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PROGRESS IN

Medical Genetics

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Medical Genetics

Volume V

Edited by

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Contents

Foreword

THE ROLE of the editors of volumes such as this is similar to the combined roles of the marriage broker, family advisor, and obstetrician. We bring the topic and the author together, and we nurture the union until the manuscript comes forth. We rejoice in and view with pride the excellent product, but can take no credit for it, and indeed deserve no credit for it. We are grateful to the authors of this volume for the excellence of their papers and for enabling us to assert that the promise, made in the Foreword to Volume 1, to maintain the high standards set in that volume has been fulfilled.

The initial chapter, by René Dubos, returns to the Nature-Nurture story. A debate raged in the early years of this century over the question of "Nature *versus* Nurture." It was eventually realized that the topic of debate was incorrect inasmuch as the two are inseparable. A more correct statement of the problem is the one posed and examined by Professor Dubos, namely, "Nature *and* Nurture." His interesting philosophical approach should stimulate much thought and research.

Much has been written about disturbances of hemoglobin synthesis and a good deal of this has concerned the thalassemias. This complex of diseases poses a challenge to the clinician as well as to the geneticist. D. J. Weatherall (Chapter 2) offers a fresh viewpoint and summary of our current knowledge. The porphyrias (Chapter 3) comprise a group of diseases of remarkable complexity. The authors (Waldenström and Haeger-Aronsen) have used them to illustrate clinical, classical genetic, population genetic, and biochemical genetic problems.

Liver diseases (Chapter 4) have, because of their seriousness, long attracted the attention of physicians and geneticists. Sheila Sherlock presents a timely and insightful review of the problems common to both disciplines. Emery and Walton present (Chapter 5) an excellent review of our knowledge and of the genetic and clinical problems associated with the muscular dystrophies. They have taken advantage of the peculiarities of these diseases to analyse important clinical and genetic problems.

The editors believe that the reviews presented in the several chapters of this volume will be of great interest to the clinician as well as to the basic scientist.

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

PROGRESS IN

Medical Genetics

CHAPTER 1

Nature and Nurture

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"MEN RESEMBLE THEIR CONTEMPORARIES even more than they do their progenitors." When R. W. Emerson wrote this aphorism one century ago, he had in mind chiefly the social and behavioral characteristics of human beings. But he could have applied it just as well to physical and physiological characteristics. Environmental factors affect so profoundly all expressions of the genetic endowment that the members of a given generation in a given place tend to exhibit a number of traits in common—not only in their behavior but also in their physique. Even the pattern of diseases changes from generation to generation. There is, of course, nothing surprising in the fact that although men are genetically singular they derive from the period during which they live a certain uniformity of appearance and of problems. What is inherited are not body parts or behavior patterns, but merely the way in which the organism responds to the total environment.

At the present time, the most obvious effect that the environment exerts on the biological manifestations of human life is probably the accelerated rate of physical and physiological maturation among children of all classes (Bloom, 1964; Meade and Parkes, 1965). In the countries that have adopted Western ways of life, as is well known, children are growing faster and reaching greater heights and weights than their parents. This acceleration has taken place during the past 50 to 100 years at first especially in the United States, then in industrial Europe; it has now become particularly striking in post-war Japan. The menarchial age is also advancing. While figures concerning this trait are less readily available than for heights and weights of children, there is good reason to

believe, nevertheless, that menarche has come earlier by 3 to 4 months per decade in Europe. Needless to say, this trend cannot be extended far back in time. As judged from statements in *Romeo and Juliet*, the age of first menstruation was 14 in Shakespeare's time. Around 1820, the menarchial age in England seems to have been 14½ for the upper classes, 15½ among the less well-off townspeople, and even perhaps later among the poor classes. In contrast, it is now 13 or less for practically all social groups.

Improvements in nutrition and in the control of childhood diseases certainly account in very large part for the general trend toward earlier physical maturation in the economically privileged parts of the world; some credit for it may be given also to hybrid vigor resulting from a wider range in the choice of marriage partners. What is clear in any case is that social factors profoundly affect the expression of the genetic potentialities of human beings for height, size, and sexual maturation. The development of the brain also is profoundly influenced by stimuli from the external world. While behavioral traits and mental skills are, of course, genetically controlled, observations on human beings suggest, and experiments with animals prove, that the "Remembrance of Things Past" conditions all activities of the organism.

An immense variety of environmental factors and of cytoplasmic agents can be shown experimentally to affect the complex processes of embryogenesis and growth through which the genotype is converted into the phenotype. The very early environmental influences, and the critical periods for each phase of physical and mental development, are of particular importance in this regard; they constitute aspects of biological knowledge that will require much investigation before it is possible to predict with some confidence the kind of phenotype likely to result from the expression of the genetic endowment in a given situation (Scott, 1957).

One of the promising approaches to the analysis of the interplay between genotype and environment has been opened by the discovery that all genes do not act all the time, but instead are set off and on by cytoplasmic agencies. Interestingly enough the existence of variability in gene action had been forecast by T. H. Morgan himself. He explicitly stated in his *Embryology and Genetics* (1934) that "different batteries of genes come into action as development proceeds," and that the activity of genes are affected by protoplasmic regions about them. The current studies on gene activators and repressors are only now beginning to provide an experimental basis for Morgan's inspired guess (Davidson, 1965).

Morgan's interest in gene activation and repression seems to have been focused exclusively on the phenomena of normal embryogenesis. It is now becoming obvious, however, that extranuclear agencies, capable of affecting gene action, are involved not only in the normal processes of differentiation but also in other phenomena which affect general development, often in a pathological manner.

Observations made during recent years suggest that certain hormones are of special importance in this regard because they appear able to rapidly alter the pattern of genetic activity in the cells that are responsive to them (Davidson, 1965; Goldberg and Atchley, 1966). Another reason for singling out hormones in the present discussion is that they may intervene in the mechanisms through which external factors can act on genes during early life. For example, hormonal disturbances caused by certain stresses in a pregnant female might affect the young in utero by altering the activity of its genes.

Experiments with pregnant animals have provided definite evidence that there is a factual basis to the old wives' tales concerning prenatal influences (Ferreira, 1965; Joffe, 1965). Even though the precise mechanisms involved have not yet been elucidated, a few facts seem to warrant speculation on a possible relevance of the interplay between hormones and genes in this regard. It has been observed, for example, that when a chicken egg is being formed, estrogen stimulates the animal's liver to produce the yolk proteins lipovitellin and phosvitin. Even though the rooster does not need these proteins, its liver also produces them in large amounts when the animal is treated with estrogen (Davidson, 1965). Testosterone can also act as a gene activator, producing a dramatic increase in RNA synthesis in various cells. In this light, it may be of interest that a *single* injection of testosterone into a newborn female rat produces profound and lasting changes in the anatomy, physiology, and behavior of the animal; for example, the ovaries are dwarfed and the adults fail to produce corpora lutea and to show the usual cycle of ovulation (Levine, 1966).

Needless to say, many kinds of substances other than hormones can alter gene expression. Suffice it to mention that streptomycin and related drugs can cause a misreading of the genetic code by altering the structure of ribosomes (Gorini, 1966). Even more intriguing, and probably more important from the practical point of view, are the recent reports that extensive chromosomal changes can be produced by certain types of intracellular infections caused by viruses, and other microorganisms. Such infections induce specific changes in the karyotype of the affected cells

and thereby alter the course of their development (Diaz and Pavan, 1965; Pavan and Basile, 1966).

When the intracellular microorganisms induce chromosomal changes in somatic cells, they naturally disturb the organism's economy and thereby cause pathological manifestations. Of potentially greater interest, however, is the fact that microbial infection can affect also the germ line. In *Drosophila robusta*, for example, a maternal factor that appears to be a virus produces a high frequency of chromosome aberrations; the new chromosomal arrangement is then transmitted to the next generation.

The mechanisms of the phenomena denoted by the phrases "cytoplasmic inheritance" and "nongenetic information mechanisms" constitute an area of knowledge still in its infancy (Karakashian, 1965; Salser, 1961). But the phenomena themselves are striking and sufficiently well-defined to make clear that the interplay between genotype and environment involves many more factors than was realized a generation ago. In many situations, indeed, viruses and other microorganisms become part of the complex hereditary apparatus of their host even when they remain in its cytoplasm, and they confer on it thereby new characteristics transmissible from generation to generation. It has even been suggested that many cells constitute in reality a close association of different genetic entities which have become integrated as a result of evolutionary development (Dubos and Kessler, 1963; Pollock, 1965).

The behavioral patterns, instinctive and learned, that regulate the social life of most animals and of man in particular, constitute another aspect of the environment affecting gene expression. Behavior contributes certainly to Darwinian fitness in a given socioecologic environment; while being a consequence of evolution, behavior is also one of its determinants. In this regard, one can take it for granted that the increase in the world population, and the universal trend toward urbanization, will have large consequences for human genetics.

Whatever the ultimate maximum size of the world population, there is no doubt that its increase will stop within a foreseeable future either through conscious planning or in consequence of biological catastrophes. With the present levels of life expectancy in the Western world, however, the population can be stabilized only if the numbers of children do not exceed 2.3 per couple. One can anticipate that such a strict limitation on birth rates would create grave problems for the human race—genetically, physiologically, and emotionally. Social institutions will find it difficult if not impossible to formulate and enforce laws restricting family size to the very low levels dictated by demographic considerations. The more likely prospect is that many couples will continue to have more children than

desirable and that others, hopefully, will choose or be compelled to have only one child or to remain childless. Many genetic and social determinants will play a role in this choice, or compulsion, concerning the number of children. But in any case, control of family size will almost inevitably alter the biological structure of the population.

The universal trend toward urbanization, and the attendant exposure to the various forms of crowding may increasingly affect reproductive physiology and the selection of human types. Wildlife studies and laboratory experiments have revealed that crowding reduces fertility through several independent mechanisms. There is suggestive evidence also that crowding acts as a form of selective pressure.

Until our times, furthermore, the populations of large cities were constantly being supplemented and therefore modified by migrants from rural areas and from underdeveloped countries. This biological transfusion will soon come to an end. If present trends continue, the majority of human beings all over the world will be born, will grow, will live, and will reproduce within the confines of large and crowded urban agglomerations. Mankind will increasingly become truly an urban species, and will probably need therefore to undergo further adaptive changes. It would be surprising if such changes involved only physiological responses and social innovations. More probably they will tend to alter somewhat the human genotype.

It can be taken for granted, however, that most human beings will not behave as passive receptors of the influences exerted on them by the modern world; they will, wisely or dangerously for the future, select some environments and ways of life and shun others on the basis of innate attributes and historically acquired tendencies. This in turn will determine evolutionary changes through the kind of feedback circuit that was outlined in the following words by C. H. Waddington: "(1) environmental stresses produce developmental modifications; (2) the same stresses produce a natural selective pressure which tends to accumulate genotypes which respond to the stresses with coordinated adaptive modifications from the unstressed course of development; (3) genes newly arising by mutation will operate in the epigenetic system in which the production of such coordinated adaptive modifications has been made easy" (Waddington, 1959, 1961).

If the world population truly reaches the appalling levels now predicted by the pessimists for the next century, and if all human groups have to become industrialized and urbanized in order to survive, then one can anticipate a progressive dominance of the genetic traits that facilitate adaptation to a crowded and regimented life. Most technologists and not a few biologists seem unconcerned by this prospect because

they believe that there is no limit to man's adaptability, and that his genetic endowment can be endlessly modified to fit the post-civilized technological world.

Admittedly, the human beings best suited to automated life in affluent societies are bound to differ somewhat genetically from those who cleared forests, drained swamps, and tilled the land with primitive tools during the 10,000 years that have elapsed since the beginning of the Neolithic Age. But it seems probable that biological structures and social attitudes irreversibly determined by history, will impose limitations on the range within which *Homo sapiens* can evolve with safety. The human species will probably find it impossible to adapt biologically to certain innovations that are technologically possible and even appear socially desirable. In this light, the frontiers of technology should be determined by the genetic frontiers of man.

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CHAPTER 2

The Thalassemias

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SINCE THE EXTENSIVE REVIEW of the genetics of abnormal hemoglobins which appeared in the first volume of this series much has been learned about the genetic control and structure of human hemoglobin. Single amino acid substitutions in the globin peptide chains have been demonstrated for many abnormal hemoglobins (Baglioni, 1963c.) and a start has been made in relating these changes in structure to abnormal function (Heller, 1965). While much progress has been made in the field of inherited *structural* variations of hemoglobin, the factors which control its *rate* of synthesis are still not understood. Since so much knowledge about the genetic control of normal hemoglobin structure has been obtained from a study of disease states, it seems likely that similar information about the control of the rate of synthesis of hemoglobin will be obtained from the study of conditions in which there is a defective rate of synthesis of structurally normal hemoglobin. This is the main reason for the current interest in the biochemical genetics of thalassemia and associated hereditary anemias.

