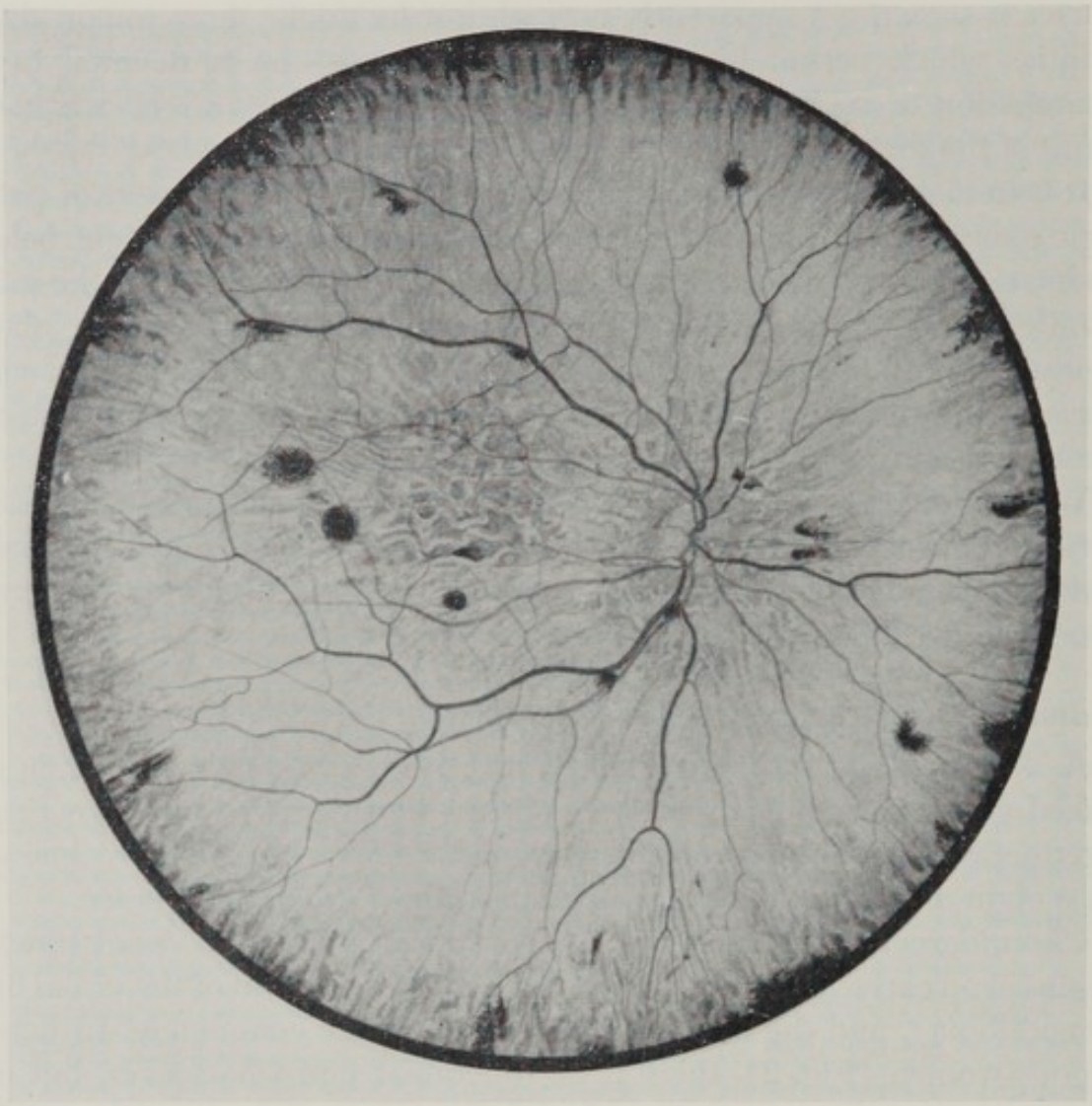


FIG. 1—Choroideremia (personal observation).

tion of this disease toward a real choroideremia (total atrophy of the choroid and the retina). It must also be pointed out that some exceptional cases of choroidal sclerosis are sex-linked with alterations of the retina in the carrier.

Sex-linked ocular albinism. In men (Fig. 5), the iris is only slightly pigmented and therefore diaphanous, and the pupillary reflex is characteristic. The fundus is depigmented and the choroidal vessels are clearly visible, whereas the macula is hypoplastic and without reflex. Nystagmus, head nodding and bad vision also occur. There are no signs of albinism outside the eyes; the hair is brown and the complexion normal (Falls, 1951; François and Deweer, 1952).

In heterozygous women (Fig. 6), the fundus shows two different regions with regard to pigmentation. The posterior pole, particularly the macular region, looks reasonably normal. There is a pigmentary peppering, however, which is quite obvious, regular, and homogeneous, and the

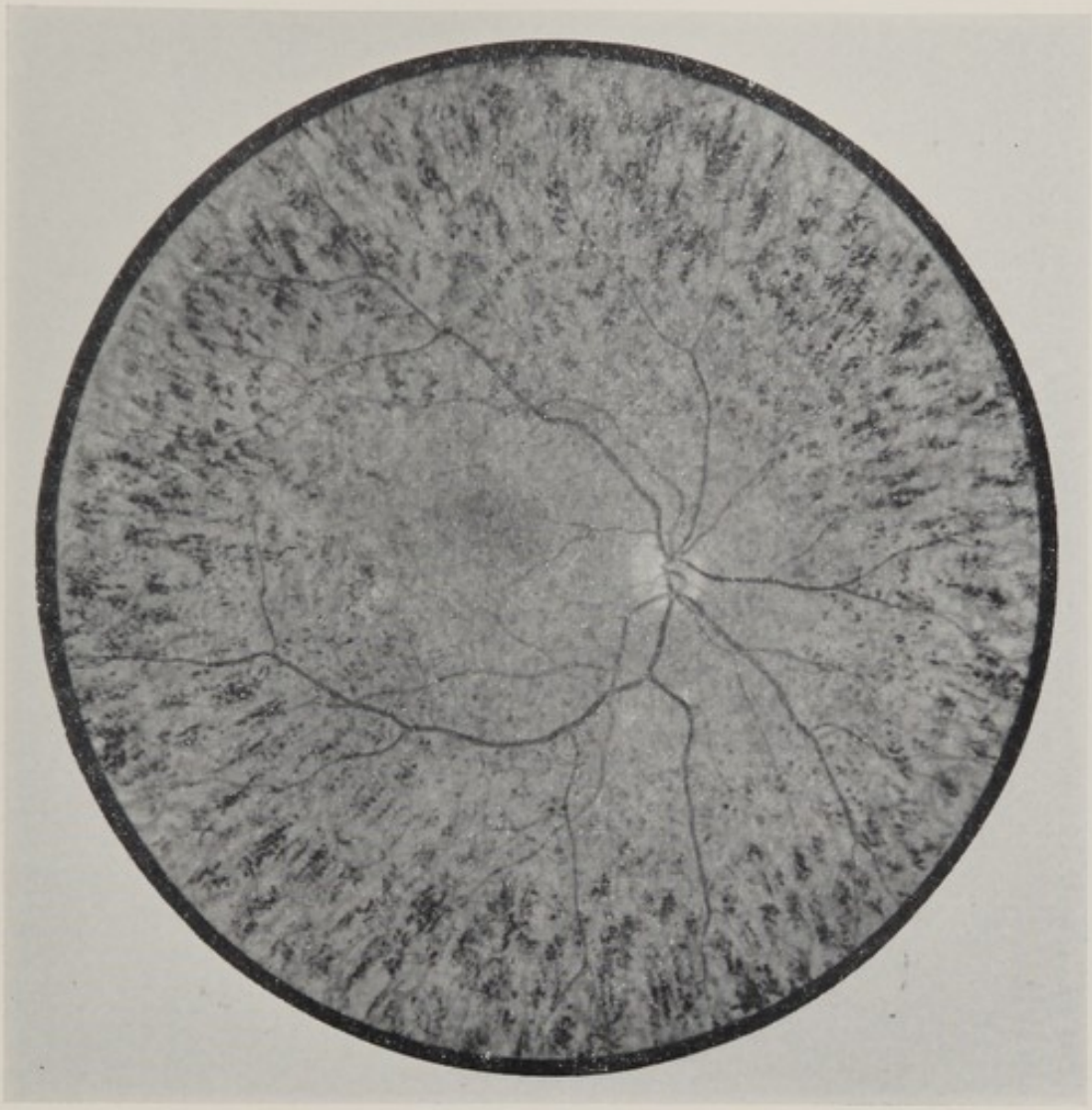


FIG. 2—Fundus of the eye of a woman carrying a gene for choroideremia (personal observation).

choroidal vessels are not visible. Outside the posterior pole and all around the periphery is pigmentary dust, brown-black and contrasting with a clear background, but the vessels of the choroid are still not particularly visible. The pigment granules are accumulated into small heaps, irregularly distributed, with hypopigmented areas in between. The alteration of clear and dark patches resembles a geographic map.

Heterozygous women show a transparent iris; despite the apparently normal pigmentation, the iris transmits light with transscleral illumination (Waardenburg, 1947). Furthermore, there is no functional disorder.

Keratosis follicularis spinulosus with ophiasis. In men, there is alopecia with loss of eyebrows, eyelashes, and beard, thickening of the eyelids with blepharitis and ectropion, corneal degeneration (characterized by diffused epithelial mottling and peripheral superficial vascularization in the form of arches), photophobia, and lacrimation (Siemens, 1925; Falls,

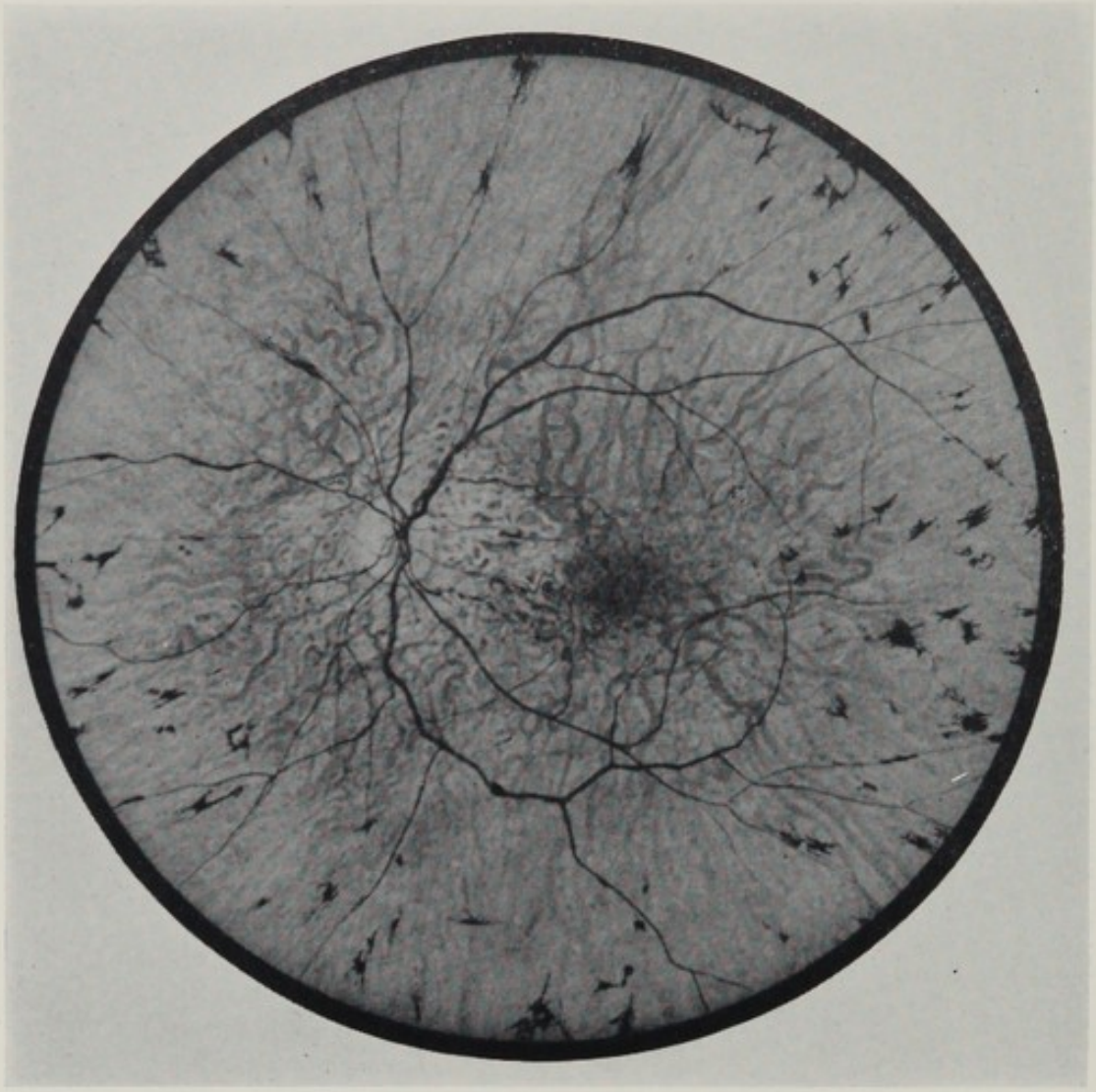


FIG. 3—Pigmentary sex-linked retinopathy (personal observation).

1954). There can be other mucosal lesions, and the skin of the neck, ears, and extremities, especially the palm of the hand and sole of the foot, are thickened (follicular keratosis). In heterozygous women, only discrete cutaneous changes at the extremities (discrete follicular keratosis) appear and sometimes slight changes in the corneal epithelium.

External congenital ophthalmoplegia with myopia. In men, there is complete bilateral ptosis, complete or incomplete ophthalmoplegia with strabismus, corectopia with dyscoria or anisocoria, and myopia with degenerative and progressive changes in the retina and choroid. This may be accompanied by other abnormalities, such as absence of the rotular and Achilles reflexes, spina bifida, flatfoot, enuresis, cardiopathy, hernia and dental and thoracic malformations.

In heterozygous women there is an absence of the rotular and Achilles reflexes but no ocular lesions (Salleras and de Zarate, 1950).

FIG. 4—Tapeto retinal reflection in a carrier of the gene of pigmentary sex-linked retinopathy (personal observation).

Megalocornea. In some families this disease is transmitted as an autosomal dominant, but in others it is sex linked. In the latter case, heterozygous women show a slight increase in the corneal diameter (Riddell, 1941).

Dyschromatopsia. The usual dyschromatopsias of the protan and deutan types are associated with a recessive gene carried by the X-chromosome. Heterozygous women may present some abnormalities of color vision, e.g., Schmidt's sign.

Congenital Ectodermal Anhidrotic dysplasia. This disease is characterized by absence of teeth, lanuginous hair, hypotrichosis, ozena, absence of sweat glands and sometimes absence of lacrimal glands and eyelashes. It is transmitted sometimes as an autosomal dominant and sometimes as a sex-linked gene. In the latter case, men present the complete clinical pic-

FIG. 5—Sex-linked ocular albinism (personal observation).

ture, whereas heterozygous women show only reduction or malformation of the teeth and abnormalities of the sweat glands and breasts (Grant and Falls, 1944).

When this disease is transmitted as an autosomal dominant, signs may be restricted to abnormalities of the nails (Franceschetti, 1953).

Recessive autosomal inheritance. In some persons, the gene is not fully recessive, and in consequence the heterozygote is not entirely normal but shows a minor or intermediate abnormality.

Fuch's gyrate atrophy of the choroid and retina. This is a very rare heredodegenerative disease with slowly progressive atrophy of the choroid, the pigment epithelium, and the retina. In a case we investigated personally (a girl born of a consanguineous marriage), we found, moreover, a constitutional and hereditary anomaly of the leukocytes. This anomaly was present not only in the girl, but also in both parents and is

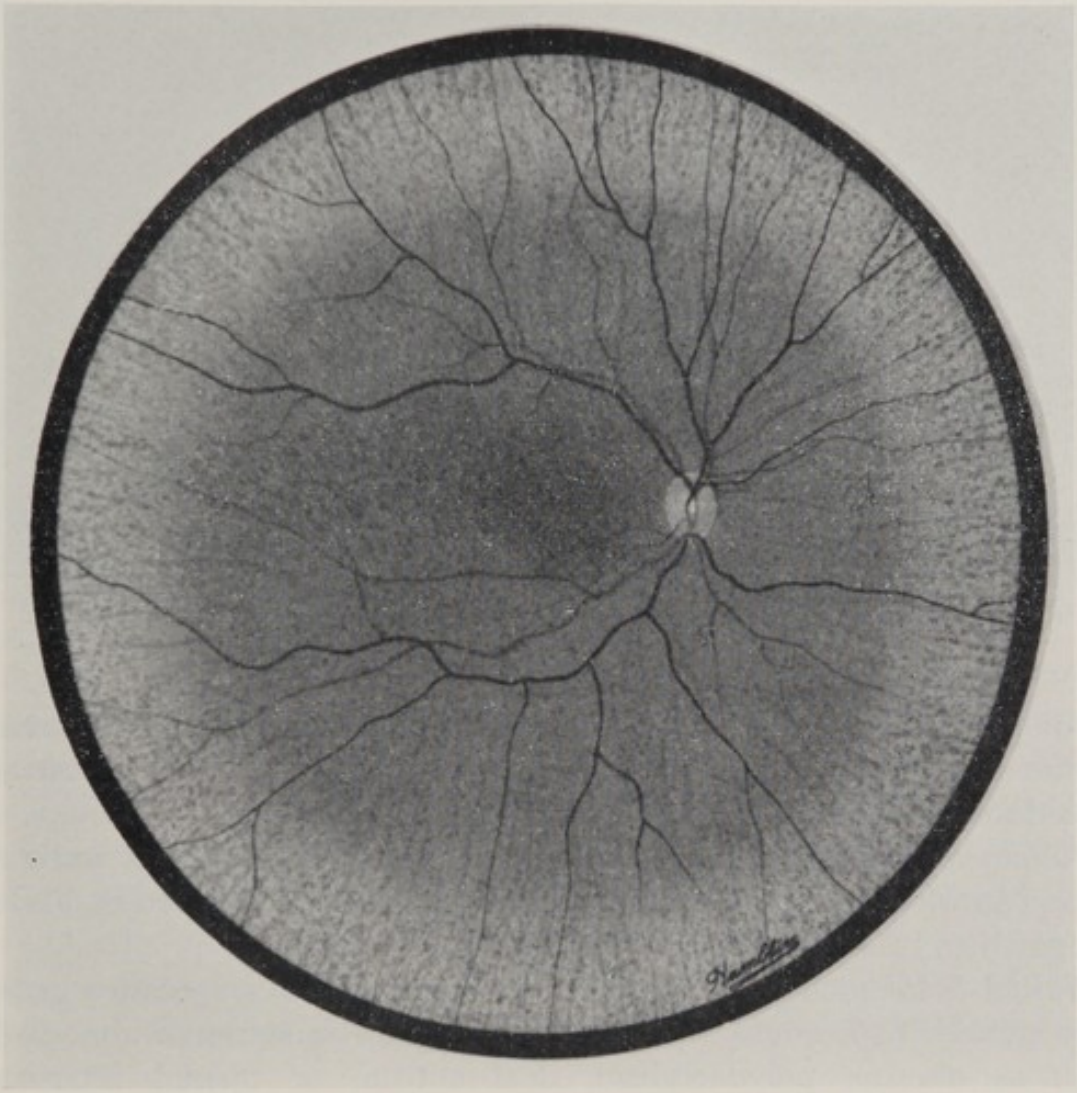


FIG. 6—Fundus of the eye of a woman carrying a gene for ocular albinism (personal observation).

transmitted as a dominant through four generations. The polynuclear leukocytes, as well as the basophiles and eosinophils, contain numerous azurophilic granulations (Alder's anomaly). It is suggested that Alder's anomaly may represent the effect of the gene for gyrate atrophy in heterozygous state.

Juvenile amaurotic idiocy. In the heterozygote there is an increase of vacuolated lymphocytes in the blood (Rayner, 1952).

Albinism. The heterozygote shows only a transparent iris which is traversed by light in transscleral illumination (Waardenburg, 1947). This is entirely independent of the apparent degree of iris pigmentation. Occasionally, one finds hypoplasia of the fovea and some reduction of visual acuity.

Wilson's disease or hepatolenticular degeneration. This disease is characterized by Kayser-Fleischer rings (Fig. 7) and a decreased synthesis of

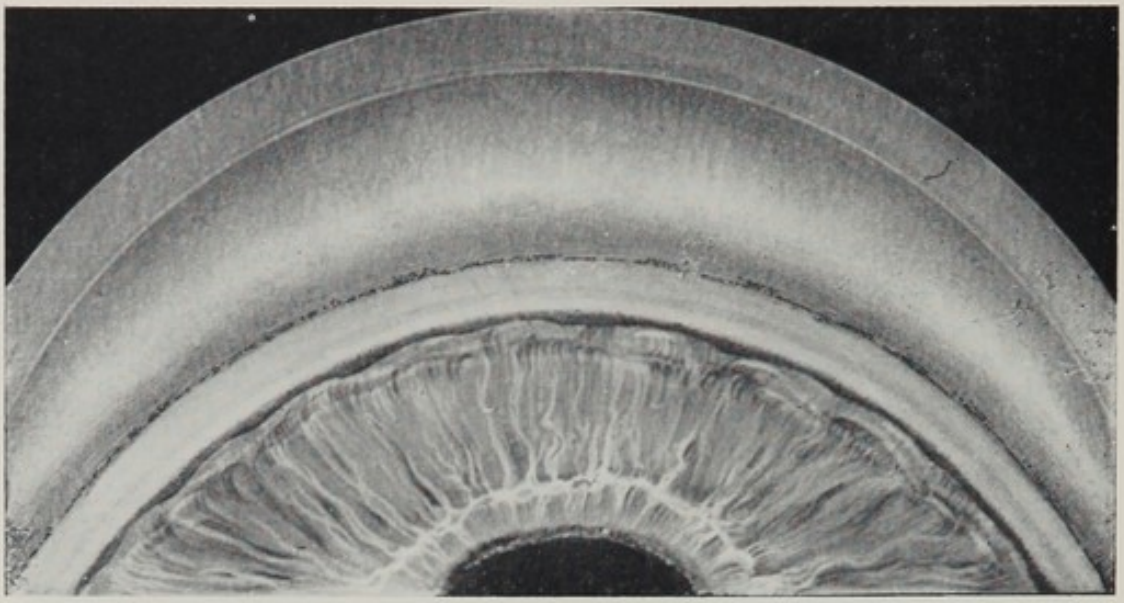


FIG. 7—Kaiser-Fleischer ring in Wilson's disease (personal observation).

ceruloplasmin (Bearn, 1953; Turpin and collaborators, 1953). An increased amino acid excretion also may occur. The heterozygote may exhibit a reduced serum ceruloplasmin concentration (Bearn, 1960).