INTRODUCTION

This historical background to current ideas about thalassemia has been recently reviewed (Bannerman, 1961; Weatherall, 1965) and need only be briefly mentioned here. A form of severe anemia, occurring early in life and associated with splenomegaly and bone changes, was first described by Cooley and Lee (1925). The condition was later named "thalassemia"—after "the sea," since early cases were all of Mediterranean

background (Whipple and Bradford, 1936). It was only in the period after 1940 that the true genetic character of this disorder was fully appreciated. It became clear that the disease described by Cooley and Lee (1925) was the homozygous state for a partially dominant autosomal gene, while the heterozygous state was found to be associated with much milder hematological changes (Wintrobe et al., 1940; Valentine and Neel, 1944; Smith, 1948). The severely affected homozygous condition became known as thalassemia major, while the heterozygous states, according to their severity, were designated thalassemia minor or minima. Many examples of individuals with moderate anemia and splenomegaly have been reported, in which the clinical picture was not severe enough to be called "thalassemia major" or mild enough to be designated "thalassemia minor." The term "thalassemia intermedia" has been used to cover this group. This is a clinical classification, having no genetic basis and, as will be seen in later sections, the intermediate thalassemias show marked genetic heterogeneity (Pearson, 1964).

The thalassemias are all characterized by an inherited defect in hemoglobin synthesis. In severe cases much of the hemoglobin which is synthesized appears to be rapidly broken down in the marrow and never appears in the peripheral blood. The defect in hemoglobin synthesis results in the formation of many grossly under-hemoglobinized cells with marked variation in size and shape and an associated reduction in survival time. There is a marked reduction in the rate of incorporation of iron into hemoglobin and iron is present in large quantities in the red cell precursors.

From this brief picture of the hematological findings in thalassemia, it is not surprising that attention was first focused on heme synthesis in this disorder. Several defects in heme biosynthesis have been clearly demonstrated (Bannerman, Grinstein and Moore, 1959; Bannerman, 1961) but more recently similar defects have been found in several forms of acquired anemia associated with ineffective erythropoeisis (Steiner, Baldini and Damashek, 1963). The defects of heme synthesis in thalassemia have been fully reviewed in a recent monograph (Bannerman, 1961).

It is currently believed that the clinical picture of thalassemia can result from several distinct inherited defects in globin production and that all the other findings outlined above are secondary to this inability to synthesize globin effectively. This concept has followed careful examination of hemoglobin patterns of patients with the clinical findings of thalassemia. Although the hemoglobin pattern in the peripheral blood may not strictly mirror hemoglobin synthesis in the marrow, there is increasing evidence from *in vitro* studies of globin synthesis in thalassemia

that the associated hemoglobin pattern can provide at least some information about the underlying defects in globin synthesis.

In order to appreciate current ideas about the genetics of thalassemia it will first be necessary to outline recent work on the structure and genetic control of normal hemoglobin. This topic has been the subject of several recent reviews (Baglioni, 1963c; Huehns and Shooter, 1965; Weatherall, 1965) and only those aspects of particular importance to the thalassemia problem will be briefly discussed here.

Genetic Control of Hemoglobin Synthesis

Structure of Hemoglobin

Hemoglobin is a conjugated protein consisting of a protein moiety, globin, and 4 heme groups. Human adult hemoglobin consists of a major component, hemoglobin A, and at least two minor components, hemoglobins A_2 and A_3 (Kunkel et al., 1957).

The globin fraction of hemoglobin A consists of 4 peptide chains, 2 α-chains and 2 β-chains, each with about 140 amino acid residues (Rhinesmith, Schroeder and Pauling, 1957). The sequential amino acid analysis of both α- and β-chains is now complete (Braunitzer et al., 1961; Konigsberg, Guidotti, and Hill, 1961; Goldstein, Konigsberg and Hill, 1963). Hemoglobin A_2 is present in trace amounts only in the neonatal period and reaches its adult level of 1.5-3.5 per cent of the total hemoglobin by the age of 6 months. Like hemoglobin A it has 2 α-chains, but the β-chains are replaced by a pair of structurally distinct chains called δ-chains (Müller and Jonxis, 1960; Ingram and Stretton, 1961). The δ-chains differ by only 8 amino acid residues from the β-chains and their structure is now fully worked out (Stretton, 1962). Hemoglobin A_3, which is probably a derivative of hemoglobin A produced by aging, has α- and β-chains similar to those of hemoglobin A, but has a glutathione bound to the β-chain (Müller, 1961). The detailed nature of this hemoglobin has not yet been worked out.

Fetal hemoglobin, hemoglobin F, is the principal pigment throughout intrauterine life and is present as about 60 to 85 per cent of the total hemoglobin at birth. The synthesis of hemoglobin F rapidly declines after birth in normal infants, and it is present in trace amounts only in adult life. The α-chains of hemoglobins A and F are identical (Weatherall and Baglioni, 1962; Schroeder et al., 1963), but in hemoglobin F are paired with 2 γ-chains (Schroeder and Matsuda, 1958) which differ from β-chains by 17 differences in amino acid composition and 39 differences in sequence (Schroeder et al., 1963). Hemoglobin F is separated by

column chromatography into 2 fractions, designated F_I and F_{II}. Hemoglobin F_I has the N-terminal end of one γ-chain covered by an acetyl group (Schroeder, 1962).

In early intrauterine life, before the age of 10 weeks, two further hemoglobin components are present which have been named hemoglobins Gower I and Gower 2 (Huehns *et al.*, 1964). Hemoglobin Gower 2 has α-chains similar to those of hemoglobins A and F, but the non α-chains seem to be structurally distinct and have been designated ϵ-chains. Hemoglobin Gower I consists entirely of ϵ-chains.

The human fetal and adult hemoglobin types are summarized in Figure 1.

$\alpha_2 \beta_2$	Hemoblobin A
$\alpha_2 \delta_2$	Hemoglobin A_2
$\alpha_2 \gamma_2$	Hemoglobin F II
$\alpha_2 \gamma\gamma^{Acetyl}$	Hemoglobin F I
$\alpha_2 \Sigma_2$	Hemoglobin Gower 2

FIG. 1—The normal human hemoglobins.

Genetic Control of the Structure of the Globin Peptide Chains

The fundamental studies of Pauling and his co-workers (1949) in the demonstration of a structural alteration of the hemoglobin of patients with sickle-cell anemia and the resulting discovery of many other structural hemoglobin variants were fully discussed in Volume I of this series. More recently, using modifications of the fingerprinting technic first applied to the study of hemoglobin structure by Ingram (1958), these hemoglobin variants have been shown to follow single amino acid substitutions in either the α-, β-, γ- or δ-chains (Baglioni, 1963c). Several critical family studies have further clarified the genetic control of these peptide chains.

A large Baltimore family was described in 1958 in which 2 abnormal hemoglobins, S and Hopkins 2, were present (Smith and Torbert, 1958). Several members of this family were heterozygous for both of these variants and their hemoglobin patterns showed both hemoglobins S and Hopkins 2 and considerable amounts of hemoglobin A. It was subsequently established that hemoglobin Hopkins 2 is an α-chain variant, while hemoglobin S is a β-chain variant (Itano and Robinson, 1960). Assuming a one-gene one-peptide chain relationship, these doubly affected individuals would have the following genotype:

$$\alpha/\alpha^{Ho2};\ \beta/\beta^{S}$$

and produce the following peptide chains:

$$\alpha_2 \quad \alpha_2{}^{Ho2};\quad \beta_2 \quad \beta_2{}^{S}$$

Random combination of these subunits would result in 4 species of hemoglobin:

$\alpha_2\ \beta_2$	hemoglobin A;
$\alpha_2{}^{Ho2}\ \beta_2$	hemoglobin Hopkins 2;
$\alpha_2\ \beta_2{}^{S}$	hemoglobin S;
$\alpha_2{}^{Ho2}\ \beta_2{}^{S}$	'hybrid' molecule.

The charge of the $\alpha_2{}^{Ho2}\beta_2{}^{S}$ molecule is such that it would have an identical rate of electrophoretic migration to hemoglobin A. These observations explain the finding of 3 hemoglobins in the individuals heterozygous for hemoglobins S and Hopkins 2, and provide good evidence for a one-gene one-peptide chain relationship in human adult hemoglobin (Itano and Robinson, 1960). This concept has received confirmation by the reports of individuals heterozygous for both hemoglobins G_a and C, all of whom carried 4 hemoglobin fractions (McCurdy, Pearson and Gerald, 1961; Weatherall, Sigler and Baglioni, 1962). Such an individual's genotype can be represented as $\alpha/\alpha^{G};\ \beta/\beta^{C}$, and therefore 4 types of peptide chains, α_2, $\alpha_2{}^{G}$, β_2 and $\beta_2{}^{C}$, would be synthesized and hence 4 hemoglobins as follows:

$\alpha_2\ \beta_2$	hemoglobin A;
$\alpha_2{}^{G}\ \beta_2$	hemoglobin G;
$\alpha_2\ \beta_2{}^{C}$	hemoglobin C;
$\alpha_2{}^{G}\ \beta_2{}^{C}$	hemoglobin G/C.

Chemical studies have confirmed that the 4 hemoglobin fractions found in such doubly affected persons do, in fact, have the predicted structure (Weatherall, Sigler and Baglioni, 1962).

Further evidence for the genetic control of the peptide chains of hemoglobin has been obtained from the study of individuals heterozygous for α-chain hemoglobin variants. In the neonatal period such persons carry 2 forms of fetal hemoglobin, hemoglobin F ($\alpha_2\gamma_2$) and a component composed of normal γ-chains and variant α-chains ($\alpha_2{}^{X}\gamma_2$) (Minnich et al., 1962; Weatherall and Baglioni, 1962). When followed into adult life the same individual carries 4 hemoglobin components: hemoglobin A ($\alpha_2\beta_2$), the α-chain variant ($\alpha_2{}^{X}\beta_2$), hemoglobin A_2 ($\alpha_2\delta_2$) and a second minor component composed of normal δ-chains and

variant α-chains ($\alpha_2{}^X\delta_2$) (Huehns and Shooter, 1961; Weatherall and Boyer, 1961).

From this evidence, a tentative model of the genetic control of human hemoglobin synthesis can be constructed (Fig. 2). In intrauterine life α-chains are combined with γ-chains to form hemoglobin F ($\alpha_2\gamma_2$) and possibly, before the tenth week, with ϵ-chains to form hemoglobin Gower 2 ($\alpha_2\epsilon_2$). ϵ-chain synthesis ceases at about 10 weeks and, in normal infants, γ-chain synthesis almost ceases after birth. During intrauterine life β-chain synthesis occurs at a very slow rate, but after birth β- and δ-chain synthesis is fully activated and α-chain synthesis continues, α-chains now combining with β-chains and δ-chains to form hemoglobins A ($\alpha_2\beta_2$) and A_2 ($\alpha_2\delta_2$), respectively.

This scheme for the genetic control of the hemoglobin chains is entirely consistent with the findings in patients with the various hemoglobinopathies. It fully explains the finding of both normal and variant hemoglobin in heterozygotes and the absence of normal hemoglobin in homozygotes (Fig. 3). It also accounts for the observation that infants with β-chain hemoglobin variants are normal in the neonatal period and are only clinically affected at 2-3 months when γ-chain synthesis ceases and β-chain synthesis takes over. Hemoglobins with altered α-chains, on the other hand, are present in both intrauterine life and in adult life, this situation probably accounting for the lack of serious clinical manifestations observed in affected individuals. Presumably those α-chain variants associated with a severe defect in hemoglobin synthesis would be rapidly lost by natural selection.

While formal genetic studies have confirmed the existence of genetic

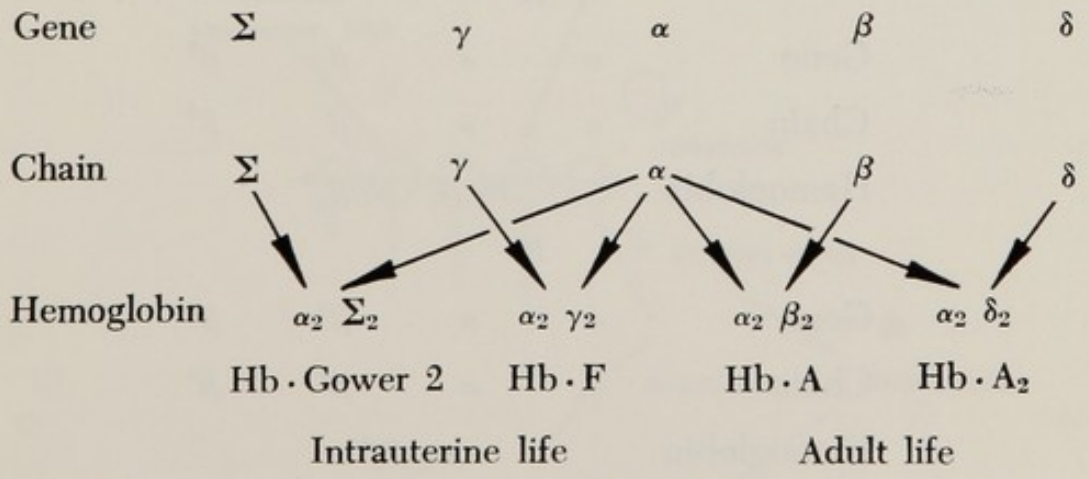


FIG. 2—The genetic control of hemoglobin synthesis. The single genetic locus directing α-chain synthesis in fetal and adult life explains why α-thalassemia is associated with defective synthesis of fetal and adult hemoglobin while β-thalassemia is only manifest after the neonatal period.

loci controlling the structure of the α-, β-and δ-chains, no such evidence is available for the γ-chain locus. It is likely that the α- and β-chain loci are at some distance apart on the same chromosome or on different chromosomes (Smith and Torbert, 1958; Bradley, Boyer and Allen, 1961) while the β- and δ-loci appear to be closely linked (Ceppellini, 1959; Boyer et al., 1963). Unfortunately there is no evidence as to the chromosomal site of the γ- or ε-loci and no definite information as to which chromosomes carry the hemoglobin genes.

Control of the Rate of Synthesis of Hemoglobin

Although much progress has been made in the elucidation of the genetic control of the *structure* of hemoglobin, the factors which control the *rate* of synthesis are poorly understood. Since these factors are probably of prime importance in the further understanding of the thalassemias they must be briefly considered here.

Healthy red cells contain similar amounts of hemoglobin and the proportions of the major and minor hemoglobin components are remarkably constant. Thus, a mechanism must exist for the "turning off" of hemoglobin synthesis and for maintaining δ-chain synthesis at about 1/40 of the rate of β-chain synthesis. Furthermore, there must be a mechanism for the "switching on" of β-chain and δ-chain synthesis and the "turning

Gene	α	α	β	β
Chain	α	α	β	β
Hemoglobin		$\alpha_2\ \beta_2$		
		A		
Gene	α	α	β	β^s
Chain	α	α	β	β^s
Hemoglobin		$\alpha_2\ \beta_2 \cdot \alpha_2\ \beta_2{}^s$		
		B		
Gene	α	α	β^s	β^s
Chain	α	α	β^s	β^s
Hemoglobin		$\alpha_2\ \beta_2{}^s$		
		C		

FIG. 3—The inheritance of sickle-cell anemia in terms of the affected peptide chain. A. Normal. B. Sickle-cell heterozygote. C. Sickle-cell homozygote.

off" of γ-chain synthesis in the neonatal period, and for the synchronization of α-chain with γ- or β-chain synthesis.

The organization of protein synthesis is summarized in Figure 4 and there are several levels at which the rate of hemoglobin production might be modified. The rate of messenger RNA (mRNA) production, the stability of mRNA, and the rate of laying down of the constituent amino acids and their binding in peptide linkage on the messenger RNA-ribosomal complex, could all modify the rate of peptide chain production (Baglioni, 1963c). Presumably the availability of the sRNA anticodons for any given amino acid can also modify the rate of chain synthesis at this level (Itano, 1965). As the chains grow in length from the N-terminal end they are thought to fold and the sequence of amino acids which allows the most rapid assumption of the tertiary configuration may well be rate limiting, as may the rate of subunit association (Brenner, 1959). Finally the level of any hemoglobin fraction will depend on its relative survival in the erythrocyte.

The ratio of normal to variant hemoglobin in heterozygotes offers a model for the examination of some of these rate limiting mechanisms.

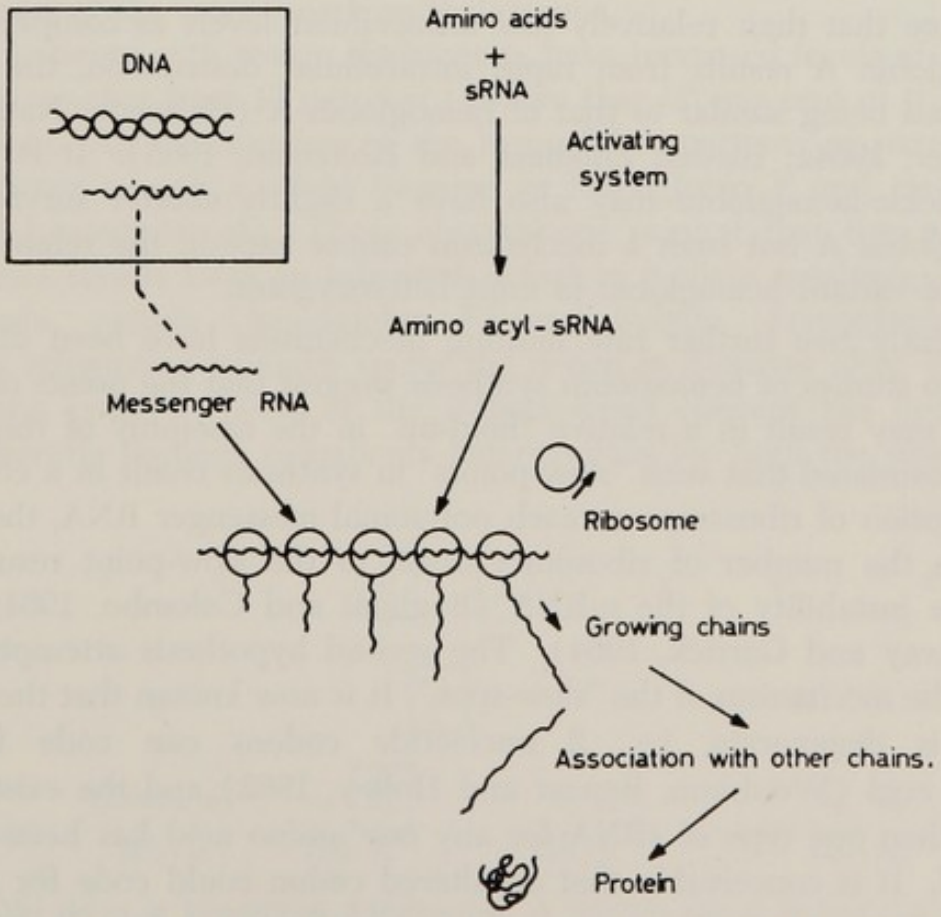


FIG. 4—The organization of protein synthesis. (With kind permission of Blackwell's Scientific Publications, Oxford.)

This approach has been discussed extensively by Itano (1957, 1965) and need only be briefly summarized here. The abnormal hemoglobin is usually present as less than 50 per cent of the total hemoglobin in heterozygotes, although there are well-documented exceptions (Thorup et al., 1956; Clegg, Naughton and Weatherall, 1965). It is not yet clear, however, how a single amino acid substitution can alter the net rate of synthesis of the affected peptide chain. It seems unlikely that a single substitution can alter the rate at which the chains are assembled since there is a marked difference in the relative proportions of hemoglobins C, E and O present in heterozygotes, yet in each case there is a lysine substituted for a glutamic acid (Baglioni, 1963c). It is possible, however, that the residues which precede or follow the altered residue are as important as the changed residue (Itano, 1965). It is also possible that substitutions in various critical parts of the chain can alter the rate at which the affected chain folds and assumes its tertiary configuration. There is some evidence that subunit association is not entirely random and may therefore be rate limiting in some cases. In most cases the relative rates of turnover of the mutant and normal hemoglobins have not been studied. In the case of hemoglobins H and Zurich there is good evidence that their relatively low intracellular levels as compared with hemoglobin A results from rapid intracellular destruction, the rate of synthesis being similar to that of hemoglobin A (Gabuzda, Nathan and Gardner, 1964a; Rieder, Zinkham and Holtzman, 1965). It is possible that sickle-hemoglobin may also have a slightly shorter survival than hemoglobin A but such a mechanism cannot explain the relatively low level of variant hemoglobin in most heterozygotes.

Recently two further rate limiting mechanisms have been discussed. *In vitro* studies of hemoglobin synthesis suggest that the points of substitution may result in a relative "hold-up" in the assembly of the chains. It is postulated that such "slow-points" in synthesis result in a change of distribution of ribosomes on each polysomal messenger RNA, the reduction in the number of ribosomes distal to the slow-point resulting in relative instability of the mRNA (Baglioni and Colombo, 1964; Boyer, Hathaway and Garrick, 1964). The second hypothesis attempts to explain the mechanism of the "slow-spot." It is now known that the genetic code is degenerate, i.e., 2 nucleotide codons can code for one amino acid (Weisblum, Benzer and Holley, 1962) and the existence of more than one type of sRNA for any one amino acid has been demonstrated. It is conceivable that an altered codon could code for a sRNA which is in relatively poor supply, so producing a rate limiting step (Itano, 1965). Clearly a mutation of this type can alter the rate of syn-

thesis of protein without changing its primary structure (Itano, 1965).

It is clear, therefore, that little is really known about how the rate of hemoglobin synthesis is controlled. The further study of the thalassemias promises to answer some of these questions.

Development of the Concept of Thalassemia as a Hemoglobinopathy

Studies of hemoglobin electrophoretic patterns of patients with the clinical findings of thalassemia have suggested that at least some of those disorders result from inherited defects in synthesis of the globin chains. This idea was first suggested by Itano (1957) and elaborated by Ingram and Stretton (1959) and others. These workers suggested that the thalassemias result from defective synthesis of the globin fractions, similar in every way to the hemoglobinopathies except that the chemical abnormalities of the affected chains are such as not to alter the charge of the molecule. Ingram and Stretton (1959) also suggested that the underlying defect might be in the control of the rate of synthesis of the chains rather than in their structure. These ideas have provided a stimulus for most of the recent work on thalassemia.

Many patients with severe thalassemia have increased levels of hemoglobin F, ranging from 10 per cent to more than 90 per cent of the total hemoglobin. An examination of the hemoglobin patterns of parents of such patients reveals a slight increase in hemoglobin F and increased levels of hemoglobin A_2. These observations suggest that this type of thalassemia results from an inherited defect in β-chain synthesis, i.e., β-thalassemia, γ-chain (hemoglobin F) and δ-chain (hemoglobin A_2) synthesis attempting to make up for the deficit in β-chains (Fig. 5).

A strong point in favor of the β-thalassemia concept has been the electrophoretic findings in patients heterozygous for both the sickle-cell

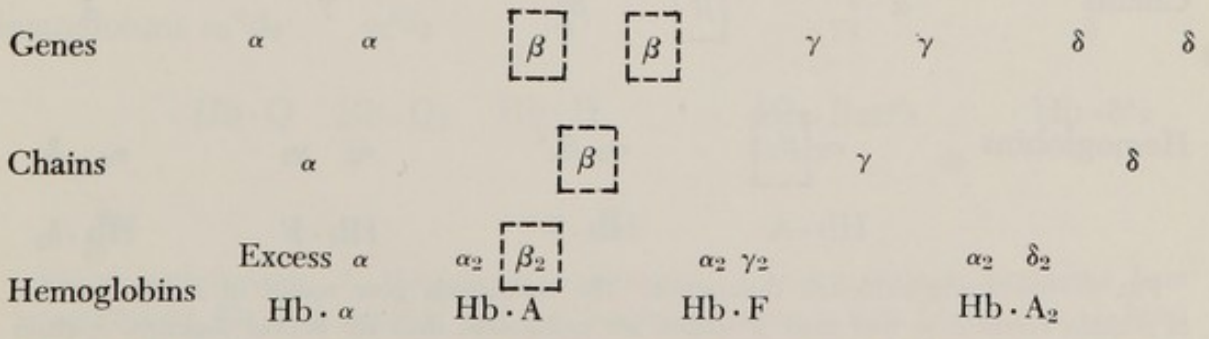


Fig. 5—The defect in homozygous β-thalassemia. A defective rate of β-chain synthesis can be partly compensated by γ-chain (hemoglobin F) and δ-chain (hemoglobin A_2) synthesis. The excess of α-chains probably are precipitated in the red cell or degraded in the bone marrow.

gene and the high hemoglobin A_2 type of thalassemia gene. In such cases the level of hemoglobin A is considerably less than that of hemoglobin S and indeed in some cases hemoglobin A is completely absent. Such interaction between the thalassemia gene and that for a β-chain hemoglobin variant suggests that the action of the thalassemia gene is to reduce the rate of normal β-chain synthesis, in doubly affected persons β^A-chains being synthesized less effectively than β^S-chains (Fig. 6). Furthermore, critical family studies have shown that the genetic determinant for β-thalassemia is allelic or closely linked to the β-structural locus. These observations suggest that this type of thalassemia is due to an inherited defect in β-chain synthesis.