Keratoconus. This ocular abnormality is sometimes dominant and sometimes recessive. In the latter case, the carrier can be recognized by his high astigmatism (Sorsby, 1951).

Bardet-Biedl syndrome. This syndrome is linked to a recessive gene of very variable expression. The carrier might show some minor abnormality such as obesity, polydactylism, deaf-mutism, or mental retardation (Sorsby, 1951; J. François, Bekaert and Stefens, 1953).

Individuals Carrying a Dominant Gene of Delayed Manifestation

In autosomal dominant inheritance with delayed manifestation, the carrier of the gene appears healthy at first but at some stage of adolescence, or later, up to old age, will become obviously ill, or persons will exhibit only a minor abnormality.

The conviction is spreading that numerous degenerative diseases of old age have a hereditary basis. We are referring not only to certain diseases of the eye such as glaucoma or senile macular degeneration (François and Deweer, 1952), but also to some general diseases such as essential arterial hypertension, gout, or rhizomelic spondylosis, the ocular complications of which are well known.

Primary glaucoma. Detailed investigations of family trees show that both open-angle and closed-angle glaucomas may be hereditary and may be due to an autosomal dominant gene (François, Deweer and van den

Berghe, 1951; Weekers, Gougnard-Rion and Gougnard, 1955). The detection of the beginning of glaucoma will be greatly facilitated by systematic examination of relatives and descendants.

It seems that open-angle glaucoma is caused by a gene which affects the outflow resistance of aqueous humor, but in congestive glaucoma (closed-angle glaucoma) the gene carries a tendency toward narrowing of the iridocorneal angle.

Fuch's epithelial and endothelial dystrophy. This dystrophic disease is due to an irregularly dominant gene with incomplete penetrance and is more pronounced in the female (Falls, 1954). Generally, it appears only after 50 years of age and is characterized by vacuoles or epithelial bullae, by thickening of the corneal parenchyma especially toward the center, and also by alterations in the endothelium. The carrier shows droplets at the center of the corneal epithelium (cornea guttata) or shagreened endothelium.

Xeroderma pigmentosum. This disease is a recessive, partially sex-linked character according to Fisher (1936) and Haldane (1936), a recessive autosomal one according to Bauer (1940), a dominant partially sex-linked one according to Anderson and Begg (1950); recent analysis indicates that it is most likely an incompletely dominant autosomal character which is sex influenced (Morton, 1957). The carrier may show numerous ephelides and sometimes pigmented nevi (Hoede, 1940).

Rhizomelic spondylosis is probably due to a dominant gene of feeble penetrance. It can be spotted early by roentgenography of the lumbosacral and sacroiliac joints.

In pleiotropic or polyphenic syndromes, it is rare for one individual to show all the specific manifestations of the gene at the same time. The carriers can be found only by careful study of all members of an affected family.

Phakomatoses. The dominant gene responsible for *Recklinghausen's neurofibromatosis* has essentially complete penetrance but variable expressivity. According to Crowe and Schull (1953), one should find at least six café-au-lait spots in a carrier, the diameter of the largest being over 1.5 cm.

Bourneville's tuberous sclerosis (Fig. 8) is due to a dominant gene whose variability of expression is very great. Isolated manifestations which might be observed in a carrier of the gene are sebaceous adenomas or psychic disorders (François and Deweer, 1953).

Von Hippel-Lindau's angiomatosis can sometimes have one symptom only, namely, retinal angiomatosis (Fig. 9).

Sturge-Weber-Dimitri's neurocutaneous angiomatosis can also be monosymptomatic, showing only a nevus flammeus of the face.

Marfan's syndrome. The subluxation of the lens, the excessive length of

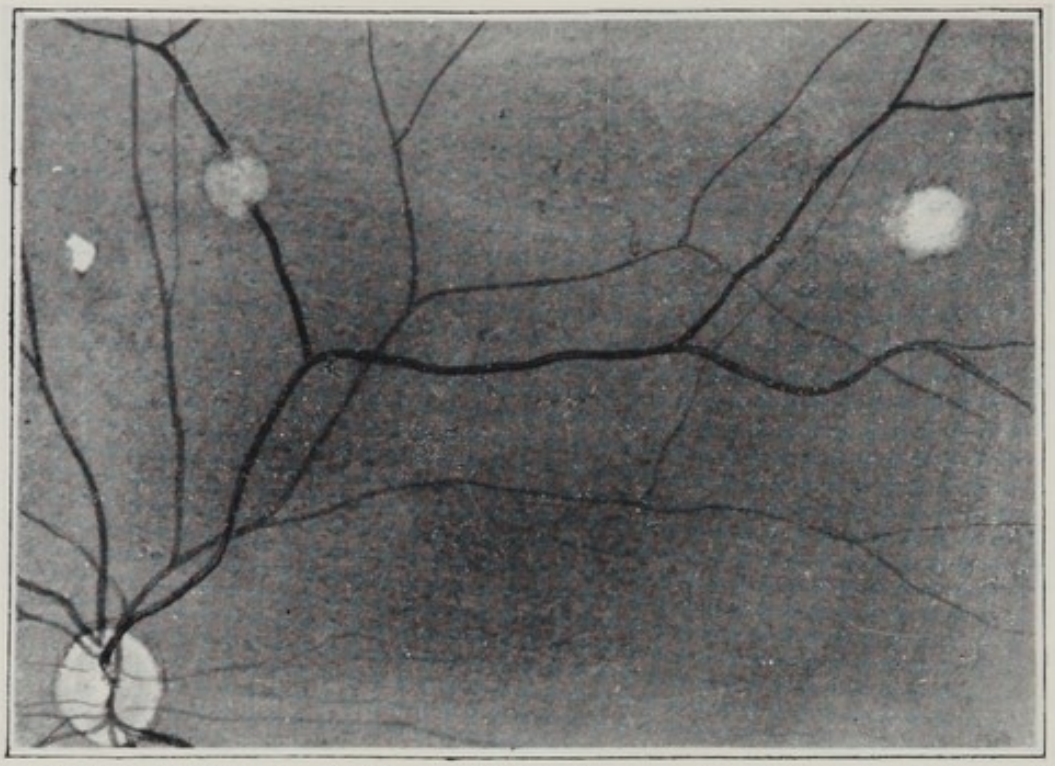


FIG. 8—Retinal phakomas in Bourneville's tuberous sclerosis (personal observation).

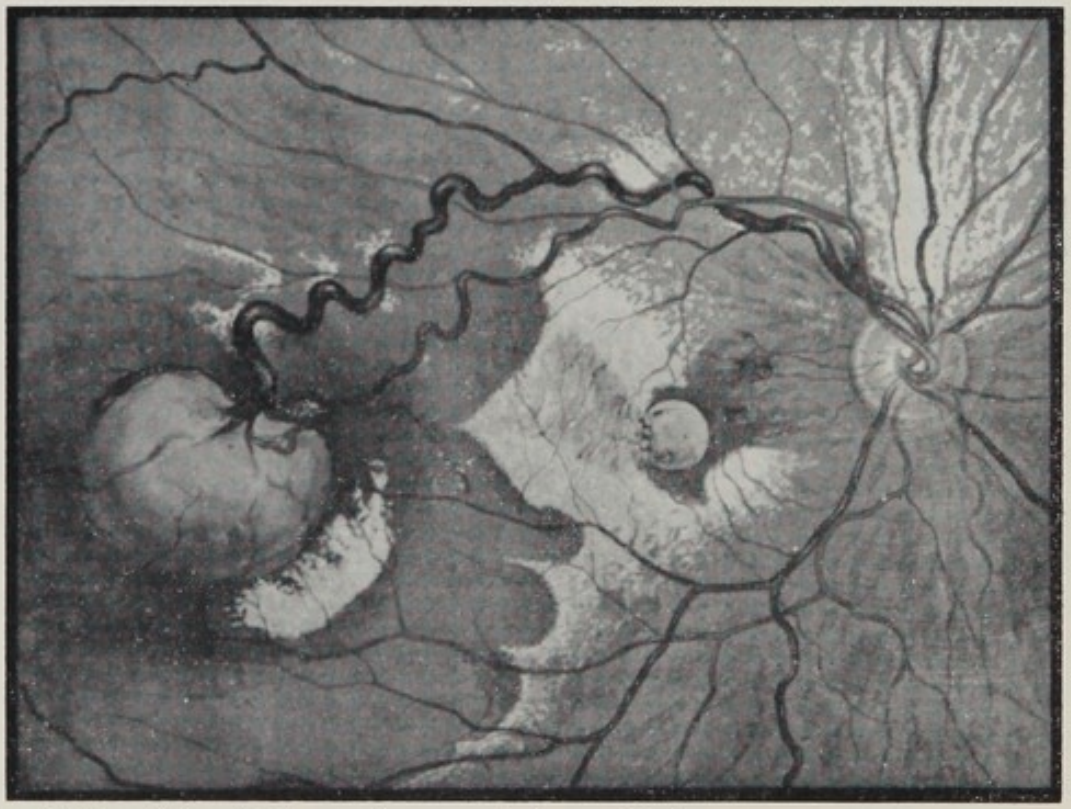


FIG. 9—Von Hippel's disease (personal observation).

the fingers, and the cariopathy constitute, alone or in association, the usual manifestations of the attenuated form of this dominant disease.

Waardenburg syndrome. This dominant syndrome (1951) is character-

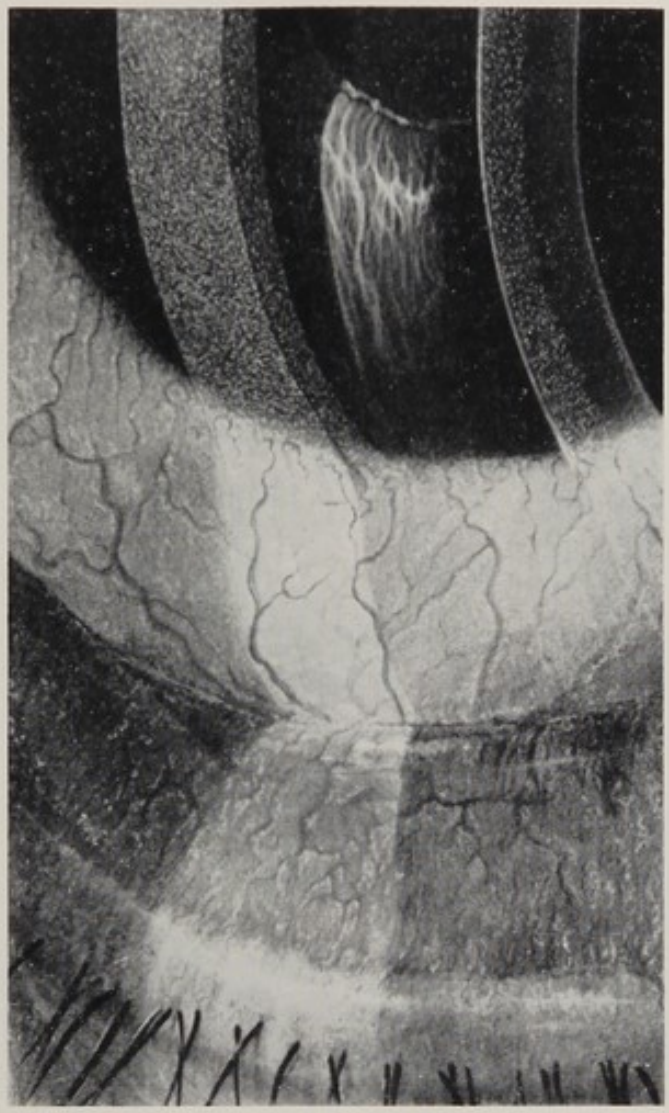


FIG. 10—Crystals of cystine in the cornea and conjunctiva (personal observation).

ized by lateral displacement of the internal canthi and lacrimal puncta, hyperplasia and enlargement of the root of the nose, hyperplasia of the medial portion of the eyebrows, total or partial heterochromia of the iris, albinism limited to the forefront (a lock of white hair), and congenital deafness in one or both ears. Not all of these symptoms are present in all affected members of a given family. The indicative symptom is the lateral displacement of the inner canthi and lacrimal puncta, with or without some of the other signs.

Bonnevie-Ullrich syndrome. The carrier of the disease is recognized by unilateral ocular abnormalities involving the anterior portion of the eye and adnexa and malformation of the hands.

Ehlers-Danlos syndrome. The carrier is recognized by the scarred, papery, or atrophic skin of the forehead and hyperelasticity of the skin of the eyelids.

Steinert's myotonic dystrophy. Typical iridescent opacities under the

FIG. 11—Franceschetti's mandibulofacial dysostosis (after Franceschetti and Klein, 1949).

anterior and posterior capsules are the main and often only manifestation of the disease in the carrier of the gene.

Van der Hoeve's syndrome. The carrier of the dominant gene is recognized by the fact that his scleras have a bluish coloration. This is the essential and most frequent sign of the disease.

Adult cystinuria and juvenile cystinosis. It can be accepted that mild cystinuria in families with a tendency to cystinosis may represent an incomplete manifestation corresponding to heterozygotes, whereas severe conditions (Fig. 10) represent homozygotes (Pfändler, 1956, personal observation).

Franceschetti's mandibulofacial dysostosis (Fig. 11) is caused by a dominant gene with incomplete penetrance and very pronounced pleiotropism. The carrier of the gene can be recognized by the palpebral abnormalities alone, these being the main sign of the syndrome.

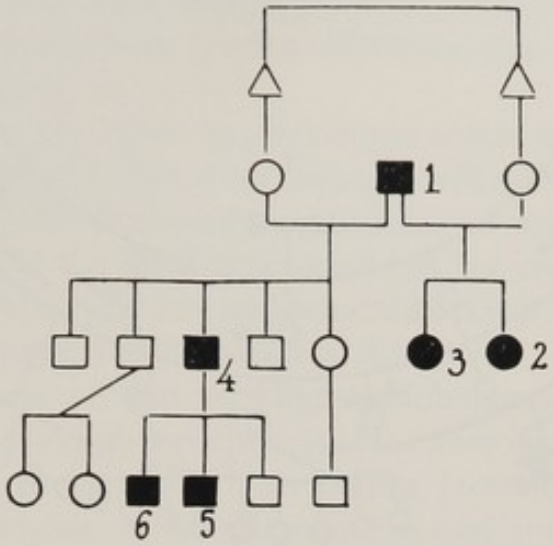


FIG. 12–1, Anophthalmia of the right side; 2, bilateral anophthalmia; 3, coloboma of the choroid of the right eye; 4, anophthalmia of the right eye and coloboma of the iris of the left eye; 5, microphthalmia; 6, coloboma of the iris and the lens of the left eye (after Fleischer, 1938).

IV. Considerations on Some Particular Eye Diseases

1. Colobomatous Defects of the Globe

A series of experiments on rabbits have shown that a great variety of malformations can be observed in one and the same group of offspring. These range from a heterotypical cone to anophthalmia, with all intermediate stages, such as an inferior cone, posterior staphyloma, colobomatous cup of the papilla, coloboma at the point of entry of the optic nerve, coloboma of the choroid or lens, persistence of the pupillary membrane, typical or atypical coloboma of the iris, dyscoria or polycoria, corectopia or ectopia of the lens, aniridia, microphthalmia, etc.

These facts show that there is an obvious correlation between all these malformations, which vary in their importance. They all represent the same fundamental lesion and all spring from the same genotypic factor which varies in its penetrance and expressivity.

Clinical observations confirm the experimental results: Siemens (1920), Pfanmüller (1894), McMillan (1921), Gallemaerts (1924), Marchesani (1933), Bell (1933), Fleischer (1938; Fig. 12), Franceschetti (1946), Beattie (1947), Kronenberg (1948).

We have observed a woman with bilateral microphthalmia and congenital cataract on the right side who has seven children (Fig. 13); one of these has bilateral coloboma of the iris with high hypermetropia. We have also described a family comprising four generations in which the following six malformations are found: unilateral anophthalmia, unilateral micro-

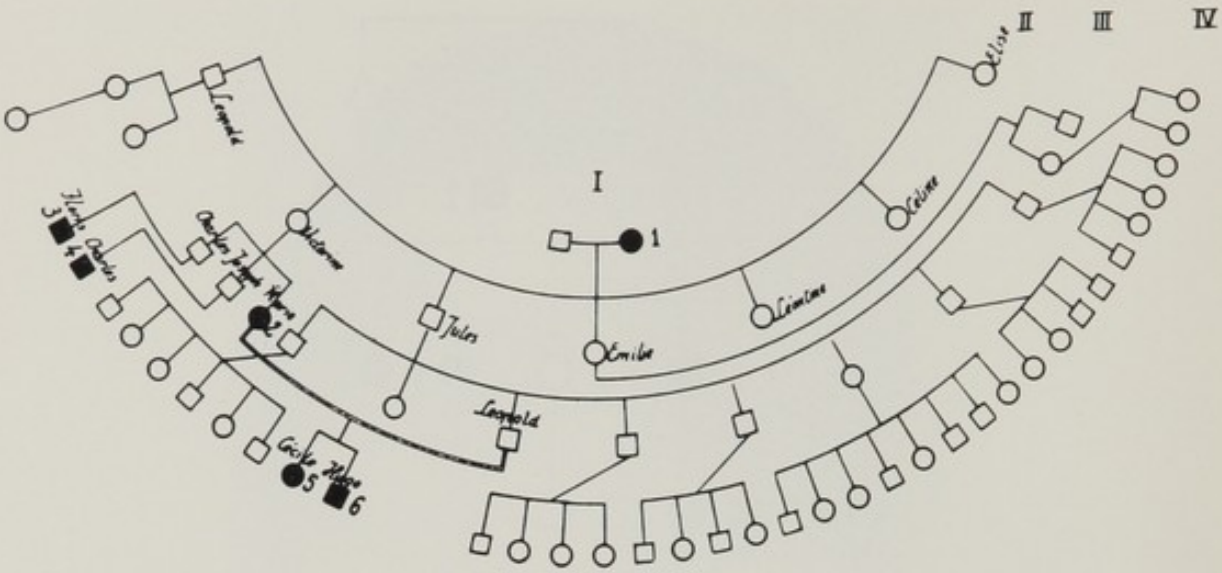


FIG. 13–1, Anophthalmia of the right eye; 2, microphthalmia of the right eye with glaucoma and cataract; 3, bilateral microphthalmia with blindness; 4, typical coloboma of the iris of the left eye; 5, bilateral anophthalmia with adiposogenital syndrome and mental retardation; 6, on the right, typical coloboma of the choroid, and on the left, microphthalmia with coloboma of the choroid and the optic nerve (personal observation).

phthalmia, bilateral microphthalmia, unilateral typical coloboma of the iris, bilateral anophthalmia with adiposogenital dystrophy and mental retardation, and typical coloboma of the choroid and microphthalmia with coloboma of the choroid and optic nerve of the other eye.

These facts are interesting from three points of view.

1. They show clearly the close genotypic relation between different colobomatous and general malformations of the eyeball.

2. They show, in addition, that abnormalities are transmitted as irregularly dominant characters. It is a common mode of inheritance for all colobomas. They often bypass a generation, and only 20 to 30 per cent of the descendants are affected (instead of 50 per cent).

As for other hereditary diseases, it is a fact that the more serious (microphthalmia, cryptophthalmia, and anophthalmia) can be recessive.

But in order to know the exact hereditary character in a given family, one must look for rudimentary colobomas (indentations of the pupillar border, colobomatous pit of the optic disc, hypoplasia of the mesodermal layer of the iris, etc.), because, just as the penetrance of a gene may occasionally be small or absent, so its expressivity can also vary a great deal.

3. One member of our family (IV-5; Fig. 13), apart from bilateral anophthalmia, presents an adiposogenital syndrome with mental retardation. This serious condition may be the expression of a homozygous genotype arising from a consanguineous marriage between two heterozygotes. It is one of the atypical forms of the Bardet-Biedl syndrome and represents a bridge

between this and colobomas, which can in addition be associated with a great variety of malformations (such as abnormalities of the skull, skeleton, face, urogenital system, etc.).

In fact one commonly observes pleiotropic action of a gene responsible for colobomatous defects. One other example will suffice: Pfändler (1954) describes a family with dominant transmission of aniridia through three generations. A boy of the third generation has not only bilateral aniridia, but also hydrocephalus, physical and mental backwardness, malformations of the ears and cranium, and neurological disorders.

Finally, let us point out that hereditary colobomatous diseases must not be confused with certain phenocopies, such as microphthalmia arising from the retinopathy of prematurely born babies (retrolental fibroplasia) or congenital toxoplasmosis or toxoplasmic macular pseudo-coloboma (necrotizing chorioretinitis). A differential diagnosis will generally be easy if one bears in mind the antecedents, associated symptoms, and absence of other cases within the family.

2. *Refractive Errors*

It is a mistake to believe that the hypermetropic eye is simply too short and the myopic eye simply too long. Steiger's studies (1913) supplemented by those of Tron (1929), Kreikar (1939) and Stenström (1948), have shown that none of the optical components, such as the curvature of the cornea, the depth of the anterior chamber, the thickness of the lens and its anterior and posterior curvatures, and the length of the axis of the globe, are constant, and that they vary between individuals—within limits, however—so that total refraction can be very different, according to the combination and interaction of different variable optical components. Thus emmetropia can be achieved equally as well from strong corneal refraction and a short anteroposterior axis as from weak corneal refraction and a long anteroposterior axis.

The values of each of these optical components are distributed in a normal binomial curve (Figs. 14–19).

If these different optical factors which determine refraction are independent of each other and could vary freely, the total refraction of the eye would follow a normal binomial curve, like those of each component separately.

But studies by Scheerer (1928), Betsch (1929) and Franceschetti (1930), based on the refraction measurements of 12,000 eyes, have shown that the curve obtained is symmetrical (when eyes with serious myopia and choroidosis are left out) but higher and narrower than the binomial curve. Therefore, there is a preponderance of values around the average, that is, emmetropia (Fig. 20).

According to Tron (1929), this is due to negative correlation between

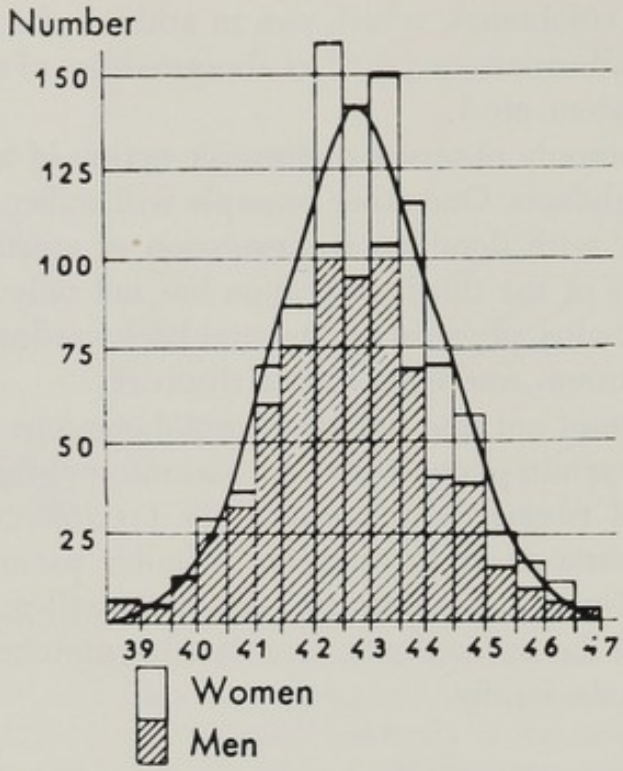


FIG. 14—Curve of frequency of refraction of the cornea, based on 1,000 cases (after Stenström, 1948).

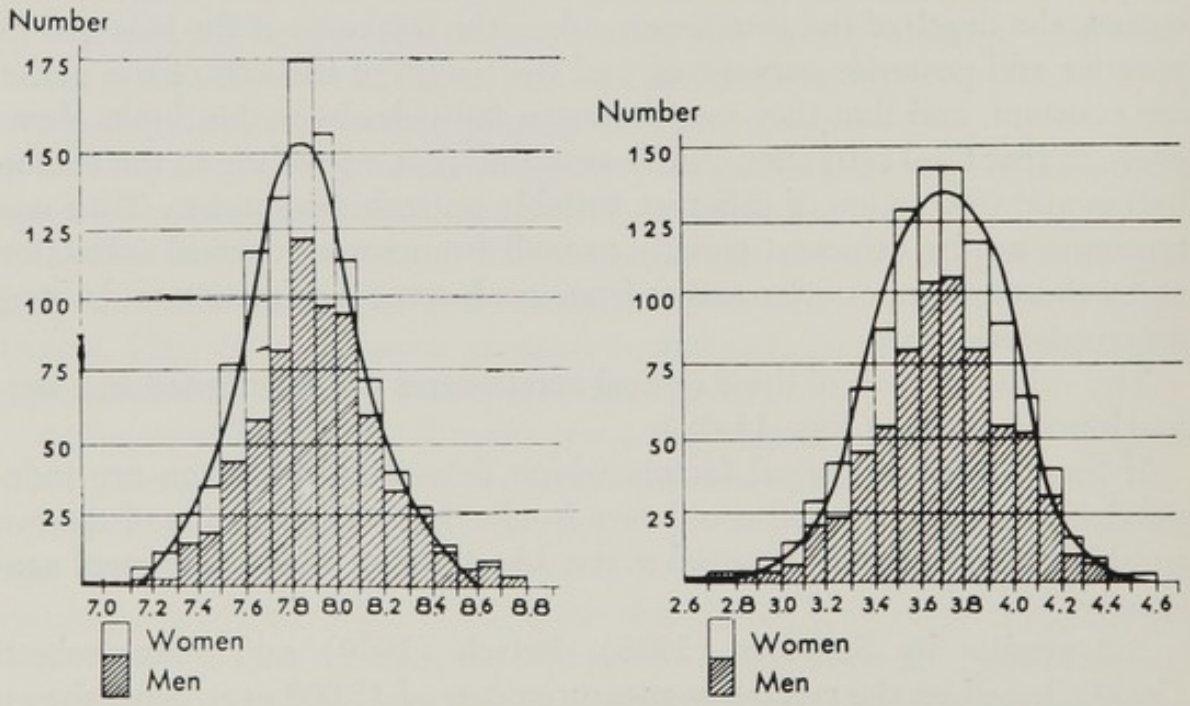


FIG. 15—Curve of frequency of the curvature radius of the cornea, based on 1,000 cases (after Stenström, 1948).

FIG. 16—Curve of frequency of the depth of the anterior chamber based on 1,000 cases (after Stenström, 1948).

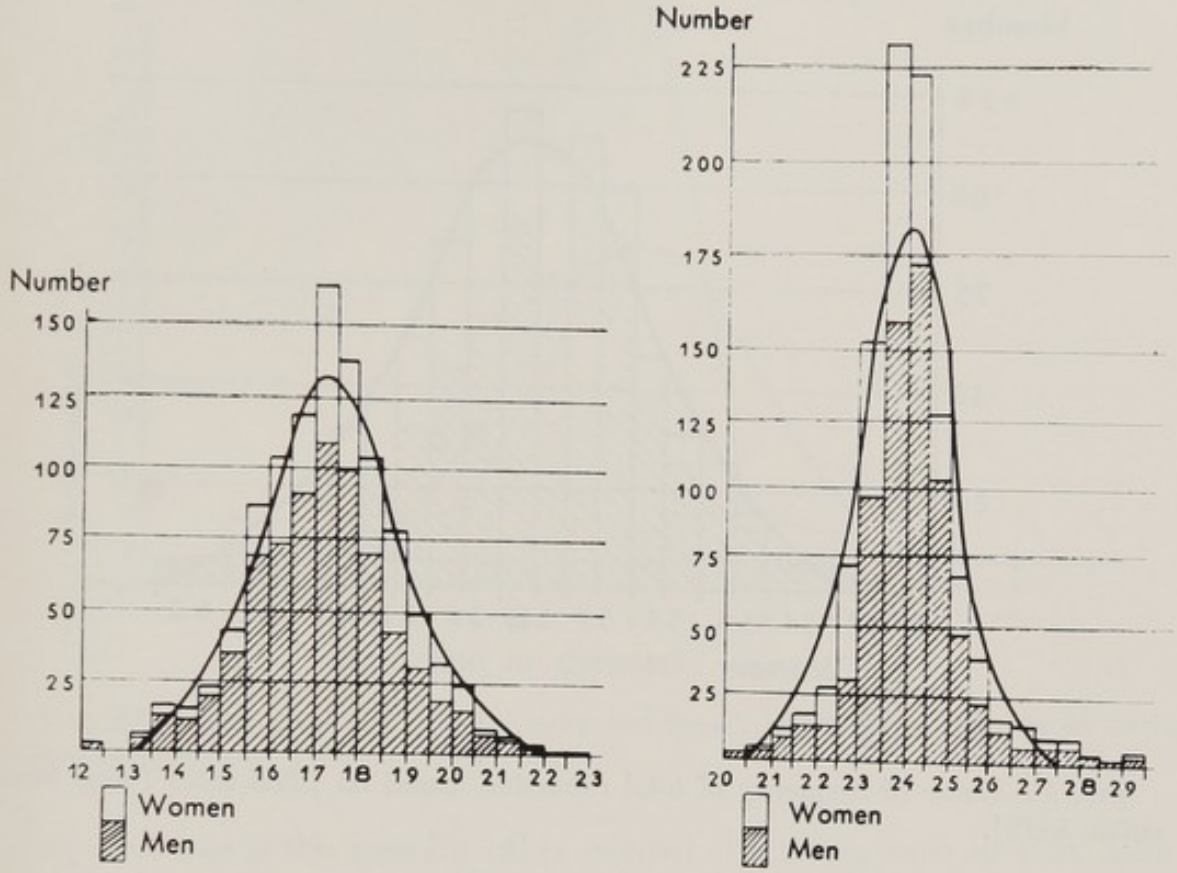


FIG. 17—Curve of frequency of refraction of the lens, based on 1,000 cases (after Stenström, 1948).

FIG. 18—Curve of frequency of the length of the anteroposterior axis, based on 1,000 cases (after Stenström, 1948).

the different factors which determine refraction, particularly between the lengths of the eye and the total refraction. As the length of the axis increases, total refraction decreases; these two factors which develop in opposite directions tend to produce emmetropia; this is the phenomenon of "emmetropization," described by Straub (1918) and also accepted by Ostroumov (1952).

In addition, the distribution of total refraction values shows another excess toward the myopic end of the curve (Fig. 21). A study of the length of ocular axes gives the explanation. Both highly myopic and highly hypermetropic eyes have an anteroposterior axis with a length exceeding the normal distribution. For instance, Stenström (1948) found that among 23 myopic eyes (between 6 and 14 D.) the axis measured between 26 and 29 mm. in 21 eyes and between 25.5 and 26 mm. in only two eyes.

From all this, we can draw the following conclusions.

1. The different optical components do not have specific values, in either emmetropic eyes or ametropic eyes. Only the anteroposterior axis

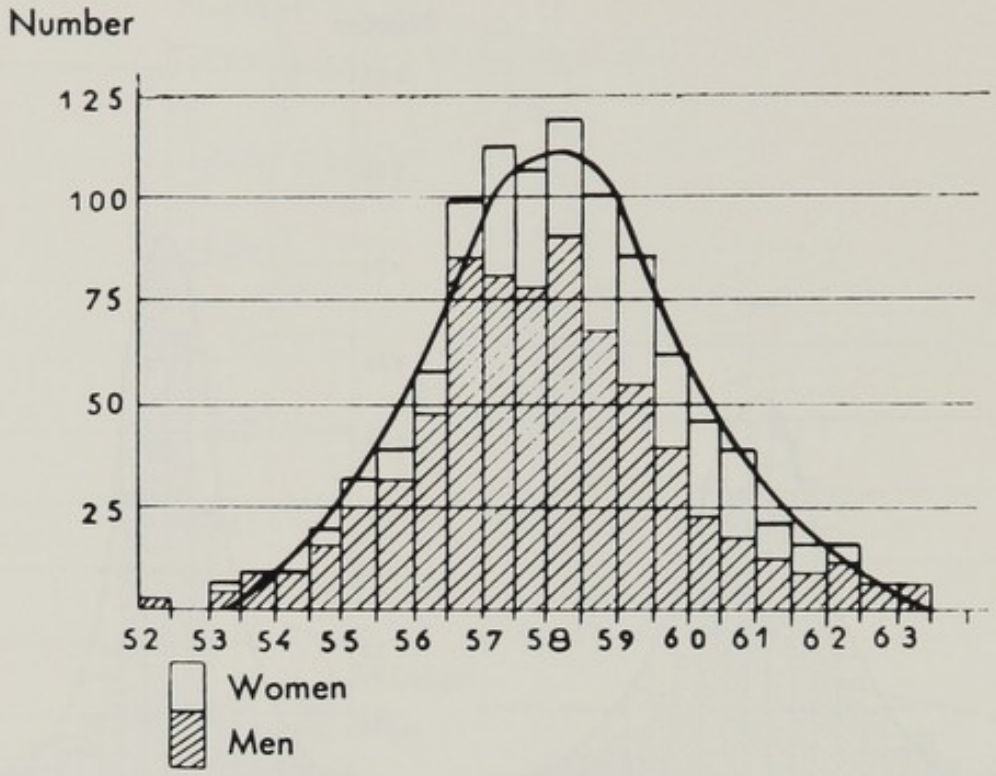


FIG. 19—Curve of frequency of total refraction, based on 1,000 cases (after Stenström, 1948).

generally tends to be longer in myopic eyes and shorter in hyper metropic eyes although in a given individual who is severely myopic or hypermetropic the length of the axis can be within normal limits.

2. Most ametropias (two thirds) do not arise from abnormal factors but from the combination of components within the normal variation.

3. In one third of the cases only (30.2 per cent of myopias and hypermetropias) is the length of the axis outside the limits of normal distribution; this percentage is actually much smaller if we exclude hypermetropias beyond 4 D. and myopias beyond 6 D. The degree of ametropia is not proportional to the length of the axis.

4. With the exception of high myopias and hypermetropias, ametropias can be considered physiologic variants.

The foregoing statements are enough to show how complex the problem is, and it is, therefore, not surprising that accurate and valid genetic data should be rare as yet. Actually, the following considerations are concerned in the problem:

1. Each optic component is probably inherited independently of the other.

2. Each optic component might be transmitted according to different patterns of heredity.

3. It is likely that several genetic factors contribute to the final optic

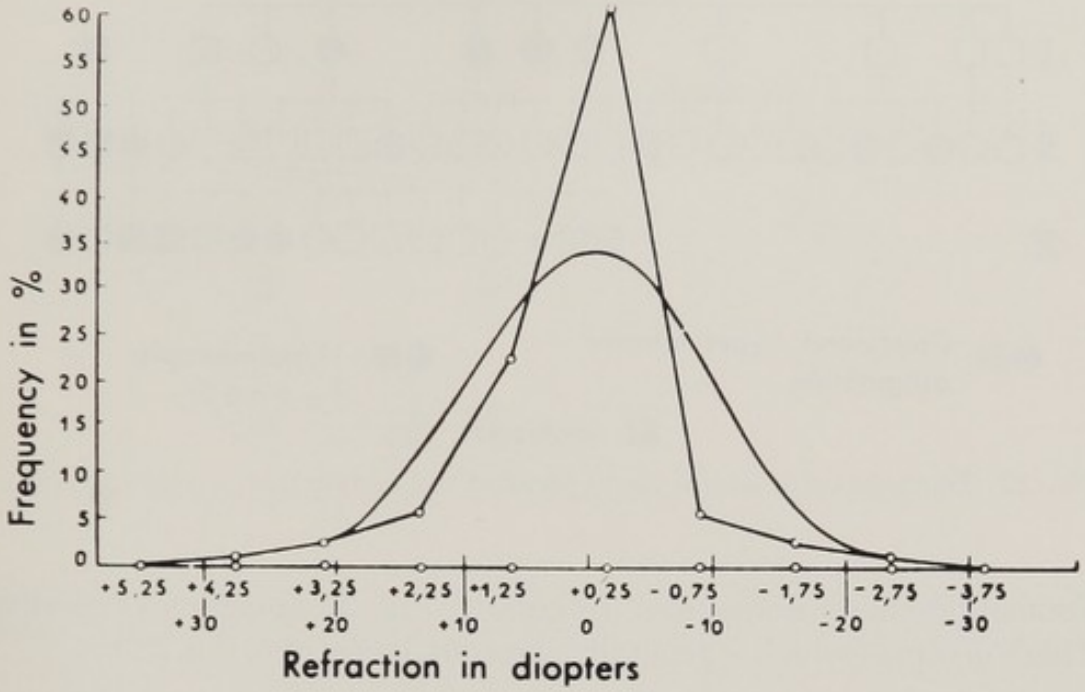


FIG. 20—Curve of refraction of Scheerer and Betsch and theoretical binomial curve (after Franceschetti, 1930).

component, as is the case for other normal characters such as size, color of skin, color of eyes (polygenic characters), etc.

4. It is impossible to study the genetic factors determining refraction by taking into account total refraction alone. Genetic analysis can only be valid if the pattern of heredity and the postnatal development of each optic component are known.

The only certain data about the inheritance of optic refraction concern corneal refraction and astigmatism (which is generally transmitted as an

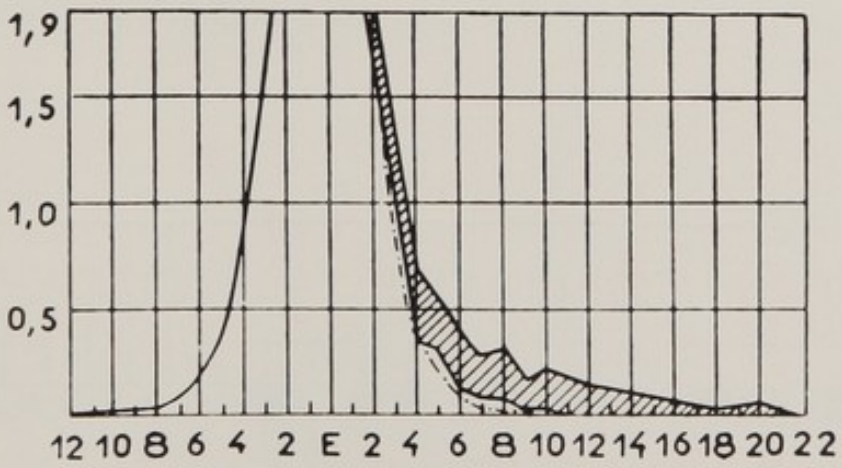


FIG. 21—Curve of refraction. The hatched part represents the cases of myopia with choroidosis. To the left of *E* are the positive values, and to the right of E are the negative values (after Scheerer, 1928).

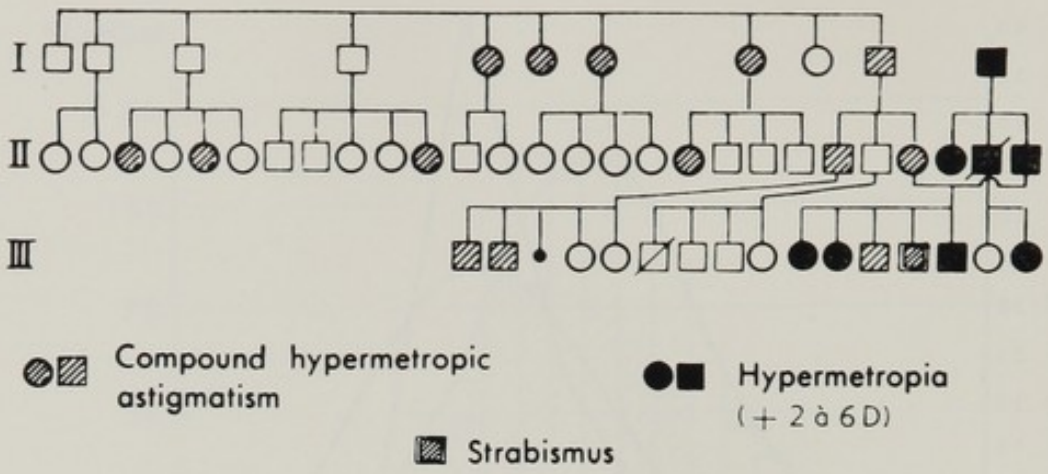


FIG. 22—Transmission of astigmatism (personal observation).

autosomal dominant, sometimes irregularly, Fig. 22, sometimes recessive) and high myopia (which is generally autosomal recessive, Fig. 23).

3. *Retinoblastoma*

Apart from sporadic cases, which are by far the most numerous, there are cases in which high incidence within a family is evident. If we take into consideration only those authors who have published all the retinoblastomas they observed, we find only 44 families in which the tumor has occurred several times as compared to 722 families in which it has occurred only once. The incidence of retinoblastoma in families would amount to only 3.92 per cent of the incidence of sporadic cases (François, 1961a). In 119 of 133 families, examined by Macklin (1960) the disease appeared to be sporadic, that is to say that the incidence of familial retinoblastoma is 10 per cent in this series.

Dollfus and Auvert (1953) considered 241 sibships in which retinoblastoma had occurred; in only 5 cases were siblings affected. Therefore,

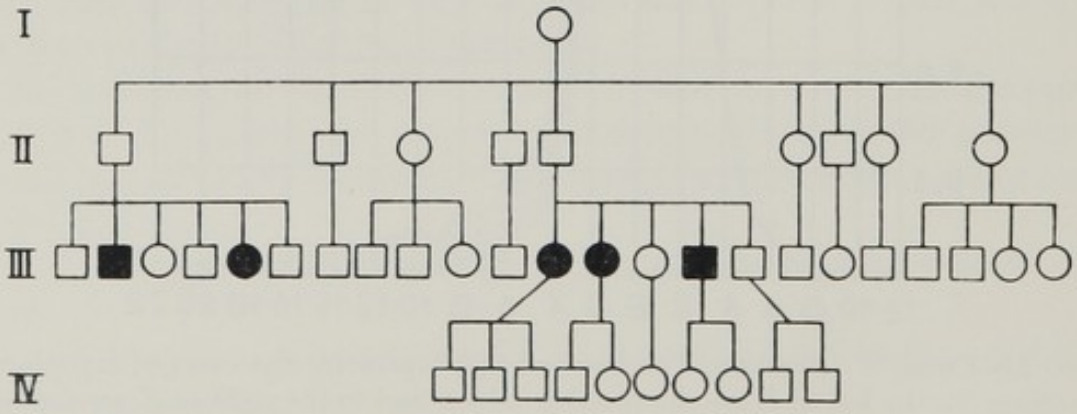


FIG. 23—Malignant myopia of −5 to −10 D (personal observation).

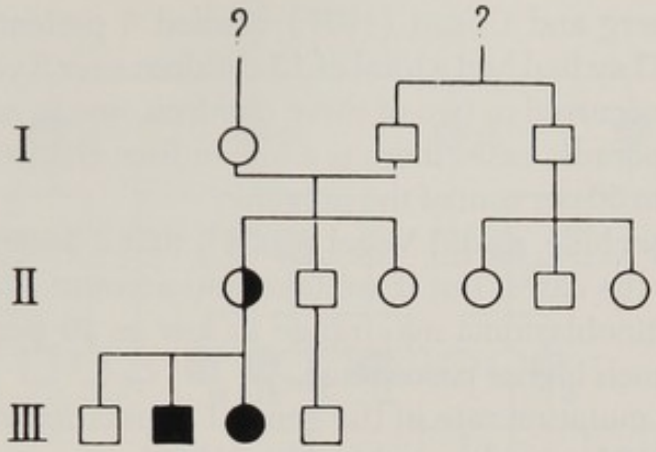


FIG. 24—Retinoblastoma (personal observation).

there is only a 2 per cent chance of an affected subject having brothers or sisters also affected. Other authors have arrived at similar figures; Reese (1949) gives 1 to 4 per cent, Falls and Neel (1951) 1.5 per cent and Macklin (1956, 1960) 6.7 to 8 per cent.

Retinoblastoma follows the pattern of autosomal dominant heredity (Fig. 24), sometimes irregularly, the penetrance of the gene being reduced.

What are the risks of a surviving person passing on the disease to his descendants? Theoretically 50 per cent of his children should be affected by retinoblastoma. However it occurs only in 40 per cent or less.

One must now consider this question: is the difference between these percentages (40 and 50) in dominant transmission due to chance or to the fact that there are individuals who are phenotypically normal (lack of penetrance) but genetically affected carriers? The following conclusions can be made:

1. The 10 per cent difference is significant because it is more than three times the average margin of error (2.4 per cent).

2. Dollfus and Auvert (1953) have proved that in 40 sibships of the second generation, transmission was effected 10 times by a person who was clinically unaffected but of necessity affected genetically since one parent and several siblings of the carrier were affected (irregular dominance).

3. These facts allow us to conclude with Franceschetti (1953) that only 75 per cent of the affected persons are actually gliomatous, with the result that in family groups we will find that 37.5 per cent of the members are phenotypically affected and 12.5 per cent are carriers.

The degree of penetrance is nevertheless differently appreciated. It is 20 per cent according to Macklin (1960), 30 per cent according to Dollfus and Auvert (1953), 60 per cent according to Böhringer (1956), 60 to 90 per cent according to Kaelin (1953) and 80 per cent according to Franceschetti and Bischler (1946).