In some persons with the clinical picture of thalassemia no increase in hemoglobins F or A_2 is found. Furthermore, this variety of thalassemia does not interact with β-chain hemoglobin variants. In some patients with this type of thalassemia small quantities of rapidly migrating hemoglobins are present. One of these, hemoglobin H, consists of 4 β-chains (β_4) (Jones et al., 1959) while the other, hemoglobin Bart's, has 4 γ-chains (γ_4) (Hunt and Lehmann, 1959). It is thought that this type of thalassemia results from an inherited defect in α-chain synthesis, excess β-chains and γ-chains aggregating to form hemoglobins H and Bart's, respectively (Fig. 7). Several instances of the interaction of this type of thalassemia with α-chain hemoglobin variants have been reported. In each case the α-chain hemoglobin variant was present as more than 50 per cent of the total hemoglobin, i.e., the synthesis of normal α-chains was reduced in the presence of this type of thalassemia gene (Fig. 7). These observations suggest that this variety of thalassemia results from

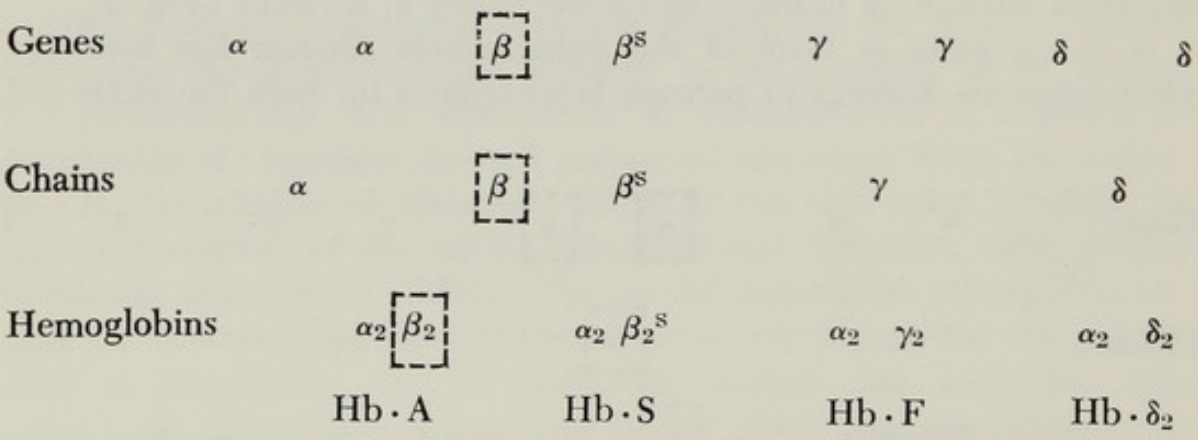
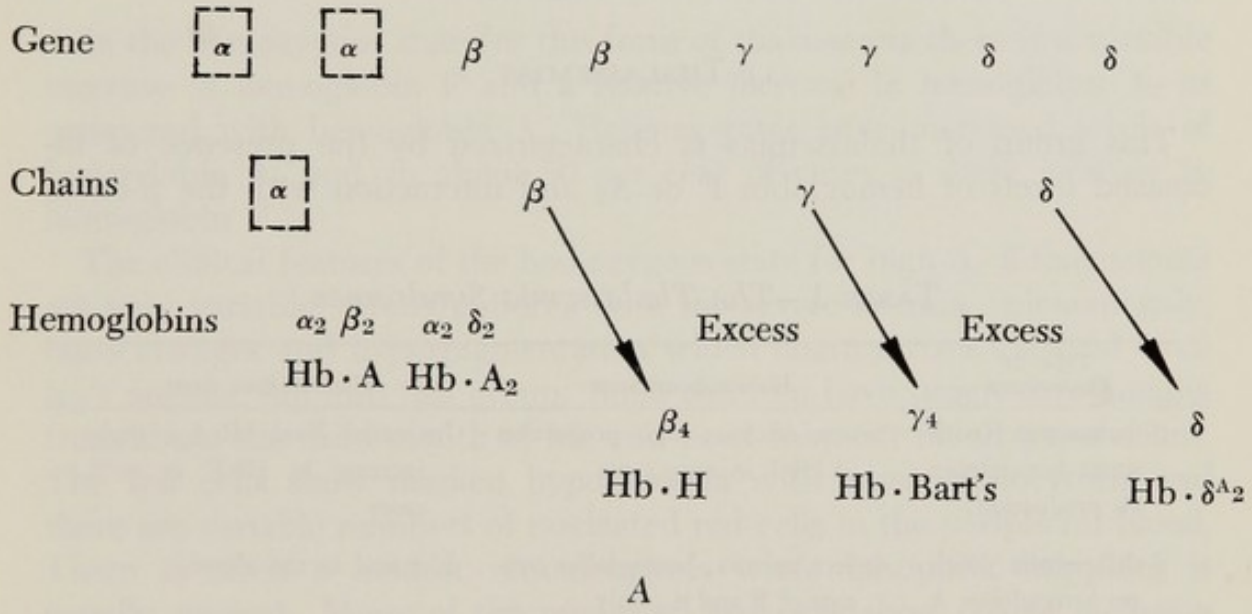


FIG. 6—Interacting sickle-cell thalassemia. The thalassemia gene results in a defective rate of β-chain synthesis so that more β^S-chains are synthesized than are normal β-chains. γ-chain and δ-chain synthesis is increased so that the final hemoglobin pattern consists of 70–80 per cent hemoglobin S with increased levels of hemoglobins F and A_2 and a small quantity of hemoglobin A. In some cases no normal β-chains are synthesized.

defective α-chain synthesis, although genetic evidence which would relate the abnormal gene to the α-chain locus has not yet been obtained.

The concept of the α- and β-thalassemias has been a useful basis for the study of these disorders, but in recent years it has become clear that there are probably several types of thalassemia secondary to defective β-chain synthesis and growing evidence for heterogeneity within the dis-



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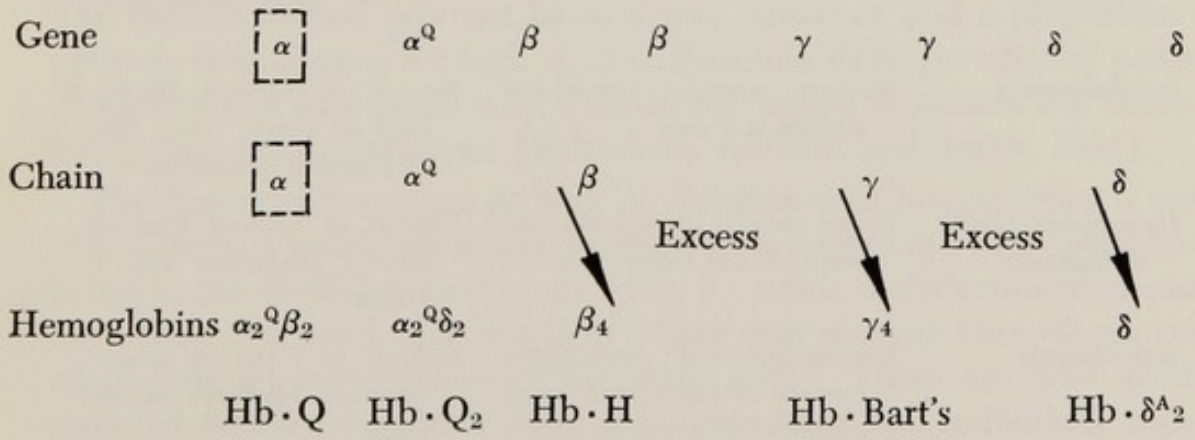


B

FIG. 7—α-thalassemia. A. Homozygous α-thalassemia. The few α-chains synthesized form hemoglobin A. The large excess of β, γ and δ-chains aggregate to form hemoglobins H, Bart's and $\delta^{A}{}_{2}$, respectively. B. Heterozygosity for an α-chain hemoglobin variant (hemoglobin Q) and α-thalassemia. There is complete suppression of normal α-chain synthesis and only hemoglobins Q, Q_2, H, Bart's and $\delta_2{}^{A}{}_{2}$ are synthesized.

orders grouped under the general heading α-thalassemia (Weatherall, 1965). Furthermore, there appear to be disorders associated with defective δ-chain synthesis, δ-thalassemia and several other conditions associated with the simultaneous defective production of β- and δ-chains which result in a clinical picture of thalassemia. These conditions, which together constitute the "thalassemia syndromes," are summarized in Table 1, and will each now be considered in further detail.

β-Thalassemias

This group of thalassemias is characterized by the presence of increased levels of hemoglobin F or A_2 and interaction with the β-chain

Table 1.—*The Thalassemia Syndromes*

Type of Thalassemia	Homozygous State	Heterozygous State
β-thalassemia (with some hemoglobin A production)	Severe anemia; high proportion of Hb.F in most cases	Increased level Hb.A_2; slight increase in Hb.F in 50% cases
β-thalassemia (with no hemoglobin A production)	Severe anemia; hemoglobin consists of F and A_2 only	Identical to the above.
β-thalassemia (with no hemoglobin A production)	Moderate anemia; hemoglobin consists of F and A_2 only	Increased level of Hb.A_2; unusually high levels of Hb.F in the range 20-80 percent
β-δ-thalassemia	Moderate anemia; hemoglobin consists of F only	Normal or reduced Hb.A_2; Hb.F in 5-20% range, heterogeneously distributed
Hemoglobin Lepore thalassemias	Severe anemia; hemoglobin consists of F and Lepore only	Normal or reduced level of Hb.A_2; Hb.Lepore and F present
α-thalassemia	Intrauterine death; hemoglobin consists of Bart's with small quantities of A and H.	Difficult to detect in adults; Increased Hb.Bart's in infancy
α-thalassemia intermedia (hemoglobin H disease)	Inheritance uncertain; associated with a mild hemolytic anemia associated with variable levels of Hb.H and Hb.Bart's	
δ-thalassemia	Not described	Reduced levels of Hb.A_2
Thalassemia-like states	Probably types of β or β-δ-thalassemia not associated with changes in levels of hemoglobins F or A_2	

hemoglobin variants. A study of the hemoglobin patterns of the parents of children apparently homozygous for β-thalassemia and of individuals heterozygous for both β-thalassemia and β-chain hemoglobin variants has provided evidence for heterogeneity within the β-thalassemia group (Table 1). Each type of β-thalassemia can be considered separately.

β-Thalassemia with Increased Levels of Hemoglobin A_2

In the homozygous state for this form of thalassemia there is a variable increase in hemoglobin F and a relative increase in hemoglobin A_2 as compared with hemoglobin A. Heterozygotes have increased levels of hemoglobin A_2 and, in about 50 per cent of cases, a slight increase in hemoglobin F.

The clinical features of the homozygous state for high A_2 β-thalassemia are very variable. Many children with the severe anemia, splenomegaly, bone changes and hyperpigmentation which characterizes classical Cooley's anemia, fall into this group. Such children have progressive anemia from about the third month of life and need frequent blood transfusions. The red cells show marked hypochromia with anisopoikilocytosis and there are variable numbers of nucleated red cells in the peripheral blood. There is often a modest reticulocytosis while basophilic stippling is usually present. Many of the nucleated red cells show coarse inclusion bodies which are best seen after staining with methyl violet and which may consist of denatured α-chains (Fessas, 1963). There is marked erythroid hyperplasia of the marrow with increased iron deposition. The clinical picture may not be so severe, however, and a proportion of patients homozygous for high A_2 β-thalassemia show the clinical picture of thalassemia intermedia with a moderate degree of anemia not necessitating frequent transfusions (Erlandson, Brilliant and Smith, 1964).

The hemoglobin pattern in high A_2 β-thalassemia homozygotes is variable. There is nearly always an increase in hemoglobin F, the usual level being in the 40-60 per cent range (Fig. 8), while well-documented cases have been described with less than 10 per cent or more than 90 per cent alkali-resistant hemoglobin (Weatherall, 1965). There are now several reported families in which there was more than one individual apparently homozygous for high A_2 β-thalassemia with no detectable hemoglobin A, electrophoretic or chromatographic fractionation of their hemoglobin showing hemoglobins F and A_2 only (Fessas, 1964; Went and Schokker, 1965). The fact that this form of thalassemia runs true within families suggests that it is a distinct entity (Table 1). The hemoglobin F found in homozygous β-thalassemia is indistinguishable by a variety of chemical technics from that of normal umbilical cord blood (Sturgeon

et al., 1963). The level is not related to the severity of the disease or to repeated blood transfusion. It is heterogeneously distributed throughout the red cells and turnover studies have suggested that cells carrying larger proportions of hemoglobin F have a long survival (Shepherd, Weatherall and Conley, 1962; Gabuzda, Nathan and Gardner, 1963).

The percentage of hemoglobin A_2 in homozygous high A_2 β-thalassemia is very variable, and is only elevated in a small proportion of cases, while in many cases it is subnormal. If expressed as a percentage of hemoglobin A, however, it is usually markedly elevated. Thus a patient with 89 per cent hemoglobin F, 10 per cent hemoglobin A and 1 per cent hemoglobin A_2, has a hemoglobin A/A_2 ratio of 10 to 1 instead of 40 to 1. This suggests that there should, in general, be a reciprocal relationship between hemoglobin F and A_2 levels in homozygotes for this type of thalassemia and this is, in fact, the case (Weatherall, 1965). In absolute terms, however, the amount of hemoglobin A_2 per red cell is not increased and there seems to be an overall defect in hemoglobin synthesis, hemoglobin A_2 being synthesized only relatively more efficiently than hemoglobin A (Weatherall, 1965).

If care is taken during the preparation of hemolysates, a slowly migrating hemoglobin can be demonstrated on starch gel electrophoresis

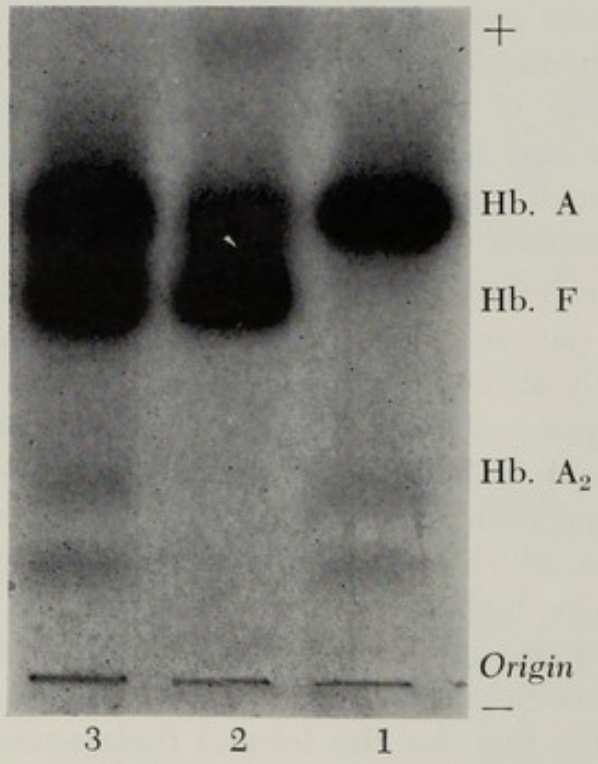


FIG. 8—The hemoglobin pattern in thalassemia major. (1) Normal adult; (2) normal umbilical cord blood; (3) 12-year-old child with thalassemia major. Starch gel electrophoresis, pH 8.5 (Tris-EDTA system)

of samples obtained from β-thalassemic homozygotes. Hybridization studies have suggested that this is free α-chain. It is not present in normal hemolysates under similar conditions (Fessas and Loukopoulos, 1964).

The clinical findings in heterozygotes for this type of thalassemia are very variable, ranging from a completely normal clinical and hematological picture to one of severe anemia and splenomegaly not unlike the homozygous state. In about 50 per cent of cases there is no elevation of hemoglobin F, while a moderate elevation, in the 2-4 per cent range, is found in the remainder. Exceptional families having more than one individual with up to 20 per cent F have been described (Weatherall, 1964a), while reports of the finding of up to 80 per cent F in high A_2 β-thalassemia "heterozygotes" must be accepted with caution, since detailed family studies have not always been presented which would rule out the presence of a second thalassemia gene. The hemoglobin F is heterogeneously distributed among the red cells (Shepherd, Weatherall and Conley, 1962). Hemoglobin A_2 levels are elevated in the 3.5–6.5 per cent range (Kunkel et al., 1957). The level of hemoglobin A_2 is unrelated to that of hemoglobin F, but is related to the mean corpuscular hemoglobin level (Weatherall, 1964a). The increase in δ-chain synthesis is the result of increased activity at both δ-loci, individuals doubly affected with this form of thalassemia and a hemoglobin A_2 (δ-chain) structural variant showing elevations of both normal and abnormal minor hemoglobin components (Ceppellini, 1961). There is a tendency for hemoglobin F and A_2 levels to be similar within families.

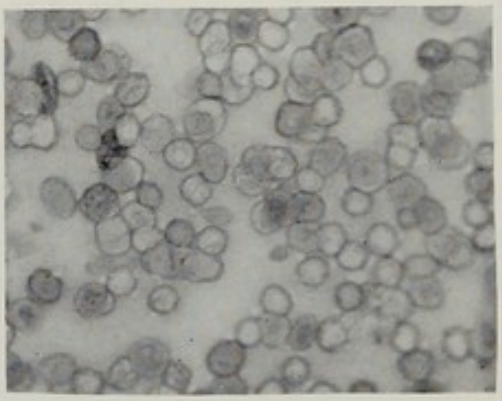
β-Thalassemia with Normal Levels of Hemoglobin A_2 and Increased Levels of Hemoglobin F (β-δ-Thalassemia)

A form of thalassemia with hematological findings similar to those of the high hemoglobin A_2 type, but in which there is no elevation of hemoglobin A_2 and relatively high hemoglobin F levels, has been observed in Greeks (Fessas, 1961), Italians (Zuelzer, Robinson and Booker, 1961), Negroes (Weatherall, 1964a), and Thais (Flatz, Pik and Sringam, 1965). This variety of thalassemia has been variously called F-thalassemia (Fessas, 1961), β-thalassemia type 2 (Fessas, 1964), normal A_2-β-thalassemia (Weatherall, 1965) and β-δ-thalassemia (Motulsky, 1964).

The homozygous state for this type of thalassemia has only been encountered on one occasion to date (Brancati, 1965). The affected person was an adult with moderate anemia and hepatosplenomegaly. The hemoglobin consisted entirely of hemoglobin F, no hemoglobin A or A_2 being demonstrable. Both parents showed mild hematological abnormalities

associated with increased levels of hemoglobin F and normal levels of hemoglobin A_2. It appears, therefore, that at least in some cases of this variety of thalassemia β- and δ-chain synthesis is completely deficient.

The hematological findings in individuals heterozygous for this condition are similar to those with high A_2-β-thalassemia. Hemoglobin A_2 levels are normal or slightly lower than normal, while hemoglobin F values range from 5-20 per cent. There is a tendency for hemoglobin F values to be similar within families. The fetal hemoglobin is heterogeneously distributed throughout the red cells (Weatherall, 1964a) (Fig. 9). One person has been described who may have been heterozygous for both this form of thalassemia and hemoglobin A_2^1, a δ-chain variant. Hemoglobin A_2 was absent, only hemoglobin A_2^1 being observed on starch gel electrophoresis (Comings and Motulsky, 1966). These results indicate a complete absence of δ-chain synthesis by the chromosome carrying the normal A_2-β-thalassemia gene. The clinical and electrophoretic findings in individuals heterozygous for both this form of thalassemia and high A_2-β-thalassemia are now well documented (Fessas,



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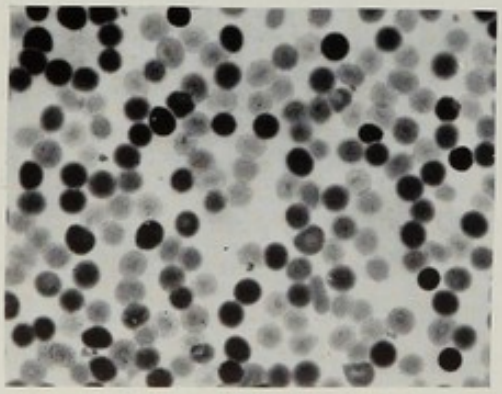

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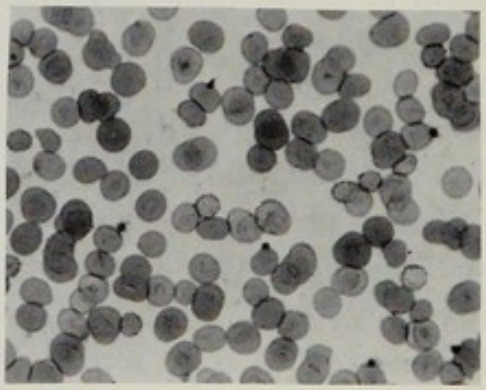

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FIG. 9—Acid elution patterns using Kleihauer technic on blood smears from the following: (A) Normal adult. (B) β-δ-thalassemia minor with heterogeneous distribution of Hb.F. (C) hereditary persistence of Hb.F with homogeneous distribution of Hb.F.

1961; Zuelzer, Robinson and Booker, 1961; Wolf and Ignatov, 1963; Gabuzda, Nathan and Gardner, 1964b). The hematological findings are those of moderately severe thalassemia, while the hemoglobin pattern consists of more than 90 per cent hemoglobin F with normal or subnormal levels of hemoglobin A_2. In several cases careful study has failed to demonstrate any hemoglobin A in these individuals.

One example of the presence, within one family, of the genes for α-thalassemia and normal A_2-β-thalassemia has been described (Weatherall, 1963). A woman received the α-thalassemia gene from her mother and normal A_2-β-thalassemia gene from her father. Both genes were transmitted to her 6-month-old baby, who carried about 10 per cent hemoglobin F and traces of hemoglobin Bart's.

At the time of writing the evidence that this type of thalassemia results from defective β- and δ-chain synthesis is fairly convincing, although further examples of homozygosity and of interaction with genes for structural alterations in the β- or δ-chains are required. It seems reasonable, therefore, to use the term "β-δ-thalassemia" to describe this condition.

Other Forms of β-Thalassemia (Thalassemia-Like States)

It seems very likely that there are types of thalassemia secondary to defective β-chain and possibly δ-chain synthesis in which there is no increase in hemoglobin A_2 levels and only minimal persistence of fetal hemoglobin synthesis (Bernini et al., 1962; Weatherall, 1965). The evidence for defective β-chain synthesis in these cases has been obtained from the study of persons affected with both the genes for such a variety of thalassemia and those for high A_2-β-thalassemia or δ-thalassemia. In the case of high A_2-β-thalassemia the genes interact to produce a moderately severe form of thalassemia with a moderate elevation of hemoglobin F (Bernini et al., 1962), while in one individual heterozygous for δ-thalassemia and a form of thalassemia with normal hemoglobin A_2 and F levels, hemoglobin A_2 was completely absent (Fessas and Stamatoyannopoulos, 1962).

From these few reports it seems likely that varieties of thalassemia secondary to defective β- and δ-chain synthesis exist which are unassociated with hemoglobin F or A_2 alterations. There has been an unfortunate tendency to label such cases as having α-thalassemia. The criteria for the diagnosis of α-thalassemia are considered later and, if not present, such cases should be labeled as "thalassemia-like states" until a more direct method for studying the underlying defect in globin synthesis is available. There is little doubt that this group will show further hetero-

geneity as more opportunities for study of interactions with β- and δ-chain structural hemoglobin variants occur.

δ-Chain Thalassemia

A reduction in the levels of hemoglobin A_2 below the 1.5–3.5 per cent range was noted in one or more family members during population surveys in Greece (Choremis et al., 1963). It is not absolutely certain that this was, in fact, a genetic abnormality since certain acquired conditions, notably iron deficiency, can reduce the level of hemoglobin A_2 as compared with hemoglobin A.

However, in 2 reported cases there has been an opportunity to study the interaction of possible cases of δ-thalassemia with other conditions. In a Negro family a child received the gene for hereditary persistence of hemoglobin F from one parent, the other parent being normal except for an unusually low level of hemoglobin A_2 (Thompson et al., 1965). The child showed complete absence of hemoglobin A_2. Since hereditary persistence of fetal hemoglobin is known to be associated with absence of δ-chain synthesis, these findings suggest that this child received a gene for defective δ-chain synthesis from the parent with low levels of hemoglobin A_2. This, to date, is the best evidence for the existence of a δ-thalassemia gene. Similar but less direct evidence has been obtained from a Greek family in which one member also showed complete absence of hemoglobin A_2 (Fessas and Stamatoyannopoulos, 1962).

β-Thalassemias in Association with β-Chain Hemoglobin Variants

As mentioned earlier, studies of the hemoglobin constitution of persons heterozygous for both thalassemia and structural hemoglobin variants have provided much information about the mode of action and location of the thalassemia genes. The best studied condition is sickle-cell thalassemia.

Sickle-cell thalassemia (Silvestroni and Bianco, 1944-45, 1955a, 1955b) was first described by Silvestroni and Bianco in 1945 and the hemoglobin patterns studied at a later date in the U.S.A. (Neel, Itano and Lawrence, 1953; Singer, Singer and Goldberg, 1955). The clinical findings in individuals with this condition are very variable, ranging from a disorder indistinguishable from sickle-cell anemia to a very mild anemia with no clinical disability. This variability is, at least in part, dependent on the associated hemoglobin constitution. The usual finding is about 60-70 per cent hemoglobin S, 25-35 per cent hemoglobin A, increased levels of hemoglobin A_2 and a variable, but small, increase in hemoglobin F (Weatherall, 1965). In some individuals, however, no detectable hemo-

globin A is present and there is about 85 per cent hemoglobin S, the remainder being hemoglobins F and A_2. The latter group generally have more marked clinical pictures than those with hemoglobin A. Hemoglobin F is quite heterogeneously distributed throughout the red cells (Shepherd, Weatherall and Conley, 1962).

It seems likely that the thalassemia genes involved in producing the "hemoglobin A-producing" as opposed to the "nonhemoglobin A-producing" type of sickle-cell thalassemia are separate entities (Weatherall, 1964a). The reported sibships have recently been summarized (Weatherall, 1965) and the production or nonproduction of hemoglobin A seems to run true within families. The hemoglobin A-producing type of thalassemia gene is more common in the Negro population. Both types of thalassemia occur in Greeks and Italians, but there is, to date, insufficient data to determine which type predominates.

The parents of individuals with sickle-cell thalassemia show either the sickle-cell trait or high hemoglobin A_2-β-thalassemia. The clinical and electrophoretic findings in the thalassemia-carrying parent appear to be the same in both hemoglobin A-producing and nonhemoglobin A-producing sickle-thalassemia. No example of the transmission of both genes from a single parent has been described. In one reported family the affected individual carried about 30 per cent hemoglobin F, while his mother had high A_2-β-thalassemia with 18 per cent hemoglobin F (Weatherall, 1964a). In another family of particular interest (Russo and Mollica, 1962) there was an individual with sickle-cell thalassemia with about 30 per cent hemoglobin A, 20 per cent hemoglobin F and

TABLE 2.—*Comparison of the Different Forms of Hereditary Persistence of Hemoglobin F (Conley et al., 1963; Fessas and Stamatoyannopoulos, 1964)*

	Negro Type	Greek Type	Swiss Type
% Hb.F in heterozygotes	18–30 (mean 26)	10–20 (mean 15)	1–3 (mean 2)
% Hb.A_2 in heterozygotes	1.0–2.1 (mean 1.6)	1.2–3.0 (mean 2.1)	Normal
Distribution of Hb.F in individual cells	Homogeneous	Homogeneous	Heterogeneous
% Hb.F when found in association with β-thalassemia	66–71 (mean 68)	26–43 (mean 34)	—
% Hb.A_2 when found in association with β-thalassemia	2.3–3.4 (mean 2.8)	3.8–5.2 (mean 4.4)	—

50 percent hemoglobin S. This individual's father had all the features of the heterozygous state for β-δ-thalassemia with normal levels of hemoglobin A_2 and 9 per cent hemoglobin F. Here, then, is an example of the sickle-cell-β-δ-thalassemia combination and it is of some interest that hemoglobin A synthesis was not completely suppressed. This is to date the only suggestion of heterogeneity of β-δ-thalassemia for the evidence cited in the previous section suggests that no β- or δ-chains are synthesized in the presence of this gene.