Tucker, Steinberg and Cogan (1957) studied 8 patients with sporadic retinoblastoma. They had had a total of 13 children over 3 years of age. Retinoblastoma had occurred in two of these children, one in each of two families, so that for sporadic cases there is a one in four chance of transmitting the disease to 40 to 50 per cent of the progeny.

The authors conclude, as did Vogel (1954), that a mutation occurs only in 25 per cent of the cases. But if we take into account that the degree of penetrance of retinoblastoma may range as low as 20 per cent, mutation may occur in a much higher percentage.

The estimated mutation rate in the general population ranges from 1.75×10^{-5} to 0.63×10^{-5} according to Macklin (1960).

Finally, some eugenic considerations might serve as conclusions:

a. A cured patient has a 25 to 40 per cent chance that nearly half of his progeny will be affected, if not clinically, at least genotypically.

b. Healthy parents who have one gliomatous child have only a 2 to 7 per cent chance of having a second affected child.

c. Apparently healthy parents who have two gliomatous children are of necessity carriers of the gene and consequently run the risk that half of their children will be affected.

4. Congenital cataracts

Constitutional congenital cataracts should, of course, be differentiated from those resulting from an intrauterine inflammatory process and from those due to embryopathies (toxoplasmosis, maternal rubella in the first three months of gestation, etc.). Congenital cataracts can be associated with the following other defects:

a. Ocular malformations: microphthalmia, colobomas, aniridia, microcornea, megalocornea, ectopia of the lens, remains of the pupillary membrane, keratoconus, pigmentary retinitis, cornea guttata and pigmentary retinitis or tapetoretinal infantile degeneration of Leber's type (Fig. 25). A family described by Pfändler (1950) illustrates all the possibilities very well: 2 patients have congenital cataract with ectopia of the lens and keratoconus, and another has ectopia of the lens, keratoconus, congenital atrophy of the optic nerve and glaucoma.

b. General malformations: several important correlations require mention.

Sjögren's syndrome is an association of oligophrenia and cataract, usually zonular or polar, but sometimes total. Sjögren (1935) has described 44 cases from 30 families. Other cases have been reported.

In regard to inheritance of this syndrome, it is generally accepted that it is due to an autosomal recessive pleiotropic gene with variable expressivity. This is based on cases in which there are no other affected members among the ancestors or the offspring of the patient, and parents of affected

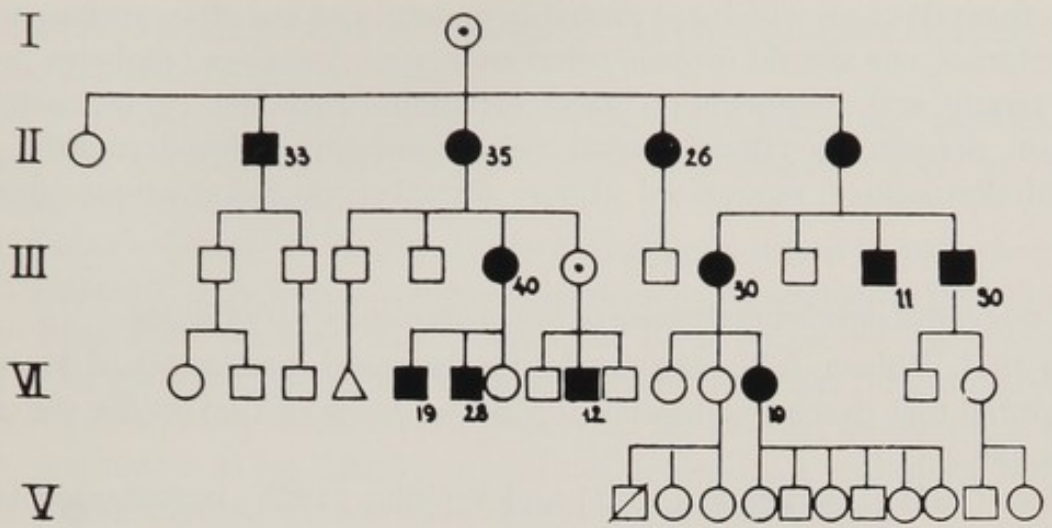


FIG. 25—Leber's disease. The numbers indicate the age at the time the disease appeared (after P. François, 1957).

persons are often related by descent. Collateral relatives are also affected, and several sibs may be affected. Franceschetti and Brugger (1944) have studied 35 cases of congenital cataract, with mental deficiency; since in the families they examined there were cases of isolated oligophrenia (in 24 brothers or sisters) and also cases of isolated cataract (in 17 brothers and sisters), they conclude that there has been a fortuitous meeting of two different hereditary diseases. Later, however (1954), Franceschetti returned to the hypothesis of a single pleiotropic gene.

Sjögren (1949 to 1950) described yet another syndrome in which cataract and oligophrenia are associated with hereditary ataxia and often with other congenital abnormalities. These include, especially, skeletal abnormalities such as small stature, scoliosis, genu valgum, restricted extensibility of the knee and defects of fingers and toes. Other authors have collected further cases.

Certain cases such as those of Garland and Moorhouse (1953), involving 2 first cousins from a consanguineous marriage, suggest recessive inheritance. Other cases, for example, 14 in 6 families collected by Sjögren (1949), show dominant inheritance.

Congenital cataract is often associated with polydactylia. Waardenburg (1932) thinks that here again there is a fortuitous coincidence.

The association of congenital cataract with microphthalmia, malformation of the head resulting in a parrot face, aplasia of the lower jaw, hypotrichosis, and dwarfism constitutes a syndrome which actually is well established (François, 1957, 1961). Nothing is known so far about the inheritance of this syndrome, although it has been seen in identical twins.

Another association of congenital cataract is with oxycephalia or plagiocephaly.

In those diseases which are probably genetic and are often accompanied by cataract, one should include some endocrine disorders (diabetes mellitus, tetany and acromegaly), some metabolic disorders (galactosemia), certain dermatoses (disseminated neurodermatitis, sclerodermatitis and poikilodermatitis), mongoloid idiocy, oligophrenia and myotonic dystrophy.

5. Congenital dyschromatopsias of the protan and deutan types

In 1911, Wilson, following up the fundamental discoveries of Morgan, suggested that dyschromatopsias depend on genes carried on the sex chromosomes.

Hess (1921), Döderlein (1922) and Wölfflin (1923) investigated families showing both deuteranomaly and deuteranopia or protanomaly and protanopia; they concluded that in women dichromasia is the recessive to the corresponding abnormal trichromasia.

In 1925, Just propounded a theory of multiple alleles for the various forms of the protan and deutan types. His theory was based by analogy, on the multiple allelic system governing eye color in *Drosophila*. This theory was taken up by Waaler (1927) who demonstrated it by means of numerous examples.

From among the authors who have contributed to the confirmation of Waaler's theory, one may mention Brünner (1930), Just (1935), Schinz (1941), Kühn (1942) and Stern (1958).

One still disputed point is the following: Are the two allelic series really at the same locus of the X chromosome (one-locus hypothesis) or at different loci (two-loci hypothesis)? As pointed out by Franceschetti and Klein (1957) and also by Stern (1958), one could decide between the two hypotheses by an observation in which a woman who is a repulsion compound heterozygote for both a protan defect and a deutan defect has numerous sons. The one-locus hypothesis is correct if there are only protan and deutan sons, whereas the two-loci hypothesis must be accepted if there are, in addition to protans and deutans also normal and achromatic sons whose phenotypes are due to crossing-over. In fact, Vanderdonck and Verriest (1960) recently described a pedigree in which a woman who is a repulsion compound heterozygote for protanomaly and deuteranopia has two deuteranopic, one protanomalous, and two normal sons (all the sons do have a male genetic sex). This observation demonstrates that the loci of the protan and deutan types of color blindness are really distinct.

6. Optic Atrophies

The most frequent hereditary optic atrophy is Leber's juvenile form, but there are other forms of hereditary optic atrophy also: congenital atrophy

(recessive), infantile atrophy (dominant), Behr's complex infantile atrophy, and a late-developing form.

Optic atrophy can also appear as a symptom of hereditary nervous diseases, i.e., ataxia or of hereditary skeletal abnormalities such as osteopetrosis and oxycephalia.

a. Leber's disease (Fig. 25). Leber had already stated in 1871 that only men seemed to be affected, and that they must be receiving the pathological gene from their mothers. His opinion has been borne out subsequently, and most published pedigrees favor sex-linked heredity. But, although an affected man always receives the gene from his mother, he does not, however, transmit it to his daughters, and the descendants will all be normal. In addition, affected women have always had normal fathers.

Therefore, Leber's disease disappears with an affected man since he no longer transmits the gene to subsequent generations, and his descendants remain perfectly healthy, genotypically as well as phenotypically, a fact which is of great importance from the eugenic point of view. On the other hand, an affected man's sister will transmit the disease to her sons and the gene to her daughters, if she is a carrier (heterozygous).

All this shows that Mendel's laws of sex-linked heredity do not apply to Leber's disease. Various hypotheses have been put forward to explain the discrepancies, particularly the absence of the disease in the descendants of affected men. None of them, however, are entirely satisfactory.

Komai (1934), Lundsgaard (1944) and Andersen (1944) rejected sex-linked heredity and adopted autosomal dominant heredity, with different expressivity in the two sexes: complete dominance in males and very incomplete dominance or incomplete recessiveness in females.

Since this hypothesis does not explain the absence of transmission by an affected man to his children and grandchildren, it has been assumed that the pathologic gene has a lethal effect on the spermatozoon. Since it cannot live when it carries the gene, the affected man cannot transmit the disease. The only source of transmission then becomes women carriers since the ova carrying the gene are viable.

The principal objection to this theory is this. A heterozygous woman, whether she is phenotypically affected or only carries the disease, must transmit the gene to 50 per cent of her sons and 50 per cent of her daughters (Xx + xy = Xx, xx, Xy, xy). The daughters will be phenotypically affected or will simply be carriers, according to the expressivity of the gene. In other words, the highest proportion of carriers will be 50 per cent. But all statistics show that, in fact, the proportion varies between 90 and 100 per cent, which means that almost all women are carriers.

It is for this reason that Rönne (1944 and 1945) rejected chromosomal heredity and believes, together with Imai and Moriwaki (1936), that this

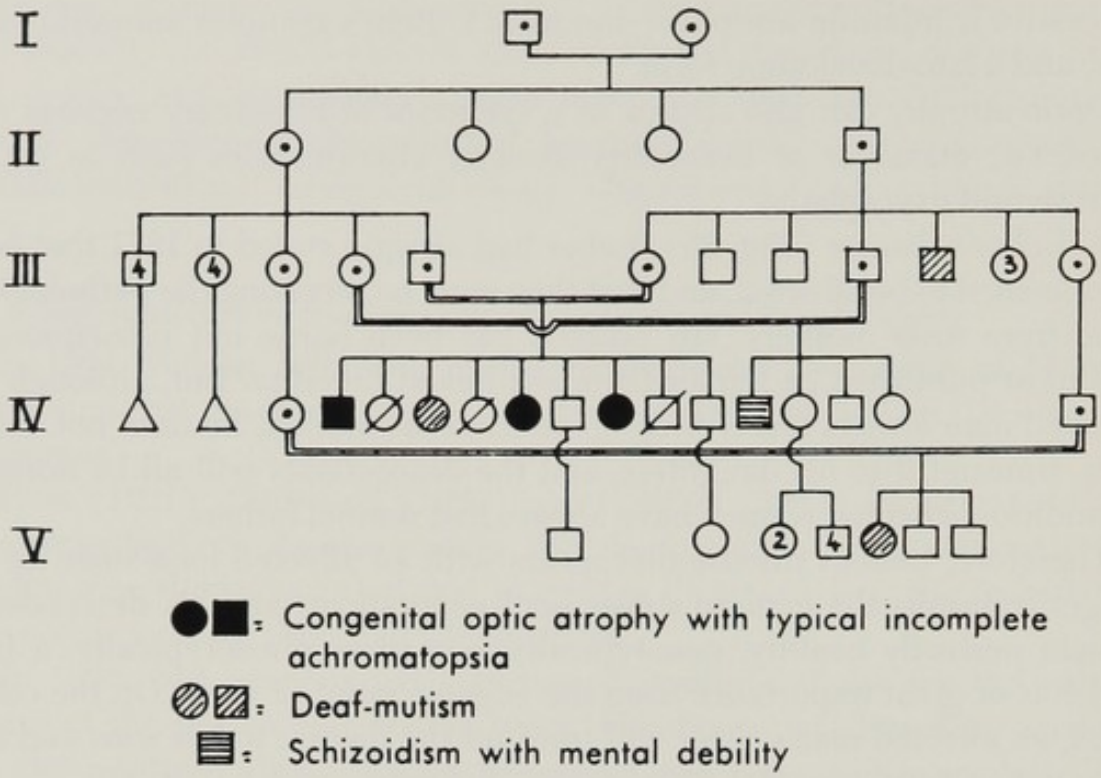


FIG. 26—Congenital optic atrophy inherited as a recessive. Consanguinity of parents (after Waardenburg, 1956).

is a case of cytoplasmic inheritance. Since the spermatozoa contain only a very small quantity of cytoplasm, they cannot transmit the disease, which can be transmitted only by the ova which have a greater quantity of cytoplasm.

Although cytoplasmic inheritance has not yet been proved in human genetics, it has now been accepted in animal and plant genetics. The main objection to applying it to Leber's disease is the failure to understand why all the sons of a woman carrier are not affected. Perhaps the expressivity of the disease is not so complete in males as had been thought.

b. Congenital recessive form (Fig. 26). Optic atrophy is present at birth or starts very early (before the age of 3 years).

c. Infantile dominant form (Fig. 27). The dominant form is milder and develops gradually from infancy onward.

Transmission in all these cases is direct and regular and is effected through both men and women. The proportion of affected men and women is similar.

7. *Albinism*

As early as 1832, Geoffroy Saint-Hilaire distinguished between perfect (Fig. 28), imperfect and partial forms; in 1909, Nettleship added a fourth,

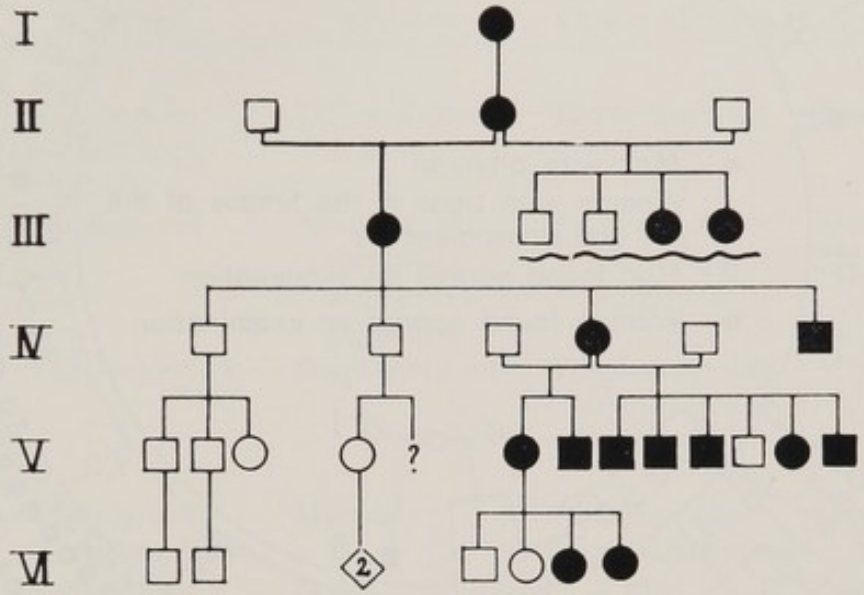


FIG. 27—Infantile optic atrophy with dominant inheritance (after Thompson and Cashell, 1935).

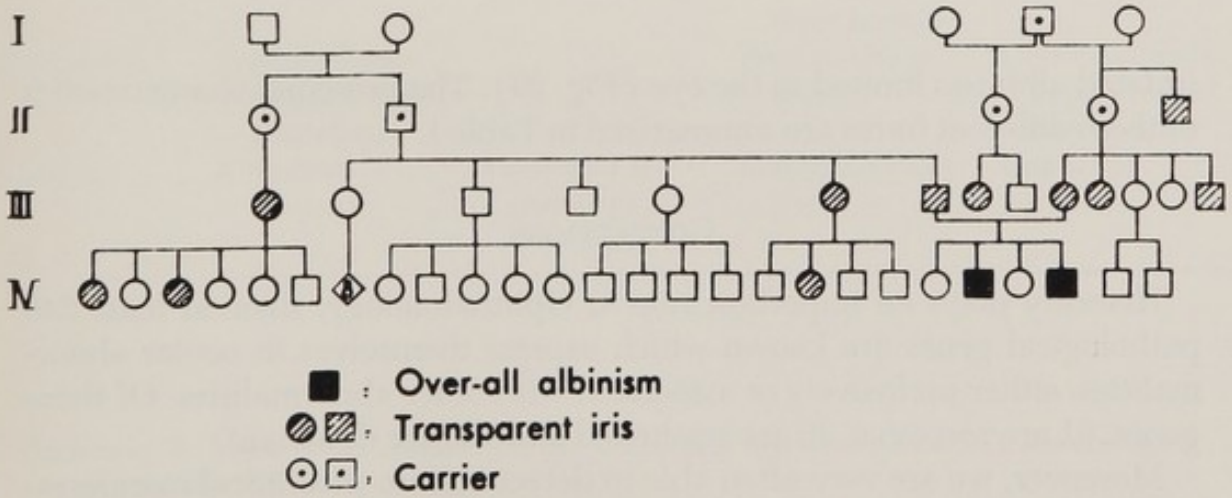


FIG. 28—Transparent iris in carriers of complete over-all albinism (after Van den Bosch, 1953).

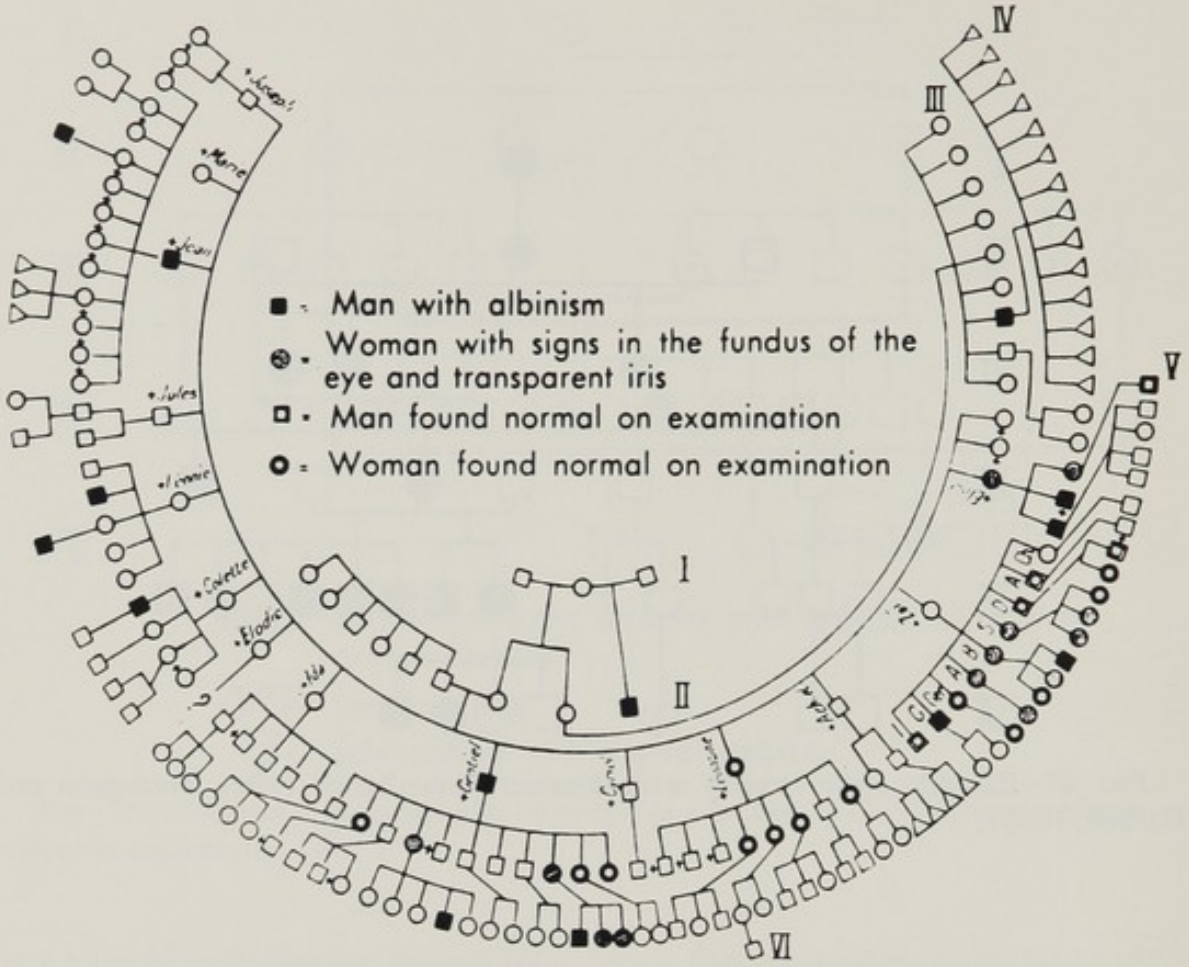


FIG. 29—Ocular albinism with intermediate sex-linked inheritance (personal observation).

isolated albinism limited to the eye (Fig. 29). The principal characteristics of these different forms are summarized in Table 1.

CONCLUSIONS

Heredity plays an important role in Ophthalmology, since at least 246 pathological genes are known which express themselves in ocular abnormalities, either exclusively or associated with other abnormalities. Of these genes, 91 are recessive, 30 are sex-linked and 125 are dominant.

Moreover, we are very often able to detect certain preclinical manifestations of hereditary diseases in the carriers of genes, thanks to more accurate methods of examination and to biochemical, radiologic, ophthalmologic and other techniques; such microsymptoms enable us to decide the actual method of inheritance followed by the gene involved. As a result, we are able to make a general prognosis and to take prophylactic measures.