The other high A_2 thalassemia–β-chain hemoglobin variant combinations are less common. Hemoglobin C-thalassemia is a well-recognized disorder occurring mainly in Negroes (Singer et al., 1954; Zuelzer and Kaplan, 1954) with sporadic reports of cases in Italians (Erlandson, Smith and Schulman, 1956), Algerians (Portier et al., 1960), and Turks (Göksel and Tartaroglu, 1961). In the Negro the disorder results in very little clinical disability, while in the Italian and Algerian cases there was a moderate degree of anemia and splenomegaly. Again, the presence or absence of hemoglobin A has run true within families and, to date, the hemoglobin A-producing variety has always been found in the Negro cases while the more severely affected Algerian cases had no detectable hemoglobin A (Weatherall, 1965). The β-thalassemia-hemoglobin E combination is usually associated with severe anemia and a clinical picture similar to thalassemia major (Minnich et al., 1954; Chernoff et al., 1956). It is interesting that electrophoretic studies of this disorder have nearly always revealed no hemoglobin A. This observation suggests that the β-thalassemia gene in the Far East is usually of the nonhemoglobin A-producing variety. This important point needs verification, particularly since the detailed studies required to exclude the presence of small amounts of hemoglobin A have not always been reported.

Several other families have been described in which the β-thalassemia gene was found in association with the gene for a β-chain hemoglobin variant. These combinations include hemoglobin G-thalassemia (Schwartz et al., 1957), hemoglobin D-thalassemia (Hynes and Lehmann, 1959; Sukumaran, Sanghvi and Nazreth, 1960) and hemoglobin J-thalassemia (Sydenstricker et al., 1961). In all these families the presence or absence of hemoglobin A in doubly affected persons ran true within sibships.

The result of receiving the genes for β-thalassemia and a β-chain hemoglobin variant will thus depend on the type of β-thalassemia gene and the properties and rate of synthesis of the hemoglobin variant. The marked difference in clinical manifestations and electrophoretic findings in such doubly affected persons and the fact that these differences tend

to run true within families provide further evidence for heterogeneity within the β-thalassemias.

Relationship of the β-Thalassemia Loci to the β- and δ-Structural Loci

There is now good evidence for close linkage between the β- and δ-structural loci. This evidence has been obtained from the study of children born of matings between normal individuals and those heterozygous for both a β-chain and a δ-chain hemoglobin variant. So far, 50 children of such matings have been studied (Ceppellini, 1959; Boyer, 1963; Pearson and Moor, 1965; Thompson, Odom and Bell, 1965) and only in one case was there a possible instance of crossing over (Thompson, Odom and Bell, 1965). Furthermore, the chemical studies of hemoglobin Lepore mentioned in a later section provide further evidence for the proximity of the β- and δ-loci.

The relationship of the β-thalassemia loci to the β- and δ-loci can be studied by examining offspring born of matings between normal persons and those heterozygous for both β-thalassemia and either a β- or a δ-chain hemoglobin variant. Such matings have recently been summarized (Weatherall, 1965). There have been 71 reported instances of children born of matings between individuals heterozygous for both sickle-cell and thalassemia genes and normal persons. In 2 cases the thalassemia was of the noninteracting type, while in 2 further families no electrophoretic studies were reported. Excluding these families we are left with one very doubtful crossover. The findings in 46 individuals born of persons with hemoglobin C-thalassemia have also been recently summarized (Weatherall, 1965) and there are at least 3 possible instances of crossing over between the β-thalassemia and β-structural loci. Unfortunately, categorical blood group data were not provided and so these reports must be regarded with caution. The β-thalassemia and β-structural variant genes have only been seen together in the repulsion phase to date. It is likely that if they occurred together on the same chromosome the abnormal β-chains would either not be produced or would be produced in very small quantities. In the former case the picture would be that of thalassemia trait while in the latter case the picture would resemble "non-interacting" sickle-cell thalassemia.

To date 4 families have been reported in which individuals heterozygous for both hemoglobin A_2^1 (B_2) and β-thalassemia have had one or more children from a mating with a normal individual (Huisman, Punt and Schaad, 1961; Weatherall, 1964a; Pearson and Moor, 1965; Thompson, Odom and Bell, 1965). In one family where the genes were clearly in the coupling phase there were 13 children, either normal or doubly

affected (Huisman, Punt and Schaad, 1961). In a second family where the genes were in coupling, a crossover could not be ruled out (Thompson, Odom and Bell, 1965). Two further families have been described in which the genes were clearly shown to be in the repulsion phase. In one family 11 children were studied with one possible example of crossing over (Pearson and Moor, 1965), while in the other family 7 children were studied, again a possible crossover being found (Weatherall, 1964a). Thus in 31 possible chances for crossing over between β-thalassemia and δ-structural loci there have been two very likely and one possible example of crossing over. Adequate blood group data are available with each of these studies.

It is difficult to interpret this type of linkage data in man, particularly since adequate blood group data and information on other genetic markers have not always been given. It appears, however, that the β-structural and δ-structural loci are closely linked and that the β-structural and β-thalassemia loci are alleles or very closely linked. To date it appears that the β-thalassemia and δ-structural loci are not so close together as the β-structural and δ-structural loci. Since the structural studies of hemoglobin Lepore suggest that the determinant for the C-terminal end of the δ-chain is adjacent to that for the N-terminal end of the β-chain, it is possible that the determinant for at least one type of high A_2-β-thalassemia lies at or beyond that for the C-terminal end of the β-chain. These findings may well be a further reflection of heterogeneity within the β-thalassemias.

α-Thalassemias

It has been more difficult to obtain firm genetic data about the α-thalassemias because of the difficulty in recognizing the heterozygous state in which, by definition, there is no elevation of hemoglobins F or A_2. Furthermore, there have been few opportunities to study individuals affected with the genes for both α-thalassemia and an α-chain hemoglobin variant and there is almost no reported information about their children.

Homozygous States

There is increasing evidence that the homozygous state for α-thalassemia is not compatible with fetal survival. There are now several reports of stillborn infants with the clinical picture of severe hydrops fetalis in the absence of ABO or Rh blood group incompatibility, in whose blood large quantities of hemoglobin Bart's was found (Lie Injo Luan Eng, 1962; Lie Injo Luan Eng et al., 1962). The electrophoretic findings in

these cases were characterized by a large proportion of hemoglobin Bart's, small quantities of hemoglobin H and variable amounts of hemoglobin A. Hemoglobin F is not usually found. These findings are compatible with a gross defect in α-chain synthesis, the large excess of γ-chains aggregating to form hemoglobin Bart's (γ_4), the smaller excess of β-chains forming hemoglobin H (β_4), and the few available α-chains binding β- in preference to γ-chains so that some hemoglobin A ($\alpha_2\beta_2$) but no hemoglobin F ($\alpha_2\gamma_2$) is found. While most reported instances of this condition have been in Orientals, the condition has recently been recognized in the Greek Cypriot population in Great Britain (Diamond, Cotgrove and Parker, 1965).

To substantiate the hypothesis that these infants were homozygous for α-chain thalassemia it should have been possible to demonstrate the heterozygous state in both parents. In each case the parents have had very mild morphological changes of their red cells with normal levels of hemoglobins A_2 and F (Lie Injo Luan Eng, 1962; Lie Injo Luan Eng et al., 1962). It has been assumed that these findings reflect a mild defect in α-chain synthesis although at the moment there is no way of confirming this supposition.

Heterozygous State

Studies in Greek (Malmos, Fessas and Stamatoyannopoulos, 1962) and American Negro (Weatherall 1963, 1964b) subjects have suggested that it is difficult to recognize the heterozygous state for α-thalassemia in adult life. The condition can only be recognized with certainty in the neonatal period when it is associated with the presence, in umbilical cord blood, of increased quantities of hemoglobin Bart's.

Electrophoretic and chromatographic studies of normal umbilical cord blood lysates reveal traces of Bart's hemoglobin (γ_4) in all normal infants (Weatherall, 1963; Huehns and Shooter, 1965). It is difficult to quantitate these trace amounts of rapidly migrating hemoglobin accurately, but where this was attempted not more than 1.5 per cent hemoglobin Bart's was found (Weatherall, 1963). In some populations a proportion of infants have been noted to carry between 5 and 15 per cent hemoglobin Bart's (Weatherall, 1965). See Table 3. In many cases traces of hemoglobin H (β_4) are present together with the hemoglobin Bart's, although it is never found in normal umbilical cord blood. Affected infants have morphological changes and diminished osmotic fragility of their red cells compatible with a mild thalassemia-like disorder. These changes persist as the infants grow older, although the hemoglobin Bart's disappears in the majority of cases between the third and sixth months of

TABLE 3.—*Incidence of Increased Levels of Hemoglobin Bart's in Umbilical Cord Blood Samples—The Technics Used in These Studies Would Have Detected Levels of 5 Per Cent or More of Hemoglobin Bart's (Weatherall, 1965; Lehmann and Huntsman, 1966)*

Racial Group	Number of Cord Bloods Studied	Incidence %
Thais	415	5.2
Chinese	1,112	4.0
Malays	54	1.5
Indonesians	480	0.5
African Negroes	110	8.2
American Negroes	900	2.0
Greeks	500	0.3
Italians	1,200	0.1
British	500	0.0

life (Weatherall, 1963). One parent and variable numbers of siblings and other family members show similar morphological changes of their red cells (Minnich et al., 1962; Weatherall, 1963). In both affected infants as they grow older and affected family members the hemoglobin A_2 and F levels are normal. In a few cases traces of hemoglobin H or Bart's can be found using a sensitive starch gel electrophoretic technic at pH 7 or an occasional cell containing inclusion bodies can be demonstrated after incubation with brilliant cresyl blue (Malmos, Fessas and Stamatoyannopoulos, 1962; Weatherall, 1963).

These observations can be interpreted in the light of the changes which occur during the neonatal switch from fetal to adult hemoglobin synthesis (Weatherall, 1963). At this time both γ- and β-chains are competing for available α-chains, and since α-chains appear to associate with β-chains in preference to γ-chains, it is not surprising that a slight excess of γ-chains occur in the red cells of normal infants. These chains will aggregate to form the trace amounts of hemoglobin Bart's found in normal cord blood samples. In the presence of the γ-thalassemia gene this situation is simply exaggerated, the greater excess of α-chains forming relatively large quantities of hemoglobin Bart's (γ_4) and a smaller excess of β-chains forming hemoglobin H (β_4). As the infants grow older the rate of hemoglobin synthesis diminishes and γ-chain synthesis ceases. There are probably sufficient α-chains to bind most of the β-chains produced in adult life and so little or no hemoglobin H is synthesized after the neonatal period. This interpretation of the findings in these infants and their families explains why the neonatal period is the

best time to diagnose heterozygous α-thalassemia and the curious fact that infants with a marked increase of hemoglobin Bart's do not go on to have similar levels of hemoglobin H in adult life.

It has recently been suggested that another form of α-thalassemia which is associated with hemoglobin Bart's levels in the 1-2 per cent range and no hematological abnormalities can be recognized in the neonatal period (Huehns, 1965). This hypothesis is based on the observation that most infants in Great Britain carry less than 1 per cent hemoglobin Bart's in the neonatal period, while in other racial groups up to 12 per cent of the infants carry between 1 and 2 per cent of this hemoglobin. This interesting observation requires further study with particular reference to the familial occurrence of these low levels of Bart's hemoglobin.