TABLE 1—*Differential Diagnosis of Different Forms of Albinism*

	Complete General Albinism	Incomplete General Albinism	Ocular Albinism	Partial Albinism
Ocular Symptoms				
Iris	Pink or pale blue; hypoplasia	Yellowish-green or blue	Hypopigmented or normal	Generally normal
Fundus	No pigments	Albinotic, but sometimes more or less pigmented	Albinotic, sometimes a little pigmented	Normal
Signs in heterozygotes	Diaphanous iris	Diaphanous iris	Diaphanous iris; fundus characteristic	
Correlations	Myopia, astigmatism, lacunae of iris	Myopia	Myopia, astigmatism, lacunae of iris	Lateral displacement of internal canthi; hyperplasia of eyebrows; heterochromia
General Symptoms				
Skin	White	More or less pigmented	More or less pigmented; sometimes normal	Restricted depigmented areas
Hair	White or straw-colored	Blond	More or less pigmented; sometimes normal	White forelock
Correlations	Psychic abnormalities; hypogenitalism		Psychic abnormalities	Deaf and dumb; hyperplasia of base of nose
Heredity	Autosomal recessive	Autosomal recessive or dominant	Sex-linked recessive	Dominant

REFERENCES

Andersen, B. 1944. Some remarks on the hereditary behavior of Leber's disease. Acta ophth. 22: 233–236.

Anderson, T. E., and Begg, M. 1950. Xeroderma pigmentosum of mild type. Brit. J. Dermat. 62: 402–407.

Bauer, K. 1940. Handbuch der Erbbiologie 4: 2, 2nd part, 1142.

Bearn, A. G. 1953. Genetic and biochemical aspects of Wilson's disease. Am. J. Med. 15: 442–449.

——. 1960. Wilson's Disease. In Stanbury, J. B., Wyngaarden, J. B., and Frederickson,

D. S., Eds.: The Metabolic Basis of Inherited Disease. New York: McGraw Hill, pp. 1295–1301.

Beattie, P. H. 1947. A consideration of aniridia, with a pedigree. Brit. J. Ophth. 31: 649–676.

Bell, J. 1933. Treasury of human inheritance, London: Cambridge University Press.

Betsch, A. 1929. Ueber die menschliche Refractionskurve. Klin. Monatsbl. Augenh. 82: 365–379.

Böhringer, H. R. 1956. Statistik, Klinik und Genetik der schweizerischen Retinoblastomfälle. Arch. Julius Klaus-Stiftg. Vererbgsforsch. 31: 1–16.

Brunner, W. 1930. Ueber den Vererbungsmodus der verschiedenen Typen der angeborenen Rotgrünblindheit. Graefes Arch. Ophth. 124: 1–52.

Crowe, F. W., and Schull, W. J. 1953. Diagnostic importance of café-au-lait spot in neurofibromatosis. Arch. Int. med. 91: 758–766.

Döderlein, G. 1922. Ueber die Vererbung von Farbensinnstörungen. Arch. Augenh. 90: 43–66, 1922.

Dollfus, M. A., and B. Auvert. 1953. Le gliome de la rétine et les pseudo-gliomes. Paris: Masson & Co., 1953.

Falls, H. F. 1951. Sex-linked ocular albinism displaying typical fundus changes in the female heterozygote. Am. J. Ophth. 34: 41–50.

——. 1954. Clinical detection of the genetic carrier state in ophthalmic pathology. Am. J. Ophth. 37: 841–854.

——, and C. W. Cotterman. 1948. Choroidoretinal degeneration: A sex-linked form in which heterozygous women exhibit a tapetal-like retinal reflex. Arch. Ophth. 40: 685–703.

——, and J. V. Neel. 1951. Genetics of retinoblastoma. Arch. Ophth. 46: 367–389.

Fisher, R. A. 1936. Tests of significance applied to Haldane's data on partial sex linkage. London: Ann. Eugen. 7: 84–104.

Fleischer, B. 1938. In Gütt's Handbuch der Erbkrankheiten 5: 11.

Franceschetti, M. A. 1930. Ueber mikrophakie und deren Erbgang. Klin. Monatsbl. Augenh. 85: 285.

——. 1946. Malformations oculaires et auriculaires familiales. Revue d'Oto-Neuro-Ophth. 18: 500–501.

——. 1953. Les dysplasies ectodermiques et les syndromes héréditaires apparentés. Dermat. 106: 129–156.

——. 1953. Discussion du rapport de Dollfus et Auvert. Bull. Soc. Franç. Opht. 66: 21.

——. 1954. Discussion of the paper by Dufour and colleagues. Bull. Soc. Franç. Opht. 67: 324.

——, et V. Bischler. 1946. Rétinoblastome et Hérédité. Arch. Julius Klaus-Stiftg. Vererbgsforsch. 21: 322–328.

——, and C. Brugger. 1944. Ueber das kombinierte Auftreten von endogenem Schwachsinn und angeborenem Star. Schweiz. med. Wchnschr. 74: 255–258.

——, and D. Klein. 1957. Two families with parents of different types of red-green blindness. Acta Genetica 7: 255–259.

François, J. 1957. Un nouveau syndrome: dyscéphalie avec tête d'oiseau et anomalies dentaires, nanisme, hypotrichose, atrophie cutanée, microphtalmie et cataracte congénitale. Bull. soc. belge Opht. 117: 569–597.

——. 1961a. Heredity in Ophthalmology. St. Louis: The C. V. Mosby Co.

——. 1961b. Syndromes with congenital cataract. XVI Jackson Memorial Lecture. Am. J. Ophth. 52: 207–238.

——, J. P. Deweer, and J. Van Den Berghe. 1951. Glaucome chronique simple á hérédité dominante. Ann. Ocul. 184: 404–422.

——, and ——. 1952. Sclérose tubéreuse cérébrale de Bourneville. Ophthalmologica 124: 321–339.

——, and ——. 1953. Albinisme oculaire lié au sexe et altérations caractéristiques du fond d'oeil chez les femmes hétérozygotes. Ophthalmologica 126: 209–221.

——, J. Bekaert, et R. Stefens. 1953. A propos de la pathogénie du syndrome de Bardet-Biedl. Ann. Ocul. 186: 665–694.

Gallemaerts, E. 1961. Anophtalmie congénitale et familiale. Ann. Ocul. 161: 490–496.

Garland, H., and D. Moorhouse. 1953. An extremely rare recessive hereditary syndrome including cerebellar ataxia, oligophrenia, cataract and other features. J. Neurol. Neurosurg. & Psychiat. 16: 110–116.

Goedbloed, J. 1942. Mode of inheritance in chorioideremia. Ophthalmologica 104: 308–315.

Grant, R., and H. F. Falls. 1944. Anodontia: report of case associated with ectodermal dysplasia of anhidrotic type. Am. J. Orthodontics 30: 661–672.

Haldane, J. B. S. 1936. A search for incomplete sex linkage in man. Ann. Eugen. London: 7: 28–57.

Hess, V. C. 1921. Die relative Rotsichtigkeit und Grünsichtigkeit. Graefes Arch. Ophth. 105: 137–153.

Hoede, K. 1940. Erbpathologie der menschlichen Haut. In Handbuch der Erbbiologie. Berlin: Julius Springer, vol. 3, pp. 456–457.

Imai, Y., and D. Moriwaki. 1936. Probable case of cytoplasmic inheritance in man: critique of Leber's disease. J. Genetics 33: 163–167.

Just, G. 1925. Zur Vererbung der Farbensinnstufen beim Menschen. Arch. Augenheilk. 96: 406–418.

Kaelin, A. 1955. Statistische Prüf- und Schätzverfahren für die relative Haüfigkeit von Merkmalsträgern in Geschwisterreichen bei einem der Auslese unterworfenen Material mit Anwendung auf das Retinagliom. Arch. Julius Klaus-Stiftg. Vererbgsforsch. 30: 263–485.

Kawakami, R. 1926. Beiträge zur Vererbung familiären Sehnervenatrophie. Graefe's Arch. Ophth. 116: 308–315.

Kreiker, A. 1939. Die Stabilisation, eine Ergänzung zur Steigerschen Lehre. Graefe's Arch. Ophth. 140: 269–277.

Leber, Th. 1871. Ueber hereditäre und congenital-angelegte Sehnervenleiden. Graefe's Arch. Ophth. 17: 249–291.

Lundsgaard, R. 1938. Ein Dänischer Stammbaum mit Vererbung von Leberscher Sehnervenatrophie. Acta. Ophth. 16: 89–100.

——. 1944. Leber's Disease. Acta Ophth. (Suppl.) 21: 1–306.

McCulloch, C., and R. J. P. McCulloch. 1948. Hereditary and clinical study of choroideremia. Trans. Am. Acad. Ophth. 52: 160–190.

Macklin, M. P. 1956. A study of retinoblastoma in Ohio families. First International Congress on Human Genetics. 754–755.

——. 1960. A study of retinoblastoma in Ohio. Am. J. Hum. Genet. 12: 1–43.

McMillan, L. 1921. Anophthalmia and maldevelopment of the eyes: Four cases in the same family. Brit. J. Ophth. 5: 121–122.

Marchesani, E. 1933. Sopra un caso di anoftalmo e microftalmo con coloboma irideo. Contributo alla conoscenza delle malformazioni congenite oculari. Boll. Ocul. 12: 225–244.

Morton, N. E. 1957. Further scoring types in sequential linkage tests with a critical review of autosomal and partial sex-linkage in man. Am. J. Human Genet. 9: 55–75.

Nettleship, E. 1909. On some hereditary diseases of the Eye. The Bowman Lecture. Trans. Ophth. Soc. U. K. 29: 57–198.

Ostroumov, V. M. 1952. Origin of spherical refraction of human eye. Vestnik Oftalm. (n° 4) 31: 12–17.

Pfändler. 1950. Une souche du Vallon de St. Imier (Suisse), manifestant par consanguinité la transmission récessive de malformations oculaires multiples (ectopie du cristallin, kératocône, cataracte congénitale, atrophie du nerf optique), associées à l'oligophrénie, manifestation d'une achromatopsie totale dans une branche collatérale. Ophthalmologica 119: 103–113.

——. 1954. Le questionnaire génétique. J. Génét. humaine 3: 149–150.

Pfanmüller, F. 1894. Beitrag zu den Kolobomen des Auges, Thesis, Giessen.

Rayner, S. 1952. Juvenile amaurotic idiocy. Acta. Genet. Basel 3: 1–5.

Reese, A. B. 1949. Heredity and Retinoblastoma. Arch. Ophth. 42: 119–122.

——. 1954. Frequency of retinoblastoma in progeny of parents who have survived the disease. Arch. Ophth. 52: 815–818.

Riddell, W. J. B. 1941. Uncomplicated hereditary megalocornea. Ann. Eugenics 11: 102–107.

Rönne, H. 1944. The inheritance of Leber's disease (cytoplasmic inheritance). Acta. Ophth. 22: 203–213.

——. 1945. Cytoplasmic inheritance in Leber's disease. Acta. ophth. 23: 89–93.

Saint-Hilaire, I. G. 1832–1836. Histoire générale et particulière des anomalies de l'organisation chez l'homme et les animaux, I: 293.

Salleras, A., and J. C. O. de Zarate. 1950. Recessive sex-linked inheritance of external ophthalmoplegia and myopia coincident with other dysplasias. Brit. J. Ophth. 34: 662–667.

Schinz, H. 1941. Ergebnisse der physiologischen Genetik in ihrer Bedeutung für die menschliche Erbforschung. Arch. Julius-Klaus-Stiftg. Vererbgsforsch. 16: 645–668.

Siemens, H. W. 1920. Ueber die Ätiologie der Ectopia lentis et pupillae. Graefe's Arch. Ophth. 103: 359–383.

Sjögren, T. 1935. Klinische und Vererbungsmedizinische Untersuchungen über oligophrenie mit kongenitaler Katarakt. Ztschr. ges: Neurol. Psychiat. 152: 263–292.

——. 1950. Hereditary congenital spino-cerebellar ataxia accompanied by congenital cataract and oligophrenia; genetic and clinical investigation. Confinia Neurol. 10: 293–308.

Sorsby, A. 1951. Genetics in Ophthalmology. St. Louis: The C. V. Mosby Co.

——, A. Franceschetti, R. Joseph, and J. B. Davey. 1952. Choroideremia: clinical and genetic aspects. Brit. J. Ophth. 36: 547–581.

Steiger, A. 1913. Die Entstehung der sphärischen Refraktionen des menschlichen Auges, Berlin: S. Karger.

Stenström, S. 1948. Variations and correlations of the optical components of the eye. In Sorsby, A., Ed.: Modern Trends in Ophthalmology. New York: Paul B. Hoeber, Inc.

Stern, C. 1958. Le Daltonisme lié au chromosome X a-t-il une localisation unique ou double? J. génét. humaine 7: 302–307.

Straub, M. 1918–1919. La part de l'hérédité et de l'étude dans le développement de la myopie. Arch. Ophth. 36: 68–84.

Tron, E. 1929. Variationsstatistische Untersuchungen über Refraction. Graefe's Arch. Ophth. 122: 1–33.

Tucker, D. P., A. G. Steinberg, and D. G. Cogan. 1957. Frequency of Genetic Transmission of sporadic retinoblastoma. Arch. Ophth. 57: 532–535.

Turpin, R., H. Jerome, and H. Schmitt. 1953. Study of variations of the coeruloplasmin by an easy technique. Proc. Roy. Soc. Med. 46: 1061–1062.

Uzman, L. L., and B. Hood. 1952. Familial nature of amino-aciduria of Wilson's disease (hepatolenticular degeneration). Am. J. M. Sc. 223: 392–400.

Vanderdonck, R., et G. Verriest. 1960. Femme protanomale et hétérozygote mixte ayant deux fils deutéranopes, un fils protanomal et deux fils normaux. Biotypologie 21: 109–120.

Vogel, F. 1954. Ueber Genetik und Mutationsrate des Retinoblastoms. Ztschr. Menschl. Vererb. 32: 308–336.

Waaler, G. 1927. Ueber die Erblichkeitsverhältnisse der verschiedenen Arten von angeborenen Rotgrünblindheit. Acta. Ophth. 5: 309–345.

Waardenburg, P. J. 1947. Herkenbaarheid van latente overdragers van Albinismus universalis en albinusmus oculi. Nederl. Tijdschr. Geneesk. 91: 1863–1866.

——. 1949. Gross remnants of the pupillary membrane, anterior polar cataract and microcornea in a mother and her children. Ophthalmologica 118: 828–842.

——. 1951. A new syndrome combining developmental anomalies of the eyelids, eyebrows and nose root with pigmentary defects of the iris and head hair and with congenital deafness. Am. J. Human Genet. 3: 195–253.

Weekers, R., C. Gougnard-Rion, and L. Gougnard. 1955. Considérations cliniques sur l'hérédité des glaucomes. Bull. Soc. Belge Opht. 110: 255–267.

Wölfflin, E. 1923. Ueber das Vererbungsgesetz der anomalen trichromaten. Pflüger's Arch. 201: 214–219.

Author Index

Ackermann, D., 136
Adinolfi, di M., 25
Akedo, H., 112, 117
Albers, R. W., 121, 132
Alexander, J., 198
Allan, J., 131, 132
Allen, D. W., 36
Allen, R. J., 120
Allfrey, V. G., 193
Allison, A. C., 40, 55, 64, 138, 145, 160
de Almeida, J. C., 306
Alsberg, C., 136
Altman, K. I., 133
Altmann, F., 317
Andersen, B., 112, 341, 351
Anderson, H. D., 35
Anderson, J. R., 215
Anderson, R., 316
Anderson, R. S., 305
Andrus, S. B., 318
Anker, H. S., 193
Antonis, A., 97
Arends, T., 53
Armstrong, M. D., 91, 141
Asatoor, A. M., 99
Ashton, G. C., 48, 49, 50, 321
Ashworth, G., 218
Askonas, B. A., 190, 193, 194
Attardi, G., 191
Atwood, 23
Audran, R., 16, 21, 22
Auerbach, C., 254 269, 270, 272, 273, 274, 275, 276, 277, 278, 279, 300, 301, 302, 303
Auld, R. M., 119
Austin, C. R., 232, 235, 237, 238, 239, 242
Austin, R. E., 36
Auvert, B., 352, 353
Awai, M., 36
Axelrod, J., 318
Baber, M. D., 146
Baldwin, E., 131, 162
Bailey, J. D., 125
Baitsch, H., 73
Balenovic, K., 134
Ball, J., 220, 222
Bangham, J. W., 246, 260, 261, 272, 279, 282, 284, 285, 286, 287, 288
Bard, L., 87
Bardawil, W. A., 214
Barnes, D. W. H., 279
Barnett, H. L., 101
Barnicot, N. A., 43, 44, 55, 56
Baron, D. M., 139, 140, 141
Bartter, F. C., 37, 126
Bateman, A. J., 251, 252, 254, 255, 256, 257, 276
Bates, H. M., 193
Battaglia, F. C., 117
Bauer, C. D., 152
Bauer, K., 341
Baumgartner-Gamauf, M., 317
Bearn, A. G., 35, 37, 41, 42, 43, 45, 46, 48, 50, 51, 58, 59, 67, 68, 69, 71, 72, 73, 74, 76, 77, 150, 151, 152, 340
Beattie, P. H., 345
Beatty, R. A., 232, 235, 236, 237, 238, 240, 241, 242, 310
Becker, E. L., 187
Becker, F. F., 138
Beckman, L., 43, 48, 52, 57, 68, 79
Beecher, H. K., 83
Beerman, W., 99
Begg, M., 341
Bekaert, J., 340
Bell, J., 345
Benditt, E. P., 65
Ben-Ishay, D., 145, 146
Bennett, D., 301
Bennett, J. C., 214
Berenbaum, M. C., 191
Berglund, F., 109
Bergman, S., 25
Bergmann, F., 105, 112
Bergren, W. R., 143
Bergstedt, J., 94, 97, 99
Berliner, R. W., 112
Berlow, 125
Bermann, H., 104
Bernhard, W., 193
Bernstein, S. E., 315 318
Berry, H. K., 88, 89, 91, 92, 98, 99, 101, 102, 103, 130, 135, 138
Berry, J. S., 160
Bessman, S. P., 120, 162
Best, W. R., 53
Bethune, J. E., 126
Betke, K., 37
Betsch, A., 347, 351
Beutler, E., 308
Bickel, H., 101, 147, 148
Biel, H., 71, 79
Bigwood, E. J., 84, 88, 89, 96, 98, 104, 130
Billett, F. S., 310
Billingham, R. E., 190, 304, 305
Binkley, E. L., 91, 145
Bischler, V., 353
Bjornboe, M., 191
Blandau, R. J., 237
Blatherwick, N. R., 130
Blattner, R. J., 152, 157
Blix, U., 188
Bloch, K. J., 213
Block, R. J., 87
Bloom, J. L., 319
Bloxam, H. R., 101
Blumberg, B., 135
Blumberg, B. S., 10, 48, 55, 64, 138
de Bobes-Sternberg, S., 152
Boccabella, A., 254
Bock, M., 254
Böhringer, H. R., 353
Bomsel-Helmreich, O., 238, 239, 241, 242
Bonetti, E., 90, 97
Bonner, D. M., 108
Bonting, S. L., 156
Borden, M., 87
Bowden, C. H., 88
Bowman, B. H., 47
Boyd, W. C., 188, 189, 190
Boyden, S. V., 195, 200
Boyer, A., 124, 132
Boyer, S., 72
Boyer, S. H., 8, 9, 10, 21, 28, 48, 50, 126
Boylan, J. W., 109
Braden, A. W. H., 48, 232, 235, 236, 237, 238, 239, 308, 309, 310, 321
Braden, A. W. H., 48
Brandt, I. K., 193
Brandtzaeg, B., 6
Braun, 323
Braun, W., 188
Braverman, N., 37
Breinl, F., 198
Brenneke, 249, 250
Brent, L., 190, 214
Bretthauer, R. K., 143
Bridges, 306
Bridges, R. A., 190, 192
Briggs, J. H., 306
Brigham, M. P., 11, 138, 149
Britten, J. S., 112
Brønnestam, R., 19
Brooks, L., 37
Brown, E., 36
Brown, J., 214
Brown, J. L. 109, 112
Brown, W. H., 314
Brugger, C., 355
deBruin, H., 156
Brummerstedt-Hansen, E., 76
Brunjes, S., 220
Brunner, R., 356
Bryan, B. A., 36
Buchanan, W., 215
Buckle, A. L. J., 89
Buettner-Janusch, V., 195
Bukantz, S. C., 189, 192
Bulman, N., 188
Bunim, J. J., 213
Burgen, A. S. V., 115
Burnet, F., 224
Burnet, F. M., 189, 197, 198, 199, 200
Burtin, P., 13, 65
Buschmann, H., 48
Bussard, H. E., 196
Butler, A. M., 93
Butler, B. A., 148, 149
Butterfield, J., 94
Cadenas, E., 112
Campbell, D. H., 187, 188, 190
Campbell, P. N., 193
Cann, J. R., 187, 197
Cannigia, A., 157
Cannon, P. R., 189
Carey, J. H., 154
Carleton, A., 37