Hemoglobin H Disease (*α-Thalassemia Intermedia*) (Huehns, 1965)

A thalassemia-like disorder of moderate severity associated with a rapidly migrating hemoglobin designated 'H' (Fig. 10) was independently described in Chinese (Rigas, Koler and Osgood, 1955) and Greek (Gouttas et al., 1955) families. Hemoglobin H consists of 4 β-chains (β_4) (Jones et al., 1959) and is unstable, tending to form red cell inclusion bodies which are removed in the spleen. The clinical manifestations of

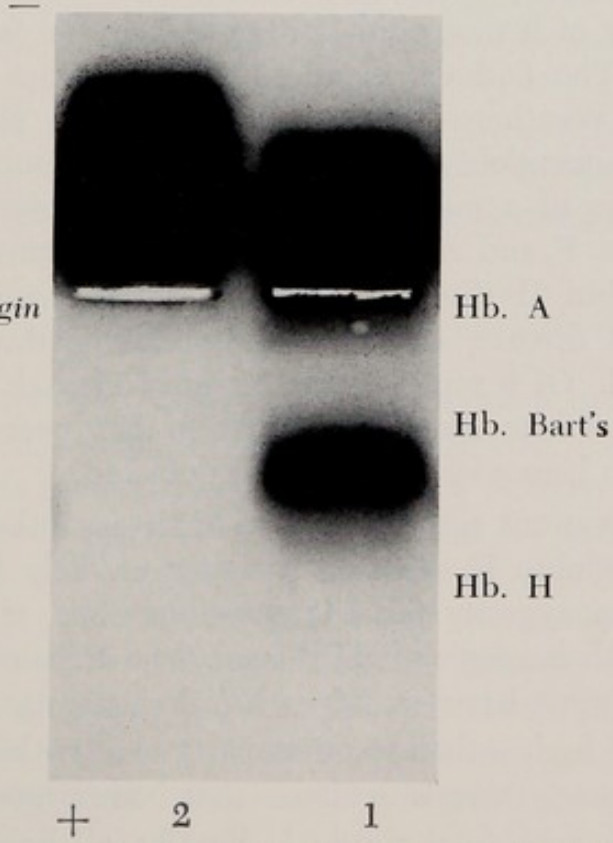

FIG. 10—The hemoglobin pattern in hemoglobin H disease. Starch gel electrophoresis, pH 7.0. (Phosphate buffer system.) 1.) Hb. H disease. 2.) Normal adult.

this disorder are, therefore, the result of defective hemoglobin synthesis and a shortened red cell survival due to splenic sequestration of older red cells in which hemoglobin H tends to precipitate (Rigas and Koler, 1961).

There seems little doubt that hemoglobin H disease results from an underlying defect in the rate of α-chain synthesis. In many cases a second hemoglobin component is present which is chemically identical to hemoglobin Bart's (Ramot et al., 1959; Sturgeon et al., 1961). In addition, another component consisting entirely of the δ-chains of hemoglobin A_2 has been isolated from hemolysates of some patients with hemoglobin H disease (Dance, Huehns and Beaven, 1963). In the presence of an overall deficit in α-chains there will be an excess of β- and δ-chains which aggregate to form hemoglobin H (β_4) and hemoglobin-$\delta^A{}_2$ (it is not yet known for certain whether this component exists as $\delta_4{}^A{}_2$). If, as in other forms of thalassemia, γ-chain synthesis persists, there will be a tendency to form γ_4 molecules since the relatively few available α-chains will preferentially bind β-chains to form hemoglobin A. In some cases where γ-chain synthesis persists to a marked degree, therefore, the hemoglobin pattern may be characterized by the presence of large amounts of hemoglobin Bart's and traces of hemoglobin H (Ramot et al., 1959).

The genetics of hemoglobin H disease have not yet been satisfactorily worked out. The findings in all reported families have recently been summarized (Weatherall, 1965). It is usual to find one parent of a patient with hemoglobin H disease completely normal while the other shows evidence of a mild thalassemia-like disorder with normal levels of hemoglobins F and A_2. Children born of matings between a parent with hemoglobin H disease and a normal individual have either had hemoglobin H disease, a mild thalassemia-like disorder or been completely normal. Of 6 reported infants born of such a mating, 4 had increased levels of hemoglobin Bart's, while there were 2 well-documented cases where no hemoglobin Bart's was detected.

These observations are compatible with several explanations for the basis of hemoglobin H disease. It seems unlikely that the disorder results from homozygosity for α-thalassemia since, if this were the case, individuals with hemoglobin H disease who transmitted the disorder to their children must have mated with α-thalassemia carriers. This would require a very high α-thalassemia carrier rate within any given population. Furthermore, there is evidence that homozygosity for α-thalassemia is incompatible with fetal survival. Finally, all babies born of a parent with hemoglobin H disease should carry relatively large amounts of

hemoglobin Bart's, but this has not always occurred. Another possibility is that hemoglobin H disease results from the interaction of an α-thalassemia gene with a second gene which segregates independently. If this were so, a quarter of the offspring of patients with hemoglobin H disease should have the disorder. To date 7 out of 29 reported offspring of a parent with hemoglobin H disease have the same disorder. The nature of this second hypothetical gene is quite obscure.

A more recent explanation of the reported family findings in hemoglobin H disease (Huehns, 1965) suggests that the disorder results from the interaction of an α-thalassemia gene with a very mild α-thalassemia gene which, alone, is completely silent. This second gene could be very common in populations where hemoglobin H disease occurs frequently, but rare in other populations, such as the Negro, where α-thalassemia occurs but in which hemoglobin H disease is not found (Weatherall, 1963). Extending the concept of β-chain synthesis being under the control of several iso-alleles (Itano, 1957) the same situation might occur for α-chain synthesis and this mild form of "thalassemia" simply represent a relatively inefficient α-chain producing allele. Since there is no α-chain hemoglobin variant which occurs with sufficient frequency to study ratios of normal to variant hemoglobin, as can be done for the sickle-cell trait (Itano, 1957), the only approach to this problem would be the very careful estimation of hemoglobin Bart's levels in subjects from a variety of racial groups. It is interesting that family studies of children with hemoglobin E and evidence of associated α-thalassemia also suggest that more than one α-thalassemia allele may exist. Furthermore, these studies were made in Thailand where hemoglobin H disease is relatively frequent (Tuchinda et al., 1964).*

α-Thalassemia in Association with β-Chain Hemoglobin Variants

A Negro family has been reported in which there was an individual with a form of sickle-cell thalassemia in whom the level of hemoglobin S was considerably less than that of hemoglobin A (Cohen et al., 1959). The associated thalassemia gene was characterized by normal levels of hemoglobins F and A_2 and segregated independently from the sickle-cell gene. The doubly affected persons had more marked morphological changes of their red cells and lower levels of hemoglobin S than is usually seen in the sickle-cell trait. More direct evidence that such "non-interacting" thalassemia results from defective α-chain synthesis has followed studies on Negro infants who had increased levels of hemoglobin

*Studies of the level of hemoglobin Bart's in their cord bloods are confirming this idea [Na-Nakorn, personal communication, 1967].

Bart's in the neonatal period and who were also heterozygous for the hemoglobin S or C genes (Weatherall, 1963). In each case the hematological changes as the babies grew older were greater than those usually seen in hemoglobin S or C carriers and the level of hemoglobins S and C were unusually low for heterozygotes.

The presence of the α-thalassemia gene thus appears to reduce the level of the β-chain hemoglobin variant as compared with hemoglobin A in heterozygotes. This observation has received further confirmation recently from a study in Thailand in which the presence of one or more α-thalassemia genes was responsible for a reduction in the level of hemoglobin E below that usually found in heterozygotes for this β-hemoglobin variant (Tuchinda et al., 1964). It is possible that, in the presence of a relative reduction in the number of available α-chains, normal β-chains are bound in preference to abnormal β-chains. A finding in support of this hypothesis was the finding of components with the structure β_4^S and β_4^C in the cord bloods of infants with hemoglobin Bart's and S or C (Huisman, 1960). This observation suggests that, in the neonatal period when there are only small amounts of hemoglobin S or C, a limited number of available α-chains preferentially bind normal γ-chains and β-chains, leaving an excess of β^S and β^C chains sufficient to form detectable amounts of β_4^S and β_4^C molecules. The existence of these so-called "Augusta" hemoglobins has not yet been confirmed (Weatherall, 1963).

α-Thalassemia Associated with α-Chain Hemoglobin Variants

There have been few chances to study individuals heterozygous for both α-thalassemia and α-chain hemoglobin variants. There are 4 reported cases of hemoglobin Q-α-thalassemia (Vella et al., 1958; Dormondy, Lock and Lehmann, 1961; ILie Injo Luan Eng and de V. Hart, 1963) and one case of hemoglobin I-α-thalassemia (Atwater et al., 1960).

In all the reported cases of hemoglobin Q-α-thalassemia there was complete absence of hemoglobin A synthesis, the hemoglobin pattern consisting of hemoglobins Q ($\alpha_2^Q\beta_2$) and Q_2 ($\alpha_2^Q\delta_2$), with variable amounts of hemoglobins H or Bart's. Thus the presence of the α-thalassemia gene resulted in the complete absence of normal α-chain synthesis, no hemoglobin A ($\alpha_2\beta_2$) or A_2 ($\alpha_2\delta_2$) being detectable. In each case one parent had a very mild thalassemia-like state with normal levels of hemoglobin A_2 and the other carried hemoglobin Q (A-Q). It is rather surprising that these thalassemia carriers had minimal hematological abnormalities in the presence of only one active α-chain locus. All these persons were of Oriental background.

One Negro woman has been found with hemoglobin I-α-thalassemia. The hemoglobin pattern consisted of about 70 per cent hemoglobin I ($\alpha_2^I\beta_2$), the rest being hemoglobin A. Of the 3 children studied, 2 had hypochromic red cells despite iron therapy, while a third carried about 20 per cent hemoglobin Bart's in the neonatal period. These findings are, to date, the only information which is available regarding the relationship of the α-thalassemia to α-chain structural loci.

It appears that, as in β-thalassemia, both hemoglobin A (α-chain) producing and nonhemoglobin A (α-chain) producing types of α-thalassemia occur. It will be most interesting to see if further cases of α-thalassemia in the Negro are of a type in which some α-chain is synthesized.

α-Thalassemia in Association with β-Thalassemia

This combination has been reported in 3 Greek individuals (Fessas and Papaspyrou, 1957; Fessas, 1961). The clinical picture was that of a mild thalassemia-like state with increased levels of hemoglobin A_2. In addition, traces of hemoglobin Bart's were present. The combination of the 2 thalassemia genes appears to result in no greater disability than that observed in β-thalassemia carriers.

The association of α-thalassemia with β-δ-thalassemia was described in an earlier section.

Thalassemia Associated with a Structural Abnormality of Hemoglobin

Hemoglobin Lepore Syndrome. In 1958 Gerald and Diamond reported the finding of about 10 per cent of a slowly migrating hemoglobin variant in the blood of one parent and several siblings of an Italian child with severe thalassemia. They named this hemoglobin "Lepore" after this patient's family name. Abnormal hemoglobins with similar characteristics to that of hemoglobin Lepore have been found in Italians (Gerald and Diamond, 1958; Pearson, Gerald and Diamond, 1959; Wolf and Ignatov, 1963), Greeks (Fessas, Stamatoyannopoulos and Karaklis, 1962), Papuans (Neeb et al., 1961), Turkish-Cypriots (Beaven et al., 1964), and Negroes (Ranney and Jacobs, 1964) and been designated hemoglobins $\text{Lepore}_{\text{(Boston or Washington)}}$, Pylos, $\text{Lepore}_{\text{(Hollandia)}}$, $\text{Lepore}_{\text{(Cyprus)}}$ and $\text{Lepore}_{\text{(The Bronx)}}$, respectively.

The carrier state for hemoglobin Lepore is clinically and hematologically indistinguishable from that for β-thalassemia. The hemoglobin pattern consists of about 10 per cent Lepore hemoglobin, a moderate increase in hemoglobin F and normal or subnormal amounts of hemoglobin A_2, the remainder being hemoglobin A. Individuals heterozygous

for both hemoglobin Lepore and high A_2 β-thalassemia have the clinical manifestations of a severe thalassemia associated with 40-70 per cent hemoglobin F, about 5 per cent hemoglobin Lepore and normal levels of hemoglobin A_2. Those heterozygous for hemoglobin Lepore and the sickle-cell genes carry about 60-70 per cent hemoglobin S, 10 per cent hemoglobin Lepore and normal levels of hemoglobin A_2, the remainder being hemoglobin F. The clinical picture is that of sickle-cell anemia or sickle-cell thalassemia. Hemoglobins Lepore and S have similar electrophoretic properties at pH 8.6, but may be separated by Agar gel electrophoresis at an acid pH or by column chromatography (Stamatoyannopoulos and Fessas, 1962).

The homozygous state for hemoglobin Lepore has been observed once in Greece (Pylos) (Fessas, Stamatoyannopoulos and Karaklis, 1962) and in 2 Papuan sisters in New Guinea (Hollandia) (Neeb et al., 1961). The clinical picture was that of severe thalassemia while the hemoglobin pattern consisted of hemoglobins F and Lepore only. In the Greek case the complete absence of hemoglobin A_2 was confirmed by several technics, while in the Papuan cases traces of A_2 hemoglobin were demonstrated on column chromatography only. No starch gel electrophoretic studies were reported in the latter case, and further studies of this hemoglobin have suggested that it is not, in fact, normal hemoglobin A_2 (Jonxis, 1965).

It seems, therefore, that the presence of the hemoglobin Lepore gene is associated with the complete absence of β- and δ-chain synthesis as judged by the absence of hemoglobins A and A_2 in homozygotes and the absence of hemoglobin A in those heterozygous for hemoglobins Lepore and S. These observations have been clarified by recent chemical studies on hemoglobins $\text{Lepore}_{(\text{Washington})}$ (Baglioni, 1962, 1963a) and $\text{Lepore}_{(\text{Hollandia})}$ (Barnabas and Müller, 1962). These studies have shown that the non-α-chain hemoglobin $\text{Lepore}_{(\text{Washington})}$ has peptides corresponding to the δ-chain at the N-terminal end and to the β-chain at the C-terminal end. The α-chains are identical to those of hemoglobin A. Further chemical studies have localized the area of fusion of δ- and β-chains to between residues 85 and 115 of the non-α-chain of hemoglobin $\text{Lepore}_{(\text{Washington})}$, while studies of hemoglobin $\text{Lepore}_{(\text{Hollandia})}$ suggest that a similar type of fusion of portions of δ- and β-chains has occurred, in this case the area of fusion being nearer to the N-terminal end of the δ-chain portion.