Caroline, L., 35
Carson, N. A., 159
Carter, T. C., 263, 269, 270, 273, 274, 275, 279, 280, 281, 282, 283, 284
Carver, M. J., 103, 104, 111
Caspari, E., 265
Cassady, G., 127
Cattanach, B. M., 244, 245, 246, 247, 254, 255, 257, 260, 261, 269, 272, 273, 275, 276, 279, 281, 284, 285, 286, 287, 288
Caylor, H. D.. 153
Cebra, J. J., 23
Chadwick, J. M., 98
Chai, C. K., 315
Chang, M. C., 232, 235
Chapelle, E. W., 85, 86
Charles, D. R., 275
Charley, P. J., 35
Chase, M., 190
Cherniak, M., 151
Cheung, M. W., 97
Chi, C. A., 3
Chiamori, N., 85
Childs, B., 87, 99, 104, 128
Chisolm, J. J., Jr., 84, 144, 146, 154
Choy, S. H., 215
Christensen, H. M., 104, 112, 113, 114, 115, 116, 117
Christensen, H. N., 117
Christensen, P. J., 104, 111
Christie, F. H. E., 126
Christophe, J., 318
Chu, E. H. Y., 246, 307
Cinader, B., 190
Clarke, J. T., 91, 94, 161
Clay, R. D., 147
Clayton, B. E., 149
Cleve, H., 67, 68, 69, 71, 72, 73, 76, 77
Cochrane, W., 123
Cochrane, W. A., 123
Coen, L. J., 112
Coffman, C. M., 251
Cogan, D. G., 146, 354
Cohen, B. L., 322
Cohen, C., 156
Cohen, M. M., 127
Cohn, E. J., 84
Cohn, G. L., 193
Cohn, M., 191
Coleman, D. L., 311, 322
Coles, H. M. T., 159
Collis, W. R. F., 97
Combes, B., 152
Comfort, M. W., 153
Condie, R. M., 192
Conley, C. L., 215
Connell, G. E., 45, 89
Connolly, J. M., 191
Consden, R., 88
Cooke, R. E., 87
Coon, M. J., 153
Coons, 323
Coons, A. H., 191
Cope, F. W., 89
Corcos, J. M., 221
Coryell, F. C., 35
Cotterman, C. W., 333
Coursin, D. B., 98
Courtice, F. C., 192
Cowie, V. A., 121
Craig, J., 225
Craig, J. M., 121
Crampton, R. F., 112
Cravioto, J., 98
Crawford, M. A., 105, 140
Crawhall, J. C., 87
Creger, W. P., 215
Creighton, A. S., 221
Crigler, J. F., 318
Crittenden, L. B., 319
Crokaert, R., 84
Cromartie, W. J., 190
Crome, L., 121
Crowe, F. W., 341
Crow, J. F., 323
Crumpler, H. R., 133
Cryssel, R., 22
Cullen, A. M., 86
Currarino, G., 127
Cusworth, D. C., 94, 98, 105, 106, 111, 126, 131, 134, 141, 143, 144, 148, 158, 159
DaCosta, F. M., 155
Dagenais, Y. M., 318
Dammin, G., 215
Dammin, G. J., 189
Dancis, J., 37, 111, 120, 121, 122, 123, 124
Darling, S., 98, 144
Darmady, E. M., 144, 147
Darwin, C., 109
Date, J. W., 104
Datta, S. P., 88, 97, 160, 161
Davidson, C. S., 96
Davidson, J. D., 157
Davies, R. K., 131
Day, M. G., 98
Dedmon, R. E., 147, 149
Defalco, A., 131, 132
Deicher, H., 213, 215, 220
DeMoss, J. A., 108
Dent, C. E., 83, 88, 90, 94, 97, 98, 105, 111, 122, 123, 124, 126, 131, 133, 134, 136, 137, 138, 139, 141, 144, 145, 147, 148
Dessauer, H. C., 48
Devriendt, A., 143
Deweer, J. P., 334, 340, 341
Diamond, L. K., 37
Dickie, H. A., 215
Dikstein, S., 105, 112
Dillon, R. T., 85
DiMinno, R. L., 255, 256
Dimson, S. B., 148, 149
Dineen, J. K., 193, 196
Dingman, W., 104, 131, 132
Dixon, F. J., 189, 190, 192, 216, 217, 218
Dixon, G. H., 89
Dixon, W. J., 254
Djaldetti, M., 142
Dobzhansky, Th., 98, 160
Döderlein, G., 356
Doell, R. G., 144
Dollfus, M. A., 352, 353
Doniach, D., 213
Donnell, G. M., 149, 150
Donnell, G. N., 143
Doolan, P. D., 121
Dorfmeijer, H., 24
Douglas, R., 55
Dresser, D. W., 190
Dreyfuss, F., 145
Drèze, A., 89
Driscoll, S. G., 99
Dubert, J. M., 190
Dubois, R., 98
Dubiski, S., 2
Dunn, L. C., 1, 264, 265, 296, 298, 302, 309
Dunn, M. S., 136
Durrum, E. L., 87, 90
Dustin, J. P., 89, 99, 103, 114, 143
Dutton, G., 122
Eastoe, J. E., 89, 156
Eckhardt, R. D., 96, 97
Edelman, G. M., 23, 195
Edozien, J. C., 97, 98
Edsall, G., 188, 189, 190
Edsall, J. T., 84
Edwards, K. D. G., 99
Edwards, R. G., 232, 235, 237, 240, 241, 242, 250, 251, 253, 254, 255, 257
Efron, M. E., 133
Efron, M. L., 90, 116, 120
Ehrenberg, A., 35
Ehrich, W. E., 191
Ehrlich, P., 197, 198, 199
Eisen, H. N., 187, 193, 194
Elian, E., 161
Elsden, D. F., 89
Elson, D., 194
Elvehjem, C. A., 132
Engel, F. L., 116
Engle, R. L., Jr., 146
Ephrussi, B., 323
Epstein, W. L., 215
Espagnon, P., 3
Evans, A. H., 108
Evered, D. F., 90, 94, 111, 120, 134
Eyquem, A., 1, 18, 22, 25, 28
Fagraeus, A., 191, 196
Fairbanks, V. F., 308
Falconer, D. S., 213, 254, 275, 278, 289, 319
Falls, H. F., 333, 334, 335, 338, 341, 353
Farr, R. S., 189
Farr, L. E., 36
Fawcett, D. W., 310
Fawcett, J. S., 98
Fazekas, J. F., 121
Feldman, J., 217, 218
Feldman, M., 194
Fellers, F. X., 148
Felton, L. D., 190
Fenner, F., 198
Ferguson, E., 3
Ferguson-Smith, M. A., 230
Field, C. M. B., 159
Filteau, G., 89
Finch, C. A., 35, 38, 58
Finch, L. R., 112, 114, 116, 138, 141
Finch, S. C., 38
Fine, J. M., 8, 16, 21, 22
Fink, K., 133
Fink, R. M., 133
Firschein, I. L., 135, 136, 160
Fischberg, M., 236, 238, 241
Fisher, R. A., 341
Fishman, M., 196
Fitch, F. W., 190, 192
Fleischer, B., 345
Florkin, M., 109
Flynn, F. V., 98, 148
Folin, O., 136
Forbat, A., 65
Ford, C. E., 279, 282, 283
Ford, D. E., 306
Fowler, D. I., 97, 101, 103, 111
Fox, W., 48
Frame, E. G., 85, 86
Franceschetti, M. A., 338, 344, 345, 347, 353, 355
François, J., 332, 334, 340, 341, 352, 355, 356
Frank, M., 142
Frankl, W., 136

Franklin, E. C., 13, 23, 221
Fraser, D., 126
Fraser, F. C., 301, 320
Fredrickson, D. S., 118
Freidman, H., 194
Freter, G. G., 199
Freund, J., 189, 190
Friedman, E. A., 214
Frimpter, G. W., 138, 159, 163
Friou, G., 223
Fudenberg, H., 6, 10, 13, 16, 18, 19, 23, 221, 225
Gagnon, A., 112
Gahne, B., 48
Gairdner, D., 121, 122
Galatius-Jensen, F., 3, 88, 196
Gallango, M. L., 53
Gallemaerts, E., 345
Garcia, J. F., 36
Garland, H., 355
Garlick, J. P., 43, 56
Garrod, A. E., 36, 83
Gartler, S. M., 98, 133, 134, 135, 136, 160
Garvey, J. S., 190
Gates, W. H., 261
Gayer, J., 112
Geiger, D. W., 151
Gerald, P. S., 37, 320
Gerber, G., 133, 156
Gerber, G. B., 133, 156
German, J. L., 221
Gerok, W., 111, 112
Gerritsen, T., 103, 159, 163
Ghadimi, H., 91, 93, 94, 95, 97, 98, 99, 120, 125, 162
Gibbs, N. K., 98
Giblett, E. R., 37, 40, 41, 42, 43, 44, 47, 48, 49, 52, 53, 55, 56, 57, 64
Gibson, B. H., 279
Gibson, R. M., 120
Gidaspow, T., 136
Gilbert, J. B., 116, 134
Giles, B. D., 4, 6, 7, 9, 10, 15, 25
Gill, T. J., 111, 114
Ginsburg, B. E., 315
del Giovane, L., 157
Girão, C. B., 140
Gitlin, D., 36, 195, 198, 225
Givol, D., 23
Glass, B., 72
Glass, H. B., 9
Glendening, M. B., 101, 104
Globerson, A., 194
Gluecksohn-Schoenheimer, S., 264, 298, 309
Gluecksohn-Waelsch, S., 264, 296, 298, 301, 302, 309, 312, 317, 320, 321, 324
Goedbloed, J., 333
Gold, G. L., 114
Goldberger, R. F., 112
Goldbloom, R., 105
Goldstein, D. A., 113
Goldstein, F. B., 121
Gomez, F., 98
Good, R. A., 192
Goodman, M., 48, 50
Gorden, A. H., 88
Gordon, H. A., 192
Gordon, M. J., 308, 309
Gorer, P. A., 305, 306
Gorham, J., 225
Gormsen, H., 191
Gorrie, G., 224
Goudie, R. B., 215
Gougnard, L., 341
Gougnard-Rion, C., 341
Gowen, J. W., 251, 262, 320
Gower, J. S., 306
Gower, J. S., 244, 245
Grabar, P., 13, 65, 67
Granboulan, N., 193
Grant, R., 338
Green, D. E., 138
Green, H., 112, 138, 189
Green, S., 37
Green, T. W., 189, 193
Greengard, O., 193
Greenstein, J. P., 84, 87
Gregory, K. F., 132
Grice, D., 225
Griffen, A. B., 244, 277, 279
Grobman, A. B., 275
Groshek, A. P., 154
Gross, J. B., 153
Grossman, L., 188
Grubb, R., 1, 2, 3, 4, 6, 12, 13, 15, 16, 18, 20, 24, 25, 26, 64
Grüneberg, H., 265, 299
Gubler, C. J., 36
Guest, G. M., 91
Guideri, R., 157
Gullbring, B., 309
Gunderson, C. H., 190, 192
Gunn, D. R., 65
Guthrie, R., 87, 155
György, P., 136
Hadorn, E., 300
Hagedorn, D., 324
Häggqvist, 240
Hagihira, H., 112, 116
Hais, I. M., 87, 88
Halberg, F., 107
Haldane, J. B. S., 241, 341
Halden, E. R., 36
Hall, R., 221
Hall, V., 109
Hamburgh, M., 313, 315
Hamerton, J. L., 279
Hamilton, P. B., 85
Hammaker, L., 132, 318
Hanan, R., 190
Hanau, C., 214
Hancock, J. L., 237
Hanes, C. S., 89
Hanna, B. L., 127
Hansen, R. G., 97, 143
Harboe, M., 4, 6, 12, 15, 21, 22, 24, 25, 28
Hare, D., 115
Hare, R. S., 94
Hargis, B. J., 189
Harlow, H., 121
Harman, P. J., 311, 312
Harper, H. A., 104, 121, 148, 150
Harris, H., 40, 42, 43, 50, 52, 57, 65, 88, 97, 118, 127, 132, 133, 135, 136, 138, 139, 144, 145, 152, 158, 160, 161
Harris, J. I., 89
Harris, M., 87
Harris, P. F., 192
Harris, S., 191, 196
Harris, T. N., 191, 196
Harrison, H., 144, 146
Harrison, H. C., 146
Harrison, H. E., 84
Hart, E. W., 139
Hartley, B. S., 89
Hartley, G., Jr., 191
Hartwig, Q. L., 49
Harvey, A. M., 215
Hasselmeyer, E., 101
Hauge, M., 3, 196
Haupt, H., 71, 79
Haurowitz, F., 190, 198
Hauschka, T. S., 307
Hausmann, E., 156
Hazzard, W. R., 311
Heffelfinger, J. C., 91, 120
Heide, K., 71, 79
Heidelberger, M., 189, 190
Heilmeyer, L., 37
Heimer, R., 221
Heinz, E., 113
Heller, G., 3
Helmreich, E., 193, 194
Hemet, Y., 16
Hempling, H. G., 115
Henderson, M. J., 112
Henderson, R. B., 133
Henion, W. F., 132
Hennessey, T. G., 36
Henningsen, K., 7
Henry, C., 8, 18
Henry, R. J., 85
Henson, J. B., 225
Hersov, L. A., 141
Hertwig, 249, 250, 267, 274, 278
Hess, E., 218
Heston, 319
Heustis, R. R., 305
Heys, F. M., 157
Hickman, C. G., 40, 41, 48, 64
Hillcoat, B. L., 92, 135
Hiller, O., 39, 40, 44
Hillman, R. S. L., 112
Himwich, H. E., 121
Hird, F. J. R., 112, 114, 116, 138, 141
Hirokawa, R., 195
Hirschfeld, J., 65, 66, 67, 68, 69, 72, 73, 75, 76, 77
Hirschmann, S. Z., 151
Hitzig, W. H., 37
Hoagland, M., 193
Hoecker, G., 304
Hoede, K., 341
Hogen, W., 126
Hollander, W. F., 251
Holley, H. L., 214
Holman, H., 213, 214, 215, 216, 220, 223
Holman, H. R., 13, 216
Holmberg, C. G., 35
Holmes, M., 224
Holmgren, G., 48, 52, 57
Holt, L. E., Jr., 84, 98, 118, 123, 124, 130, 154
Holter, H., 113, 114
Holub, M., 191, 192, 196
Holzel, A., 113, 144
Honda, M., 104
Hope, D. B., 133
Horibata, K., 191
Horsfall, 39, 43
Hotchkiss, J., 116
Hotchkiss, R. D., 108
Hrubesova, M., 193, 194
Hsia, D. Y-Y., 99, 118, 121
Hudson, R., 213
Huestis, R. R., 316
Huff, R. L., 36
Huggins, A. K., 88
Hughes, B., 136
Hughes, W. L., 190
Huisman, T. H. J., 96, 97, 99, 101, 103

Humphrey, J. H., 190, 194
Hunter, A., 120, 125
Hurel, R., 14
Hurst, P. E., 121
Hutchison, J. H., 98
Hutt, F. B., 320
Hutzler, J., 123
Ibbott, F. A., 94
Ikawa, M., 117
Iland, C. N., 188
Imai, Y., 357
Ingelman, B., 35
Ingram, V. M., 321
Inman, J. K., 35, 36
Iseki, S., 10
Ishihara, T., 245
Isselbacher, K. J., 143, 144
Jackson, C. E., 153, 154
Jackson, H., 254
Jacob, F., 99, 108, 300
Jacobs, E., 91
Jacobs, F. A., 112
Jacobs, J., 55
Jacobson, A. S., 3
Jacqueline, F., 14, 18, 22, 25
Jaffe, J., 263, 265
Jagenburg, O. R., 89, 93, 94, 96, 97, 99, 101, 102, 103, 104, 105, 107, 111, 145, 154, 157, 159
Jager, B. V., 36
Jandl, J. H., 34, 36, 37
Janeway, C. A., 36, 225
Jenssen, W., 73
Jepson, J. B., 99, 124, 139, 144
Jerne, N. K., 189, 199
Jerôme, H., 154
Jervis, G. A., 99, 102, 120, 121, 130, 322
Jessar, R. A., 215
Johnson, D. E., 89
Johnstone, R. M., 114, 116
Jones, F., 36
Jones, J. C., 133
Jones, K. W., 306
Jones, R. T., 153, 156
Jonsson, B., 66, 68
Jonxis, J. H. P., 97, 98, 144, 158
Joseph, R., 158, 159
Joy, G. E., Jr., 319
Jude, A. C., 245
Julian, R., 220
Kaelin, A., 353
Kakimoto, Y., 159
Kalf, G. F., 193
Kalushiner, A., 153
Kalow, W., 65
Kammerer, W. H., 3
Kanode, R. G., Jr., 189
Kapellar-Adler, R., 104
Kaplan, M., 213
Kaplan, W. D., 247, 307
Kaplansky, S. Ya., 98
Karpinski, F. E., 145
Karush, F., 187, 188
Katchalski, E., 23
Katz, J. H., 36
Kaufmann, 220
Keller, R. A., 89
Keller, W., 35
Kelly, E. M., 243, 251, 252
Keltom, D. E., 314
Kendall, F. E., 189
Keogh, E. V., 189
Kern, M., 193, 194, 195
Kernot, B. A., 193
Kerr, A. C., 126, 161
Kessel, D., 117
Keuning, F. J., 190
Khachadurian, A., 86
Kherumian, R., 3
Kibrick, S., 215
Kidwell, J. F., 262
Kile, J. C., Jr., 252
Kimball, A. W., 250
Kind, L., 189
Kind, L. S., 189
Kindred, B. M., 244, 245, 257
King, W., 142
Kinosita, R., 247, 307
Kirk, E., 85
Kirk, R. L., 10, 11, 26, 48, 53, 57
Kistler, P., 35
Kitagawa, L., 130
Klein, D., 344, 356
Kleinmann, H., 69
Knapp, A., 154
Knapp, J., 146
Knauff, H. G., 111
Knedel, M., 65
Knight, C. S., 88
Knobil, E., 116
Knox, W. E., 86, 112, 120, 130, 136, 161
Ko, K. W., 148
Koch, R., 91
Kocher, W., 317
Kochwa, S., 142
Koechlin, B. A., 35
Koller, P. S., 267, 269, 271, 274, 278
Kolodnoy, M. H., 3
Komai, T., 245
Komrower, G. M., 143
Korn, D., 148
Kostyo, J. L., 116
Koucky, R. W., 51
Kramer, K., 109
Kraus, B. S., 135
Krawczynski, J., 94, 107
Kreiker, A., 347
Kretchmer, M., 91, 101, 102, 126, 127, 144
Kretchmer, N., 99
Kritzman, J., 225
Ku, Y., 116
Kuhns, W. J., 215
Kujawa, J., 94
Kunkel, H., 13, 17, 18, 22, 212, 221, 225
Kunkel, H. G., 213, 225
Kupiecki, F. P., 133
Kutscher, F., 136
LaDu, B. N., 102, 130, 162
LaGro, R. J., 91, 120
Lai, L. Y. C., 7, 10, 11, 48, 50, 57
Lamont-Havers, R. W., 13
Landing, B. J., 126
Landsteiner, K., 188, 189, 198
Lane, M. R., 121, 122
Langemann, F. W., 154
Lanset, S., 6, 7, 10
Larsson, O., 220, 222
Lathem, W., 148
Laurell, A. B., 2, 3, 4, 6, 12, 18, 25, 64
Laurell, C. B., 3, 21, 35, 36, 37, 67
Laurell, S., 67
LaVia, M. F., 190, 192
Law, 319
Law, D., 216
Lawler, S. D., 20, 121
Lawrence, J. S., 220, 222
Lawson, F. A., 315
Lazebnik, J., 142
Lea, D. E., 249, 251
Leader, R., 225
Leaf, A., 145
Lederberg, J., 197, 199, 200, 324
Leduc, E. H., 191
Lee, J. M., 310
Lee, S. L., 215
Lefevre, P. G., 112
Lehmann, H., 65
Lehmann, O., 138, 157
Lehninger, A. L., 112
Lehrovaara, R., 48
Lennox, E. S., 191
Lenoir, J., 6, 7, 9, 10, 11, 16, 19, 26
Leonard, S. L., 311
Leonhardt, T., 220, 222, 224
Leoschke, W. L., 132
Levenson, S. M., 105
Leverett, R., 93
Levin, B., 131, 132
Levine, L., 188
Levine, S. Z., 101
Levitz, M., 120, 121, 123
Levy, H., 35
Lewis, A. J., 220
Lewis, B. D., 121
Li, C. C., 9, 72
Lifshay, A., 221
Lim, T. W., 7, 10, 11
Lin, E. C. C., 99, 112, 116, 129, 162
Lind, J., 37
Lindholm, H., 225
Lingtang, I., 153
Link, J. K., 153
Linnet-Jepson, P., 3, 19, 196
Lippman, R. W., 118
Lipton, S. H., 103
Little, C. C., 304
Loeb, H., 98, 143
Loeb, L., 304
Loosli, R., 312
Loughridge, L. W., 99, 140
Loutit, J. F., 279
Louttit, R. T., 120
Low, N. L., 99, 145
Lowe, C. U., 149
Lubchenco, L. O., 94
Lubin, M., 117, 126
Luck, J. M., 85, 86
Luder, J., 146
Lugg, J. W. H., 89, 135, 136, 160
Lund, C. C., 89
Lundevall, J., 4, 6, 24, 25, 28
Lundquist, F., 89, 191
Lundsgaard, R., 357
Lush, D., 189
Lustgraaf, E. C., 202
Lutz, W., 213
Lyon, M. F., 263, 264, 265, 280, 281, 282, 283, 287, 302, 308, 313
Ma, T. S., 94
MacDougall, J. A., 139
Maçek, K., 87, 88
MacFayden, D. A., 85
Mackay, H. M. M., 131
Mackensen, J. A., 318
Mackenzie, D. Y., 120, 121, 122, 123
Macklin, M. P., 352, 353, 354
MacLachan, E. A., 149
Maclachlan, M. J., 221
Major, M. H., 251, 252, 253, 257, 258, 259
Mäkelä, O., 48, 194
Malkiel, S., 189
Mansa-Olsen, 73
Marchesani, E., 345
Marko, A. M., 118
Marone, A., 25
Marshall, D. C., 214
Martel, F., 89
Mårtensson, E. H., 57
Mårtensson, L., 20, 22

Martin, A. J. P., 88
Martin, C. M., 34
Martin, G. M., 65
Martin, G. R., 156, 157
Martinez, A., 313
Marver, H. S., 154
Masouredis, S. P., 3
Massel, B. F., 215
Matson, G. A., 51
Matte, C., 21, 22
Mauer, I., 317
Mauerhofer, M., 35
Maurer, P. H., 190
Mayeda, A., 10
Mayer, J., 318
Mayer, R. L., 188
Mazur, A., 37
McAllister, R., 65
McCall, K. B., 35
McCance, R. A., 126
McCarthy, J., 225
McCarty, M., 215
McCulloch, C., 333
McDonald, H. J., 90
McDougall, E. L., 48
McEvoy-Bowe, E., 89, 135, 136, 160
McEwen, C., 221
McFadden, M. L., 195
McKay, E., 153
McKenna, J., 146
McKenna, J. M., 191, 196
McKhann, G., 121, 128, 132
McKusick, V. A., 84, 149, 156, 157, 220
McLaren, A., 224, 245, 246, 247
McLean, J. R., 193
McMasters, P. D., 190, 191
McMenamy, R. H., 89, 96, 97, 107
McMillan, L., 345
McNamara, H., 99, 101
Medawar, P. B., 190, 214
Medes, G., 102, 130
Meier, H., 318
Meiselas, L. E., 215
Meister, A., 84, 87, 89
Mellors, R., 216, 225
Menano, H., 155
Menkes, J. H., 91, 102, 120, 121, 122, 123, 124, 130
Mergenhagen, S. E., 156
Merler, E., 195, 198
Merrill, J. P., 214
Mertens, E., 191
Messer, M., 155
Metcoff, J., 105, 112
Meyer, H., 86
Meyeserian, M., 213
Michelson, A. M., 311
Mickelson, O., 121, 132
Milgrom, F., 2, 12
Miller, D. S., 315
Miller, J. M., 215
Miller, S., 120
Milne, M. D., 99, 105, 138, 140, 141
Mirsky, A. E., 193
Mitchell, J., 36
Mitchison, N., 190
Mitchison, N. A., 190
Mitoma, C., 119, 155, 156, 157
Mittwoch, U., 137
Mizuhara, S., 159
Mohr, J., 6
Monaghan, E. A., 191
Monod, J., 193, 200
Monod, J., 99, 108, 300
Monto, R. W., 191
Moore, J., 212, 213
Moore, S., 89, 97, 99, 122, 138, 163
Moorhouse, D., 355
Morell, A. G., 65, 151, 152
Morgan, J. F., 155
Morgan, P., 190
Moriwaki, D., 357
Morris, L., 81, 122
Morris, M. D., 121, 122
Morrison, A. B., 126, 141
Morrison, G. A., 87
Mortensen, O., 98
Morteo, O. G., 221
Morton, N. E., 121, 341
Moscona, A. A., 324
Moses, V., 88
Motulsky, A. G., 52, 55, 305, 316
Moullec, J., 3, 8, 12, 19, 20, 21, 22
Mudge, G. H., 105, 142
Mueller, P. S., 97
Muirhead, E. E., 36
Müller-Eberhard, H. J., 13
Murakami, W. T., 188
Nachtsheim, H., 312, 317, 319
Najjar, V. A., 318
Nakai, H., 126
Nash, D. G., 262
Neame, K. D., 121
Neel, J. V., 55, 57, 72, 353
Neil, M. W., 138
Neill, D. W., 116
Nelson, E. L., 190, 191
Nelson, W. O., 254
Nemes, M., 216
Nester, M., 194
Neuhauser, E. B. D., 126
Neuman, W. F., 156
Neville, G. J., 89
Nicolaidou, M., 148
Niederöst, Ch., 35
Nilsson, S. B., 7, 19
Nitschmann, Hs., 35
Noall, M. W., 116, 117
Noell, W. K., 99, 299
Norby, D. E., 245
Norman, A. P., 94, 95, 99, 100, 101, 102, 103, 135
Northrop, J. H., 85
Norton, P. M., 97, 101, 122, 123
Nossal, G. J. V., 191, 194, 196
Novikoff, A. B., 109, 115, 117
Nyhan, W. L., 87, 96, 120
Oakberg, E. F., 245, 251, 255, 256, 278
Oates, J. A., 142
Oberholzer, V. G., 131
O'Brien, D., 94, 97, 99, 101, 102, 103, 154
Odor, D. L., 237
Ogata, K., 195
Ogata, M., 116
Oglesby, R. B., 213
Ohno, S., 247, 307
Olitsky, P. K., 319
Olivelli, F., 25
Omori, S., 195
Ortega, L. G., 216
Osawa, S., 193
Osborne, R. H., 160
Osterland, C. K., 23
Ostroumov, V. M., 349
Otis, E. M., 275
Ottensooser, F., 11
Ouchterlony, O., 65
Oudin, J., 196
Owen, R. D., 193, 198, 303, 305, 309, 322
Owen, S. G., 221
Oxender, D. L., 113, 115, 117
Oyama, J., 190
Page, E. W., 104
Paget, O. E., 317
Paigen, K., 299
Paigen, W., 99
Paine, R. S., 118, 120, 153
Painter, Th. S., 261
Palmer, G., 121
Pappas, G. D., 312
Pappenheimer, A. M., Jr., 200
Pare, C. M. B., 98, 107, 135
Park, C. R., 112
Parker, H. M., 113
Parker, P., 136
Parker, W. C., 35, 41, 42, 43, 45, 46, 48, 50, 51, 52, 53, 56, 58, 59, 72, 74
Parry, T. E., 88
Partington, M. W., 120, 125
Partridge, S. M., 89
Pasieka, A. E., 155
Paska, R., 103, 111
Patrick, A. D., 121, 122, 123, 149, 163
Paterson, P., 217
Pauling, L., 188, 198
Payne, W. W., 123
Pearce, L., 314
Pease, D. C., 109
de la Peña, C., 98
Pennell, R. B., 35
Penrose, L. S., 132
Perry, B. T., 193, 196
Perry, T. L., 91, 118, 133, 153, 156
Pesonen, 232
Peters, T., Jr., 193
Petit, M. D., 126
Pfanmüller, F., 345
Pfändler, 347, 354
Philips, E. J., 97
Philips, J. H., 188
Phillips, R. J. S., 263, 264, 265, 280, 281, 282, 302, 313
Phythyon, J., 221
Piel, C., 148, 150
Piez, K. A., 89, 122, 158
Pikó, L., 237, 238, 239, 241, 242
Pirani, C. L., 126
Pitts, R. F., 109
Plescia, O. J., 187, 188
Podliachouk, L., 14, 18, 22, 25, 28
Polani, P. E., 306
Pollak, V. E., 156
Polosa, P., 25
Pontecorvo, G., 298
Porter, R. R., 23, 194, 195, 198
Poulik, E., 40, 48, 50
Poulik, M. D., 23, 39, 42, 43, 72, 151, 195
Pratt, E. L., 97, 101
Pressman, D., 188
Prockop, D. J., 155, 156, 157
Puck, 323
Putnam, F. W., 21
Puttanna, C. R., 263
Quastel, J. H., 114, 115
Quinn, R. W., 215
Race, R., 215
Raitt, J., 215
Ramot, B., 73
Randle, P. J., 117
Ranney, H., 78
Ranney, H. M., 320, 321
Rantz, L. A., 215
Rasmuson, M., 66, 68
Rasmussen, H., 98
Rath, C. E., 35
Rathburn, J. C., 126

Ratner, S., 128, 131
Rausen, A. R., 37
Raven, A. M., 154
Ravenni, G., 157
Rayner, S., 339
Rebuck, J. W., 191, 192
Redlich, D., 99
Reed, G., 87
Reinhart, R., 35
Reinskou, T., 73
Reinwein, D., 112
Rendel, J., 48
Renwick, J. H., 121
Reyersbach, G. C., 126
Reyniers, J. A., 192
Rhodin, J., 109
Rich, A. R., 217
Richards, W., 149
Richie, R. H., 92
Richman, S., 215
Riddell, W. J. B., 337
Riddle, J. M., 191
Riegel, C., 37
Riggs, T. R., 113, 115, 116, 117
Rittenberg, M. B., 190, 191
Rivat, L., 6, 7, 9, 10, 16, 19, 26
Robbins, W., 213
Robbins, W. C., 221
Roberts, B. M., 36, 43
Roberts, E., 98, 126, 128
Roberts, J. C., 191, 192
Roberts, K. B., 191
Robinson, A. R., 51
Robinson, H. B. G., 157
Robson, E. B., 40, 55, 56, 98, 107, 127, 135, 136, 137, 138
Rodnan, G. P., 221
Rodnight, R., 141, 142
Roffler, S. K., 189
Rogers, L. L., 114
Rogoff, B., 221
Roitman, E., 98, 122, 124
Roitt, I., 213
Roldan, E. C., 153
Rönne, H., 357
Ropartz, C., 3, 6, 7, 9, 10, 11, 16, 20, 26, 28
Rose, G. A., 98, 107, 127, 135, 136, 138, 161
Rose, N. R., 217
Rosenberg, L. E., 96, 97, 152
Rosenthal, I. M., 126
Ross, C. F., 122
Ross, M. H., 312
Rothschild, M., 37
Rousseau, P.-Y., 7, 9, 10, 16, 26
Rowley, P. T., 98, 152
Roy, C., 105
Royer, M., 117
Ruppli, Von H., 215
Russell, E. S., 311, 312, 315, 320
Russell, J. A., 85, 86
Russell, L. B., 243, 244, 245, 246, 247, 248, 250, 251, 252, 253, 255, 257, 258, 259, 260, 261, 262, 272, 277, 279, 282, 284, 285, 286, 287, 288, 306, 307
Russell, W. L., 243, 244, 245, 246, 247, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 261, 262, 267, 271, 272, 274, 275, 277, 278, 283, 284, 306
Rutstein, D. D., 84
Ryan, E., 65
Sabatini, Z., 71
Sabin, F. R., 191
Saint-Hilaire, I. G., 358
St. Whitelock, O. V., 303
Sakai, K., 130
Salleras, A., 336
Saltman, P., 35
Samiy, A. H., 109, 112
Sandler, M., 98, 120
Sass-Kortsak, A., 151
Sawchuck, G. H., 304
Saylors, C. L., 244, 247, 248, 284
Scaglioni, P. R., 126
Scandlyn, B. J., 279
Schabert, P., 111
Schacht, L. E., 69
Schachter, D., 112
Schade, A. L., 35
Schafer, I. A., 116, 127, 133
Scharff, M., 200
Schedl, H. P., 37
Scheerer, 220, 351
Scheffer, E., 216
Scheidegger, J. J., 67
Scheinberg, I. H., 65, 151, 152
Schendel, H. E., 97, 98
Schinz, H., 356
Schlamowitz, M., 126
Schmid, D. O., 37, 48
Schmid, F. R., 220
Schmid, K., 71
Schmid, R., 132, 318
Schneider, H. A., 319, 320
Schoen, E. J., 149
Schoenrich, E. H., 215
Schöheyder, F., 104
Scholefield, P. G., 114, 115, 116
Scholten, H. G., 150
Schram, E., 84, 89
Schwarz, V., 143, 144
Schull, W. J., 72, 341
Schultze, H. E., 35, 71, 79
Schweet, R. S., 193, 198
Schweitzer, M. D., 211, 219
Scott, S. J., 89
Scowen, E. F., 87
Scribner, D. H., 105
Scriver, C. R., 98, 99, 105, 116, 118, 127, 128, 133, 140, 141, 155, 158
Searle, A. G., 245, 262
Searle, A. G., 160
Seastone, C. V., 215
Segalove, M., 85
Senior, B., 139
Sereni, P., 99, 101
Sgouris, J. T., 35
Shaw, K. N. F., 91, 92, 99, 140, 141
Sheldon, W., 146
Sheppard, C. W., 251
Sherliker, J., 55
Shibuya, M., 99
Ship, I. I., 213
Shirkey, H. C., 120
Shreffler, D. C., 322
Shulman, L. E., 215
Shwackman, H., 91, 92, 94, 95, 97, 98, 99, 161
Siegel, M., 215, 221
Siemens, H. W., 335, 345
Sierts-Roth, U., 313
Silides, O. J., 318
Silk, E., 65
Silman, H. J., 23
Silverie, Ch., 8
Silvers, W. K., 304, 305, 312
Simmons, R. L., 36
Simon, S., 99
Simpkiss, M. J., 123
Simpson, M. V., 193
Singer, R., 56
Singer, S. J., 187
Singh, R. B., 7, 10, 11
Siniscalco, M., 25, 40
Sirlin, J. L., 239, 242, 255, 313
Sisken, B. F., 320, 324
Sjoerdsma, A., 142, 155, 157
Sjögren, T., 153, 354, 355
Sjöquist, J., 21
Skoog, N., 67
Slater, R. J., 151
Slizynski, B. M., 254, 263, 268, 269, 270, 271, 272, 273, 274, 275, 277, 278, 279, 281, 282
Smart, G. A., 221
Smellie, J. M., 150
Smith, A. J., 120, 124
Smith, E. L., 195, 198
Smith, H., 109, 160
Smith, I., 87, 88, 155
Smith, L. J., 315, 321
Smith, P. A., 144
Smith, R. T., 190
Smith, T. E., 155
Smithies, O., 34, 38, 39, 40, 41, 42, 43, 44, 45, 48, 52, 58, 64, 322
Smyth, D. H., 112
Snell, E. E., 117
Snell, G. D., 249, 267, 269, 270, 274, 279, 280, 281, 284, 298, 304, 305
Snyderman, S., 84, 118, 123, 130, 132
Snyderman, S. E., 98, 111, 118, 122, 123, 124, 154
Sobel, C., 85, 86, 126
Sobel, E., 85
Sokoloff, L., 121, 132, 319
Solomon, A. K., 113, 114
Søndergaard, G., 98
Sonnet, J., 73
Sörbo, B., 89
Sorieul, S., 323
Sorkin, E., 195, 200
Sorsby, A., 331, 332, 333, 340
Soupart, P., 84, 104, 111
Southard, J. L., 311, 312
Spackman, D. H., 89, 111, 122
Spear, R. J., 258, 259
Spencer, R. P., 112, 117
Spiro, M. J., 139
Sponholz, R. R., 121
Sporn, M. B., 128, 131, 132, 156
Stacey, M., 188
Stacey, R. S., 120
Stadler, J., 262
Stanbury, J. B., 118
Stanbury, P. J., 160
Staron, N., 65
Stauffer, R., 4, 6, 7, 8, 9, 10, 15, 20, 21, 22, 25, 29, 72
Staveley, J. M., 55
Stavitsky, A. B., 193
Stearns, G., 94
Stecher, R. M., 220
Stefens, R., 340
Steiger, A., 347
Stein, W. D., 112, 136, 152
Stein, W. H., 89, 97, 111, 113, 115, 129, 132, 158
Steinberg, A. G., 4, 6,

7, 8, 9, 10, 11, 14, 15, 16, 17, 19, 20, 21, 22, 25, 26, 43, 44, 56, 64, 72, 121, 304
Steinberger, E., 254
Steiner, D. F., 193
Stenström, S., 347, 348, 350
Stern, C., 310, 323, 356
Sternlieb, I., 151, 152
Sterzl, J., 193, 196
Stevens, K. M., 191, 196
Stevens, L. C., 318
Stewart, G. H., 89
Stirman, J. A., 36
Stockell, A., 195
Stollerman, G. H., 219
Stone, M., 126
Stranack, F., 109, 144, 147
Strandskov, 249
Strang, L. B., 120, 124
Straub, M., 349
Strecker, H. J., 128, 155
Stretton, A. O. W., 321
Strong, F. M., 103
Stuart, C., 157
Suda, M., 112, 116, 117
Studer, M., 35
Sugawa, T., 112, 117
Summer, G. K., 157
Surgenor, D. M., 34
Sussdorf, D. H., 195
Sutcliffe, K. W., 144
Sutherland, B., 91, 98, 101, 120, 130, 132
Sutherland, B. S., 120
Sutton, E., 3
Sutton, H. E., 40, 43, 47, 48, 50, 51, 52, 58, 88, 118, 120, 133, 134, 135
Suyama, Y., 108
Swahn, B., 4, 13, 26
Szent-Gyorgyi, A., 156
Szilard, L., 201, 202
Tada, K., 120
Takedatsu, N., 116
Talbert, P. C., 153
Taliaferro, W. H., 193
Tallan, H. H., 128, 132, 133, 156
Talmage, D. W., 187, 189, 192, 197, 198, 199, 201
Tanner, M., 220, 221
Taranta, A., 219
Tarjan, G., 118
Tashian, R. E., 118, 120, 123, 130, 144
Tenenhouse, A., 114, 115
Terres, G., 190
Terrey, M., 149
Terslev, E., 149
Thibault, C., 232, 237, 238
Thom, H., 153
Thomas, D., 37
Thomas, D. H. H., 132
Thompson, D. W., 109
Thompson, P. E., 266
Thomson, A., 199
Thorbecke, G. J., 190, 192
Thuline, H. C., 245
Tieckelmann, H., 87, 155
Tihen, J. A., 275
Tiilikainen, A., 25
Tomlinson, S., 131, 132
Torosdag, S., 219
Tower, D. B., 121, 132
Triantaphyllopoulos, E., 117
Tron, E., 347
Trnka, Z., 193, 196
Tuba, J., 117
Tumulty, P. A., 215
Turnbull, A., 47, 48, 49, 58
Turpin, R., 340
Tyburczyk, W., 94
Tyzzer, E. E., 304
Uchida, I., 219
Udenfriend, S., 119, 142, 155, 157
Ueno, N., 11
Uhr, J. W., 200
Ulmann, T. D., 145
Ulrich, J. A., 153
Ullrich, K. J., 109
Urbin, M. C., 90
Ussing, H. H., 112
Uzman, L. L., 151
VanDen Berghe, J., 340
Vanderdonck, R., 356
Van Geffel, R., 143
Van Slyke, D. D., 85
Van Vunakis, H., 188
Vaughan, J. H., 2
Vawter, G. F., 146
Vazquez, J., 216, 217, 218
Venge, O., 48
Verriest, G., 356
Vestergaard, P., 93
Vicari, E., 315
Villee, C. A., 109
Vincent, M., 98
Vis, H., 84, 98, 143
Visakorpi, J. K., 98
Vogel, F., 354
Volvqvartz, K., 104
Vos, G. H., 7, 10, 11, 26
Vossen, R., 215
de Vries, A., 142, 144
Waaler, G., 356
Waardenburg, P. J., 335, 339, 355, 358
Wagner, M., 192
Waisman, H. A., 103, 121
Waksman, B. H., 214, 217
Waldenstrom, J., 215, 225
Walker, L. M., 91, 94, 115, 116, 117
Walker, N. F., 39, 64
Wallach, D. F. H., 89
Wallaston, 136
Waller, M. V., 2
Waller, R., 215
Wallis, K., 153
Wallis, L. A., 145, 146
Walshe, J. M., 113, 152
Wang, H. L., 121
Warburton, F. G., 98
Warkany, J., 91
Warren, F. L., 137
Washington, J. A., 105
Wasserman, R. H., 154
Watkin, D. M., 97
Watson, D. W., 190
Watson-Williams, E. J., 9
Watts, R. W. E., 87
Webb, B., 146
Webber, W. A., 109, 116, 138, 163
Weekers, R., 341
Weigle, W. O., 189, 191
Weijers, H. A., 144
Weiner, I. M., 105, 109
Weiner, J. S., 56
Weissbach, H., 142
Weissman, I. L., 202
Welshons, W. J., 244, 246, 247, 249, 306
Wermer, P., 300
Werner, G., 90
Wesslen, T., 191
Westall, R. G., 89, 98, 107, 120, 121, 122, 123, 124, 131, 132, 147, 159, 161
Weyers, H., 141
Whitaker, M., 65
Whitehead, J. K., 89
Whiting, A. R., 256
Wickham, L., 277
Widdas, W. F., 113
Wiel, Th. W. M. van de, 24
Wilbrandt, W., 112, 114
Wilhelmi, A. E., 85, 86
Williams, C. A., 65, 67
Williams, C. A., Jr., 65
Williams, J., 47, 48
Williams, J. W., 187
Williams, R., 216
Williams, R. D. B., 153
Williams, R. L., 116
Williamson, M. B., 90
Wilson, R. E., 214
Wilson, T. H., 99, 115, 131
Wilson, W. A., 116
Winberg, J., 94
Winblad, S., 215
Winitz, M., 84, 87
Wintrobe, M. M., 36
Winzler, R. J., 35
Wiseman, G., 115
Wilson, J., 6, 7, 10, 29
Wissler, R. W., 190, 192
Witebsky, E., 217
Wohl, M. J., 213
Wolf, B., 193
Wolf, J. K., 221
Wölfflin, E., 356
Wollaston, W. H., 83, 86
Woolf, L. I., 94, 95, 98, 99, 100, 101, 102, 103, 120, 121, 122, 123, 135
Worthen, H. G., 145, 147, 148
Wostman, B., 192
Wozniczko, G., 2
Wrenshall, G. A., 318
Wright, G. W., 189
Wright, M. E., 282
Wright, S., 99
Wright, S. W., 118, 121, 153
Wrong, O., 141
Wyngaarden, J. B., 142
Wyttenbach, A., 35
Yamamoto, R. S., 319
Yeh, H. L., 136
Yeh, M., 308
Yendt, E. R., 126
Yerganian, G. A., 318
Yoffey, J. M., 192
Yokoyama, M., 11
Yoshikawa, S., 117
Young, G., 149
Young, W. J., 28, 48, 50
Yuwiler, A., 120
Zak, S. J., 192
Zamecnic, P. C., 193
Zamis, M. J., 315
Zannoni, V. G., 102
de Zarate, J. C. O., 336
Zelakowska, M., 94
Zellweger, H., 118
Ziff, M., 218, 220
Zike, K., 220
Zinder, N. D., 319
Zingale, S. B., 215
Zoutendyk, A., 56
Zuazago, G., 94
Zweig, G., 87

Subject Index

Aborigines
  gamma globulin factors in, 11, 27
  Gc types in, 73
  transferrins in, 39, 54
Accumulase, and antibody synthesis, 200–201
Agammaglobulinemia, 211
Agglutinating factors in sera, 1–2, 12–18, 64
  diseases related to, 26
Alanine
  excretion in newborn, 103
  quantitative data, 110
  in transferrins, 45
Albinism, 331, 334–335, 339, 358–360, 361
Albumin, serum, heritable variation in, 65
Alcaptonuria, 118, 130
Alder's anomaly, 339
Alzheimer's disease, 314
Amaurotic idiocy, juvenile, 339
Amino acids, 84
  cellular transport, 112–117, 119–136
  membrane transport, symposium on, 162
  quantitative data, 110–111
  renal transport, 108–112, 136–152
  in saliva, 161
  in transferrins, 45
Amino nitrogen levels in urine, 85–86, 94
Aminoaciduria, 83–186
  acidosis with, 154
  age affecting, 99–103
  amino acid determinations, 86–91
  central-cluster, 153
  citrullinuria, 163
  convulsions with, 158–159
  cystinuria, 163
  dermatological changes with, 155
  detection of, 85–95
  dietary influence, 96–99
  diurnal variations, 107
  excretion coefficients, 92–95
  and Fanconi syndrome, 145–149
  and galactosemia, 143
  generalized, 153, 154
  genetic factors in, 159–161
  and glycinuria, 155–159
  growth failure with, 152
  histidine excretion, 162
  homocitrullinuria, 163
  hyperparathyroidism with, 153
  hypophosphatemic rickets with, 158
  imidazoleaminoaciduria, 162
  and lactase deficiency, 144
  Marfan syndrome with, 156
  masked, 154
  mechanisms of, 108–118
  muscular dystrophy with, 154
  and occulo-cerebro-renal syndrome, 149–150
  prerenal, 108
  and renal function, 97, 105–107, 108
  screening programs, 91–92
  sex factors in, 103–104
  syndromes with, 152–155
  total amino nitrogen methods for, 85–86
  types of, 118–159
  unusual ninhydrin reacting compounds, 159
  and vitamin D deficiency, 144–145
  and Wilson's disease, 150–152
  see also specific amino acids
α-Aminobutyric acid, 110
γ-Aminobutyric acid, in urine, 98
β-Aminoisobutyric acid
  excretion of, 97, 102, 133–136, 160
  quantitative data, 110
  transport of, 116
Ammonia, in transferrins, 45
Anemia
  acquired hemolytic, 211
  aregenerative red cell, 211
  macrocytic, experimental, 315–316
Aneuploidy, 243, 248–259
Angiomatosis
  Hippel-Lindau, 332, 341
  Sturge-Weber-Dimitri, 332, 341
Animals. *See* Mammalian genetics
Anti-D, gamma globulin with, 24–25
Antibody synthesis, 187–209
  cellular sites of, 190–193
  clonal selection theory, 199–200
  direct template theory, 198
  feedback control mechanisms, 201
  indirect template theory, 198–199
  natural selection theory, 199
  permease-accumulase theories, 200–201
  side chain theory, 197
  subcellular site of, 193–196
  theories of, 197–202
  in vitro studies, 196
Antigens
  antibody reactions with, 187–190
  differences between males and females, 305
  hyperreactivity to, 215–216
  immunoelectrophoresis of mixtures, 65
Arginine
  in argininosuccinic-aciduria, 131
  in cystinuria, 136
  excretion in newborn, 103
  in Hartnup disease, 141
  metabolism of, 128
  quantitative data, 110–111
  in transferrins, 45
  transport of, 116
Argininosuccinase, 132
Argininosuccinic acid, 131
Argininosuccinic-aciduria, 130–132, 163
Arthritis
  osteoarthritis in mice, 319
  rheumatoid, 210

agglutinating sera in, 1–2, 12, 64
gamma globulin factors in, 25
relatives of patients, 220
Asparagine, 111
Aspartic acid
quantitative data, 110
in transferrins, 45
transport of, 116
Asthma, bronchial, gamma globulin factors in, 25
Audiogenic seizures, 314–315
Auto-immune diseases, 210–229
Autosomes, 242–244
recessive inheritance, 338

Bardet-Biedl syndrome, 332, 340
Betaine, transport of, 129
Biochemical variations in mammals, 320–323
Blood dyscrasias, experimental, 315–317
Bonnevie-Ullrich syndrome, 343
Bourneville cerebral tuberous sclerosis, 332, 341

Calculi, urinary, 142, 161
Cancer
agglutinating sera in, 1
gamma globulin factors in, 25
susceptibility of mice, 319
Cataracts, 317, 332, 354–356
Caucasians
gamma globulin factors in, 6–8, 27
Gc types in, 73
transferrins in, 40, 52
Cells
dissociation and aggregation, 324
hybridization in vitro, 323
Cellular transport of amino acids, 112–117, 119–136
Ceruloplasmin, in Wilson's disease, 151
Chinese
gamma globulin factors in, 10, 27
Gc types in, 73
transferrins in, 41, 53
Chorea, Huntington's, 314
Choroid, atrophy of, 338
Choroideremia, 333
Chromatography, and aminoaciduria, 83–186
Chromosomes
aberrations in mammals, 230–294
autosomes, 242–244, 338
deficiencies, 261–262
dominant lethals, 243, 248–259
duplications, 260–261
gene action in X-chromosome, 307–308
heteroploidy, 231–259
inversions, 262–266
mammalian genetics, 295–330
monosomy, 242–248
rearrangements, 262–289
sex-chromosomes, 244–248, 305–308
t-locus of mouse, 264–266, 298, 309
translocations, 266–289
trisomy, 242–248
Citrullinuria, 163
Colchicine, heteroploidy from, 238–240
Cold shock, heteroploidy from, 238
Collagen metabolism, 156
Coloboma, 332, 345–347
Color blindness, 356
Congenital malformations, 301
ophthalmic, 332
Connective tissue diseases, 156, 210
experimental, 218
Convulsions
aminoaciduria with, 158–159
experimental, 312
Cornea
dystrophy, 341
megalocornea, 337
Creatinine excretion, 93–94
Cystathionine excretion, 98, 132–133
Cysteine, urinary, 138
Cystine
excretion of, 86, 99, 103, 118, 136–139, 142, 159, 161, 163
in Hartnup disease, 141
in maple syrup urine disease, 123
quantitative data, 110
in transferrins, 45
transport of, 116
Cystinosis, 145–147, 163
Cytogenetics, and translocations, 278–283

Deafness, hereditary, 317
Deoxyribonucleic acid (DNA)
and antibody synthesis, 195, 198
serum factors reacting with, 213
Dermatologic disorders
and aminoaciduria, 155
and ophthalmic disorders, 332
Dermatomyositis, 210
Diabetes
gamma globulin factors in, 25
genetic basis of, 318
Diet, effects on aminoaciduria, 96–99
N-Dimethylglycine, transport of, 129
Dominant lethals, 243, 248–259
Dreher mutant of mouse, 313
Drugs, reactions to, 215, 217
Duchenne muscular dystrophy, 312
Dyschromatopsia, 337, 356

Ehlers-Danlos syndrome, 344
Embryonic lethals, 301–302
Emmetropia, 347–349
Encephalomyelitis, experimental, 217, 319–320
Enzymes
and antibody synthesis, 200–201
cellular systems in aminoaciduria, 119–136
in muscular dystrophy, 311
Epilepsy, photogenic, 314
Erythema nodosa and multiforme, 210
Eskimos
gamma globulin factors in, 10, 27
Gc types in, 73
transferrins in, 52
Estrogens, effects on amino acid transport, 116, 117
Ethanolamine excretion, 97, 103
Experimental disorders. *See* Mammalian genetics
Expressivity of genes, 297–298

Fanconi syndrome, 145–149, 154
Fertility, and translocated chromosomes, 267–273
Franceschetti mandibulofacial dysostosis, 344
Fuchs atrophy of choroid and retina, 338
Fuchs dystrophy of cornea, 341

Galactosemia, 118, 130
and aminoaciduria, 98, 143–144
Gametes
genes affecting, 308
unbalanced, fate of, 273–274

Gametogenic stage
and lethal dominants, 254–257, 276
and translocations, 276–278
Glaucoma, 332, 340–341
β-Globulins, in mice, 322
γ-Globulins, 1–29
agglutinating factors, 12–18
in Caucasoids, 6–8, 27
detection methods, 3–5
diseases related to, 25–26
genetics of factors, 5–11
incomplete anti-serum, 24–25
inherited serologic group types, 225
inhibitors, 18–23
in Mongoloids, 10–11, 26–27
in Negroids, 8–10, 27
population studies, 26–27
in rheumatic disease, 212–214
Glomerulonephritis, 211
experimental, 218
Glucosamine, in transferrins, 45
Glutamic acid
metabolism of, 128
quantitative data, 110
in transferrins, 45
transport of, 116
Glutamic acid decarboxylase
in maple syrup urine disease, 123
in phenylketonuria, 120–121
Glutamine, 111
Glycine
in argininosuccinic-aciduria, 131
excretion of, 103, 142, 155–159, 160
in Hartnup disease, 141
hyperglycinemia, idiopathic, 124–125
in hyperprolinemia, 128
quantitative data, 111
in transferrins, 45
transport of, 116
Groenblad-Strandberg-Touraine syndrome, 332
Group specific component of serum, 64–82
distribution of, 72–75
inheritance mode, 67–68
methodology of, 67
nature of, 76–80
phenotypes of, 66
phylogenetic aspects of, 75–76
variants of, 69–72
Guillain-Barré syndrome, agglutinating sera in, 1

Hartnup disease, 99, 112, 129, 139–142
Heat shock, heteroploidy from, 238
Hematopoietic system diseases, gamma globulin factors in, 25
Hemochromatosis, and transferrin deficiency, 38
Hemoglobins
iron requirements for synthesis, 36
multiple, in mice, 320–322
Hepatolenticular degeneration, 118, 150–152, 339–340
Heteroploidy in mammals, 231–259
aneuploidy, 242–259
causes of, 232–234
euploid, 232–242
experimental induction, 237–241
genetic control of, 236–237
monosomy of chromosomes, 242–248
spontaneous occurrence, 235–237
trisomy of chromosomes, 242–248
viability studies, 241–242
Hexosamine, in transferrin, 35
Hexose, in transferrin, 35
Hippel-Lindau cerebroretinal angiomatosis, 332, 341
Histidine
excretion of, 97, 162
hyperhistidinemia, 120, 125
quantitative data, 111
in transferrins, 45
Histocompatibility loci in mice, 304–305
Hodgkin's disease, 211
Homocitrulline, in urine, 103, 159, 163
Homocystine excretion, 159
Huntington's chorea, 314
Hydantoin derivatives, syndrome from, 215, 217
Hydralazine, syndrome from, 215, 217
p-Hydroxyphenyl-lactic acid excretion, 101
p-Hydroxyphenylpyruvic acid excretion, 101
p-Hydroxyphenylpyruvic acid oxidase deficiency, 130
Hydroxyphenyluria, 101, 118, 130
5-Hydroxypipecolic acid excretion, 98
Hydroxyproline
excretion of, 102
in glycinuria, 142
in hyperprolinemia, 127
metabolism of, 155–159
quantitative data, 110
transport of, 116
Hypophosphatasia, 118

Imidazoleaminoaciduria, 162
Iminoaciduria and glycinuria, 155–159
Immunoelectrophoresis of antigen mixtures, 65
Immunology, 187–229
antibody synthesis, 187–209
rheumatic disease, 210–229
Indians, American
gamma globulin factors in, 10, 27
Gc types in, 70, 73
transferrins in, 41, 52
Indoluria, in Hartnup disease, 140
Inhibitors, gamma globulin, 18–23
Insulin, effects on amino acid transport, 117
Intestinal bacteria
affecting amino acids, 99
in Hartnup disease, 140
Iron-protein complex, 34–63
Isoleucine
in maple syrup urine disease, 122
quantitative data, 110
in transferrins, 45
transport of, 116

Japanese
gamma globulin factors in, 10, 27
Gc types in, 73
transferrins in, 41, 53
Jaundice, hereditary, 318

Keratoconus, 340
Keratosis follicularis, 335
Kidneys
amino acid transport, 108–112, 136–152
function in aminoaciduria, 97, 105–107, 108
glomerulonephritis, 211
experimental, 218
transamidinase, and muscular dystrophy, 311
Kwashiorkor, aminoaciduria in, 98
Kynurenine excretion, 140

Lactase deficiency, and aminoaciduria, 144
Latex particle test, absorption studies with, 13–14, 17–18

Leber's disease, 332, 354, 357
Lethals
  dilute, 313–314
  dominant, 243, 248–259
    agents inducing, 252–254
    early studies, 249–250
    in females, 257–259
    and gametogenic stage, 254–257, 276
    mechanisms of, 250–252
  early embryonic, 301–302
Leucine
  in maple syrup urine disease, 122
  quantitative data, 110
  in transferrins, 45
  transport of, 116
Leukemia
  agglutinating sera in, 1
  β-aminoisobutyric acid excretion in, 134
  lymphatic, 211
Liver disease, β-aminoisobutyric acid excretion in, 135
Lupus erythematosus, systemic, 210
  relatives of patients, 220
Lurchers, in mice, 313
Lymphocytes, and antibody synthesis, 190–193
Lymphoid differentiation of thymus, 302–303
Lysine
  in argininosuccinic-aciduria, 131
  in cystinuria, 136
  excretion of, 103, 154, 160
  in Hartnup disease, 141
  quantitative data, 110
  in transferrins, 45
  transport of, 116

Macroglobulinemia, 211
Macrophages, and antibody synthesis, 190–193
Macula
  coloboma, 332
  senile degeneration of, 331
Mammalian genetics, 295–330
  aminoaciduria, 160
  anemia, macrocytic, 315–316
  antigenic differences in males and females, 305
  audiogenic seizures, 314–315
  biochemical variations, 320–323
  blood dyscrasias, 315–317
  cancer susceptibility, 319
  cataracts, 317
  cell dissociation and aggregation, 324
  chromosome aberrations, 230–294
  complex genetic loci, 298–299
  congenital malformations, 301
  deafness, 317
  developmental systems, interaction of, 300–301
  diabetes and hyperglycemia, 318
  dilute lethal, 313–314
  dreher, 313
  embryonic lethals, 301–302
  encephalomyelitis, 217, 319–320
  Gc types, 75–76
  hemoglobins, multiple, 320–322
  hybrid cells in vitro, 323
  jaundice, 318
  lurcher, 313
  muscular dystrophy, 311–312
  neurological diseases, 312–315
  osteoarthritis, 319
  pathology, 310–319
  Pelger anomaly, 316–317
  penetrance and expressivity, 297–298
  phenylalanine hydroxylase in dilute mice, 322
  pleiotropic effects of genes, 299–300
  radiation genetics, 323
  reeler, 312–313
  reproduction genetics, 308–310
  rheumatic diseases, 217–219
  senescence, premature, 314
  serum factor variation, 322–323
  sex chromosomes, 305–308
  spherocytosis, 316
  thymus and lymphoid differentiation, 302–303
  transferrins, 48–50, 322
  transplantation genetics, 303–305
  tremor and convulsions, 312
  vitamin A utilization, 317–318
  zig-zag, 313
Maple syrup urine disease, 120, 121–124
Marfan syndrome, 156–157, 341
Megalocornea, 337
Methionine
  in maple syrup urine disease, 122
  quantitative data, 110
  in transferrins, 45
Methyl malonate semialdehyde metabolism, 133
Metropic eyes, 347–352
Microsomes, and antibody synthesis, 193–196
Mongolism, tryptophan metabolism in, 154
Mongoloids
  gamma globulin factors in, 10–11, 27
  Gc types in, 73
  transferrins in, 41, 53
Mononucleosis, infectious, gamma globulin factors in, 25
Monosomy of autosomes, 243–244
Mother-child pairs
  gamma globulin factors in, 19
  Gc types in, 68
  placental transferrin, 37
Muscular dystrophy
  aminoaciduria with, 154
  experimental, 311–312
Myasthenia gravis, 211
Myeloma, multiple, 211
Myeloma serum, gamma globulin in, 20–21
Myopia, 336, 347–352

Negroes
  gamma globulin factors in, 8–10, 27
  Gc types in, 73
  transferrins in, 39, 55
Neomycin, effects of, 140, 141
Nephritis, 211
  experimental, 218
Neuraminidase, transferrin treated with, 45–47
Neurofibromatosis, Recklinghausen, 332, 341
Neurological diseases
  experimental, 312–315
  and ophthalmic disorders, 332
Newborn, amino acid excretion in, 99
Ninhydrin reacting compounds in urine, 159
Nitrogen, urinary, 85–86, 94, 95
Nitrogen mustard
  β-aminoisobutyric acid excretion after, 134
  dominant lethals from, 253
  translocations from, 275

Oasthouse urine syndrome, 120
Oculo-cerebro-renal syndrome, 149–150
Odontogenesis imperfecta, 157

Ophiasis, keratosis follicularis with, 335
Ophthalmic genetics, 331–365
abiotrophies, 332
albinism, 331, 334–335, 339, 358–360, 361
Alder's anomaly, 339
amaurotic idiocy, juvenile, 339
atrophy of choroid and retina, 338
Bardet-Biedl syndrome, 332, 340
Bonnevie-Ullrich syndrome, 343
Bourneville cerebral tuberous sclerosis, 332, 341
carriers of genes, 332–345
cataracts, 317, 332, 354–356
choroideremia, 333
coloboma, 332, 345–347
congenital defects, 332
dominant gene of delayed expression, 340–344
dominant gene, incompletely penetrant, 344
dyschromatopsia, 337, 356
dystrophy, corneal, 341
ectodermal anhidrotic dysplasia, 337
Ehlers-Danlos syndrome, 344
Franceschetti mandibulofacial dysostosis, 344
functional disorders, 332
glaucoma, 340–341
heterozygous carrier for incompletely recessive gene, 333–340
Hippel-Lindau angiomatosis, 332, 341
keratoconus, 340
keratosis follicularis, 335
Leber's disease, 332, 354, 357
Marfan's syndrome, 341
megalocornea, 337
ophthalmoplegia with myopia, 336
optic atrophies, 356–358
patterns of inheritance, 332
phakomatoses, 332, 341
pigmentary retinopathy, 331, 332, 333
recessive autosomal inheritance, 338
Recklinghausen's neurofibromatosis, 332, 341
refractive errors, 347–352
retinoblastoma, 352–354
Steinert's myotonic dystrophy, 344
Sturge-Weber-Dimitri angiomatosis, 332, 341
syndromes, 332
Van der Hoeve syndrome, 344
Waardenburg syndrome, 343
Wilson's disease, 339–340
xeroderma pigmentosum, 341
Ophthalmoplegia, with myopia, 336
Ornithine
in argininosuccinic-aciduria, 131
in cystinuria, 136
excretion in newborn, 103
in Hartnup disease, 141
in hyperprolinemia, 127
quantitative data, 110
transport of, 116
Ornithine-urea cycle disorders, 132
Osteoarthritis in mice, 319
Osteogenesis imperfecta, 157
Oxalosis, 118, 142
Oxygen lack, dominant lethals from, 252

Parathyroid disorders, aminoaciduria with, 153
Pelger anomaly, 316–317
Penetrance of genes, 297–298
Peptic ulcer, agglutinating sera in, 1
Phakomatoses, 332, 341
Phenylalanine
in phenylketonuria, 91, 118, 119–121, 322
quantitative data, 110
in transferrins, 45
in tyrosyluria, 130
Phenylalanine hydroxylase, in dilute mice, 322
Phenylketonuria, 91, 118, 119–121, 322
Phosphatase, serum alkaline, 126–127
Phosphaturia, rickets with, 158
Phosphoethanolaminuria, 126–127
Phosphoglucomutase, in muscular dystrophy, 311
Pick's disease, 314
Pinocytosis, 113, 114
Pipecolic acid excretion, 102
Plasma cells, and antibody synthesis, 190–193
Plasma proteins. *See* Proteins, plasma and serum
Plasma transferrins. *See* Transferrins
Pleiotropic gene effects, 299–300
Polyarteritis, 210
Polymyositis, 210
Proline
excretion of, 102, 160
in glycinuria, 142
hyperprolinemia, familial, 127–129
metabolism of, 155–159
quantitative data, 110
in transferrins, 45
transport of, 116
Propionic acid, impaired formation of, 134
Protein, dietary, and aminoaciduria, 96–97
Proteins, plasma and serum
Ag-system, 65
antibody synthesis, 187–209
gamma globulin types, 1–33
Gc system, 64–82
Gm and Inv system, 1–33
group specific component, 64–82
and transferrins, 34–63
see also Globulins
Prurigo, Besnier's, gamma globulin factors in, 25
Pseudocholinesterase, plasma, 65
Psoriasis, gamma globulin factors in, 25

Radiation
β-aminoisobutyric acid excretion after, 135
dominant lethals from, 252–253
heteroploidy from, 240
and mammalian genetics, 323
translocations from, 275
Ragg sera, agglutinator in, 12–13, 16–18
Recklinghausen's neurofibromatosis, 332, 341
Reelers, studies of, 312–313
Refractive errors, ophthalmic, 347–352
Reproduction, mammalian, genetics of, 308–310
Retina, atrophy of, 338
Retinitis, pigmentary, 331, 332, 333
Retinoblastoma, 352–354
Rheumatic disease, 210–229
animal studies, 217–219
gamma globulins in, 212–214
immunologic abnormalities in, 216–217
reactions to foreign antigens, 215–217
relatives of patients, 219–225
skin reactivity to autologous cells, 214–215
Rheumatic fever, 210
relatives of patients, 219
Rheumatoid arthritis. *See* Arthritis, rheumatoid
Rheumatoid factor, 212
Rickets, hypophosphatemic, 158

Saliva, amino acids in, 161
Sarcosine, transport of, 129
Scleroderma, 210
Senescence, premature, experimental, 314

Serine
quantitative data, 110
in transferrins, 45
Serotonin, plasma levels in phenylketonuria, 120
Serum sickness, 217
Sex chromosomes, 244–248, 305–308
loss of, 248
phenotypes, 244–246
sex determination, 246
spontaneous abnormalities, 246–248
Sialic acid, in transferrin, 35, 45
Sjögren's syndrome, 213, 354
Skin reactivity to autologous cells, 214–215
Smith and Strang's patient, 124
SNagg sera, agglutinator in, 16–18
Sorsby's syndrome, 332
Spermatogenesis, and dominant lethals, 243, 248–259
Spermatozoa
of blood group A or B, 309
genetic transformation of, 310
separation in electrical field, 308–309
and t-locus mutants, 309–310
Spherocytosis, experimental, 316
Spondylitis, gamma globulin factors in, 25
Spondylosis, rhizomelic, 341
Steinert's myotonic dystrophy, 344
Sterility
complete, 271–273
partial, 267–271
Streptococcal infection, and rheumatic disease, 210, 213
Sturge-Weber-Dimitri neurocutaneous angiomatosis, 332, 341
Sulfonamides, sensitivity to, 217
Syphilis, gamma globulin factors in, 25

T-locus in mouse, 264–266, 298, 309
Taurine
excretion of, 97, 102, 160
quantitative data, 111
transport of, 116
Thalassemia
β-aminoisobutyric acid excretion in, 135
gamma globulin factors in, 25
Threonine
excretion of, 160
quantitative data, 110
in transferrins, 45
Thrombocytopenia, idiopathic, 211
Thymine, and β-aminoisobutyric acid, 133
Thymoma, 211
Thymus, lymphoid differentiation of, 302–303
Thyroiditis, 211
experimental, 217
relatives of patients, 221
Toluidine blue, dominant lethals from, 253
Transamidinase, kidney, and muscular dystrophy, 311
Transferrins, 34–63, 64
animal variants, 48–50
atransferrinemia, congenital, 37–38
geographic distribution of, 50–58
inherited types, 38–58
in mice, 322
physico-chemical characteristics, 34–35
physiological properties, 35–37
placental, 37, 46
radioiodine-labelled, 36
spinal fluid, 46
variants, 45–58, 59
Transfusion reactions, gamma globulin factors in, 26
Translocations of chromosomes, 266–289
in cytogenetic studies, 278–283
gametes formed by bearers, 267–274
genetic and cytological maps, 282–283
genetic localization of breaks, 279–281
independence of linkage groups, 281
induction of, 274–278
position effect, 283–289
Transplantation genetics of mammals, 303–305
Tremor, experimental, 312
Trichorrhexis nodosa, 131
Triethylenemelamine (TEM)
dominant lethals from, 254
translocations from, 275
Trisomy of autosomes, 242–243
Trypaflavine, dominant lethals from, 253
Tryptophan
in Hartnup disease, 140
metabolism disorders, 120, 154
in transferrins, 45
Tuberculosis, β-aminoisobutyric acid excretion in, 135
Tyrosine
excretion of, 160
quantitative data, 110
in transferrins, 45
Tyrosinosis, 130
Tyrosyluria, 98, 101, 118, 120, 130

Ukari syndrome, 146
Urea–ornithine cycle disorders, 132
Urinary amino acid content, 83–186
Urocanic acid formation, 162
Urticaria, gamma globulin factors in, 25

Valine
and β-aminoisobutyric acid excretion, 133
in argininosuccinic-aciduria, 131
excretion of, 160
in maple syrup urine disease, 122
quantitative data, 110
in transferrins, 45
transport of, 116
Van der Hoeve syndrome, 344
Vitamin A utilization in mice, 317–318
Vitamin deficiency, aminoaciduria with, 98, 144–145
Vitamins, effects on amino acid transport, 117

Waaler-Rose test for rheumatoid agglutinating factor, 2, 3, 12
Waardenburg syndrome, 343
Wilson's disease, 118, 150–152, 339–340

Xanthinuria, 142
Xeroderma pigmentosum, 331, 341

Zig-zag, in mice, 313

LONDON SCHOOL OF HYGIENE
AND TROPICAL MEDICINE
LIBRARY
24 JAN 1963