It has been suggested that these "fusion" genes result from a process of misalignment associated with nonhomologous crossing over at the closely linked δ- and β-loci (Baglioni, 1962). The difference in structure

between hemoglobin $Lepore_{(Washington)}$ and $Lepore_{(Hollandia)}$ depends, therefore, in the site of the unequal crossing over. Frequent crossing over does occur in areas of chromosome duplication as at the *Bar* locus in *Drosophila melanogaster*. It has been suggested that the δ-chain gene has arisen as a duplication of the β-chain gene (Ingram, 1961) and thus the similarity between the β- and δ-loci may increase the chances of nonhomologous crossing over. A similar situation has been observed in the human haptoglobin loci, unequal crossing over probably being responsible for the unequal length of the α-chains of human haptoglobin (Smithies, Connell and Dixon, 1962).

One of the most interesting questions which arises from this work is that of the reasons for the low rate of synthesis of the composite δ-β-chain. If it is assumed that the final level of a hemoglobin reflects, at least in part, its rate of synthesis, then the levels of Lepore hemoglobin found in heterozygotes suggests that the δ-β-chain is synthesized at a rate intermediate between that of normal δ- and normal β-chains. This in turn suggests that, since the non-α-chain of hemoglobin $Lepore_{(Hollandia)}$ contains the β T5 tryptic peptide, the determinants for the rate of synthesis of the δ-chain lie within the region of the first 5 tryptic peptides.

Conditions Closely Related to the Thalassemias

Hereditary Persistence of Fetal Hemoglobin

A condition associated with the persistence of hemoglobin F synthesis into adult life in the absence of any hematological abnormalities was first described by Edington and Lehmann in 1955. The condition has been recognized in several parts of Africa and studied intensively in the American Negro (Conley et al., 1963). A similar abnormality has been observed in Italians (Manganelli, Dalfino and Tannoia, 1962), Portuguese-Indians (Barkhan and Adinolfi, 1962), and Creeks (Fessas and Stamatoyannopoulos, 1964). The condition as found in Greece shows many differences from that found in the Negro population and is probably a separate entity. In addition there is a disorder which has been studied in detail in Switzerland (Marti, 1963) which is characterized by the persistence of low levels of hemoglobin F into adult life. This condition is also quite different from hereditary persistence of hemoglobin F as seen in Africa and Greece, and is almost certainly due to a different genetic abnormality. The hereditary persistence of fetal hemoglobin abnormality can, therefore, be best discussed in terms of "Negro," "Greek" and "Swiss" types, the main differences being summarized in Table 2.

Negro Type of Hereditary Persistence of Hemoglobin F
(Conley et al., 1963)

This abnormality is found in approximately one per thousand Negroes. The heterozygous state is characterized by the presence of about 20 to 30 per cent fetal hemoglobin in adult life in the absence of any hematological abnormalities. The fetal hemoglobin is distributed evenly throughout the red cells. The level of hemoglobin A_2 is significantly lower than that in normal subjects.

The homozygous state has been found on one occasion to date, follow-up studies on this child up to the age of 5 years having been reported (Weatherall, 1965). The hemoglobin consisted entirely of hemoglobin F, no hemoglobin A or A_2 being demonstrable by a variety of technics. Extensive chemical studies have revealed no differences between the hemoglobin F of this child and that of normal umbilical cord blood (Conley et al., 1963). At the age of 5 years these findings were unchanged, the child being healthy and active with no abnormal physical signs. Peripheral blood examinations revealed no anemia, but there were mild morphological changes of the red cells including some target forms. Electrophoretic studies of this child's red cell lysates have revealed, in addition to the absence of hemoglobin A and A_2, normal adult non-heme protein patterns. In particular carbonic anhydrases B and C, which are absent in the neonatal period and which assume their adult levels at the age of 2-3 years (Weatherall and McIntyre, 1966) were present at normal adult levels. The child's father was heterozygous for hereditary persistence of hemoglobin F, while his mother carried both the same gene and that for β-thalassemia. One brother carried the β-thalassemia trait, while a baby sister showed no hemoglobin A in an umbilical cord blood sample. Follow-up studies on this child are not yet available.

The heterozygous state for hereditary persistence of hemoglobin F has been found in association with those for hemoglobin S (S-F), hemoglobin C (C-F), β-thalassemia (β-thal-F), α-thalassemia (α-thal-F) and hemoglobin $A_2{}^1$ (B_2). The S-F condition has been recognized in 23 individuals to date (Conley et al., 1963). The hemoglobin pattern consists of about 30 per cent hemoglobin F, the remainder being hemoglobin S and A_2. Careful electrophoretic and chromatographic studies have failed to reveal any hemoglobin A in this condition. Despite the high level of hemoglobin S affected individuals do not have the severe hemolytic anemia with recurrent crises which occur in sickle-cell anemia with similar levels of hemoglobins S and F. The reasons for this observation follow the difference in intracellular distribution of hemoglobin F

in the two conditions, being homogeneous in the S-F condition and quite heterogeneous in sickle-cell anemia (Thompson, Mitchener and Huisman, 1961; Shepherd, Weatherall and Conley, 1962). The high level of fetal hemoglobin in *each* cell apparently protects against sickling, this hypothesis being confirmed by extensive viscosity studies on S-F and sickle-cell anemia blood samples (Charache and Conley, 1964). The C-F condition is associated with hemoglobin F levels in the 35 per cent range, again hemoglobin A being absent (Conley et al., 1963). There are no clinical abnormalities and hematological studies have shown no anemia, mild morphological abnormalities of the red cells with many target forms being the only abnormal findings. In the Thal-F condition the hemoglobin pattern consists of 60-70 per cent hemoglobin F and 2.5–3.5 per cent hemoglobin A_2, the remainder being hemoglobin A. In the one reported instance of heterozygosity for hereditary persistence of hemoglobin F and hemoglobin A_2^1 (B_2) there was complete absence of hemoglobin A_2, only hemoglobin A_2^1 being present (Conley et al., 1963). This suggests that no δ-chain synthesis was directed by the chromosome containing the persistent fetal hemoglobin gene. Similarly in an individual heterozygous for hereditary persistence of hemoglobin F and δ-thalassemia (Thompson et al., 1965) there was complete absence of hemoglobin A_2 synthesis.

To date there have been 49 chances of studying children born of matings between normal or unstudied persons and those heterozygous for hereditary persistence of hemoglobin F and either hemoglobin S or C (Weatherall, 1965). In each case the child has been heterozygous for either the β-chain hemoglobin variant or the persistent fetal hemoglobin gene, no normal or doubly affected persons having been observed. These observations suggest allelism or close linkage between the β-chain locus and that for the persistent fetal hemoglobin gene.

The Negro form of the persistent fetal hemoglobin gene is characterized, therefore, by the complete absence of β- and δ-chain synthesis, on the chromosome which carries the abnormal gene. This gene behaves as though allelic or closely linked to the β-chain locus. The absence of β- and δ-chains is completely compensated for by the persistent γ-chain synthesis which occurs in every red cell.

The Greek Form of Hereditary Persistence of Fetal Hemoglobin
(Fessas and Stamatoyannopoulos, 1964)

To date it has not been possible to define this abnormality as completely as the Negro form because of the lack of opportunity for studying its interaction with genes for β- and δ-chain hemoglobin variants which

are rare in Greece. Furthermore, no individuals homozygous for this disorder have yet been found.

The heterozygous state is characterized by levels of hemoglobin F in the 15 per cent range, while hemoglobin A_2 levels are not significantly reduced. The fetal hemoglobin is homogeneously distributed throughout the red cells which are morphologically normal. These findings are quite different from those of β-δ-thalassemia in which similar hemoglobin F and A_2 levels occur.

Individuals heterozygous for both β-thalassemia and the Greek persistence of fetal hemoglobin gene carry about 30 per cent hemoglobin F and slightly elevated levels of hemoglobin A_2. The degree of anemia and the morphological changes of the red cells are in excess of those found in the same condition in Negroes.

In the absence of examples of interaction with β-chain and δ-chain hemoglobin variants, it is not yet possible to say whether β- and δ-chain synthesis is completely absent in the Greek form of this disorder. The information summarized above is more compatible with a *partial* suppression of β- and possibly δ-chain synthesis. Thus, in heterozygotes with 10-15 per cent hemoglobin F, complete absence of one active β-chain locus would mean that, to maintain a normal mean corpuscular hemoglobin level, the active locus would have to direct the synthesis of 85-90 per cent of the β-chains normally directed by 2 loci. Furthermore, it seems unlikely that the relatively high levels of hemoglobin A found in those heterozygous for β-thalassemia and the Greek persistent fetal hemoglobin gene results from activity at a *single* β-locus which is under the influence of the β-thalassemia gene.

The Swiss Type of Hereditary Persistence of Fetal Hemoglobin (Marti, 1963)

This condition is characterized by the inherited persistence of low levels (1-3 per cent) of hemoglobin F into adult life in the absence of any hematological abnormality, and has been found in more than one member of a single family. In addition to the Swiss studies, the condition has also been recognized in Great Britain (Woodrow, personal communication), Thailand (Flatz, Pik and Sringam, 1965) and in American Negroes (Weatherall, 1965). In Great Britain and Switzerland population surveys suggest that this condition occurs in about 1 per cent of the population. The significance is completely unknown.

Population Genetics of Thalassemia

The thalassemias have now been described in most racial groups and while it is clear that there is a particularly high incidence in the Medi-

terranean region, Middle-East and Orient, technical difficulties have discouraged many really precise incidence surveys.

Surveys in Italy using an osmotic fragility screening procedure (Silvestroni and Bianco, 1959) have given incidence levels as high as 7-15 per cent in the Po Delta region and Sicily. Although this method includes a few individuals with iron deficiency and does not distinguish between different types of thalassemia, it seems likely that a large proportion of the samples associated with reduced osmotic fragility represented different forms of β-thalassemia. In a survey of 1,600 Greek males utilizing starch gel and block electrophoresis in association with full hematological studies, 85 were found to have increased levels of hemoglobin A_2 with associated hematological evidence of thalassemia in all but one case (Malmos, Fessas and Stamatoyannopoulos, 1962). There is probably a high incidence of β-thalassemia in India (Chatterjea, 1959), Sudan (Vella, 1964), Turkey (Aksoy, 1959), and parts of Israel (Ramot et al., 1964). Recent surveys in Thailand (Flatz, Pik and Sringam, 1965) have demonstrated an incidence of 4.8 per cent for high A_2 β-thalassemia and the incidence rate in Malaya and Indonesia is probably also high, although these areas need further surveys. The high A_2 β-thalassemia gene was thought to be very rare in Africa, but the incidence in American Negroes has been estimated as 0.8 per cent (Goldstein, Patpongpanij and Minnich, 1964). Further surveys using quantitative hemoglobin A_2 estimations are required in various parts of Africa. While these populations have a high frequency of β-thalassemia, sporadic reports of high A_2 β-thalassemia have appeared from practically every racial group (Weatherall, 1965).

Far less information regarding the incidence of β-δ-thalassemia is available. This condition is readily recognized on starch gel electrophoresis and, in a survey of 1,500 adult Negroes in the United States, 2 instances of this condition were found (Weatherall, 1965). The condition was found in 11 of 1,600 Greek males (Malmos, Fessas and Stamatoyannopoulos, 1962) and once in a survey of 2,790 individuals in Thailand (Flatz, Pik and Sringam, 1965). It also occurs in Italy although the incidence is unknown.

The incidence of α-thalassemia is even more difficult to assess because of the apparent difficulty in recognizing the carrier state in adult life (Weatherall, 1964b). In a survey in Greece (Malmos, Fessas and Stamatoyannopoulos, 1962) utilizing hematological studies and a search for red cell inclusion bodies and starch gel electrophoresis as an indication of the presence of hemoglobin H, an incidence of 0.6 per cent for α-thalassemia carriers was found. If it is assumed that the presence of

high levels of hemoglobin Bart's in the neonatal period results from the presence of an α-thalassemia gene, the figures in Table 3 provide an estimate of the incidence of α-thalassemia in several different populations.

Since the homozygous states for the thalassemias are not usually compatible with survival into reproductive age, the very high gene frequencies outlined above can only be maintained if the heterozygotes have a marked advantage in any given population. Presumably the thalassemias have arisen in different areas by mutation and reached a high frequency where such heterozygote advantages exist. The selective factors in this state of balanced polymorphism have not yet been fully worked out.

It has been suggested that thalassemia carriers may be more resistant to malaria than normal persons (Haldane, 1949). Certainly the high frequency areas are those where malaria was very common and, in Sardinia, a much higher incidence of thalassemia was noted in the low-lying, marshy areas where malaria was common, than in the mountainous regions (Carcassi, Ceppellini and Pitzus, 1957). Similar findings have been reported from New Guinea and New Borneo (Motulsky, 1964). The relationship of the frequency of the sickle-cell trait and glucose-6-phosphate dehydrogenase deficiency to malaria is now well established, but studies in Greece have revealed a nonparallel distribution between enzyme deficiency and thalassemia in the Arta region (Choremis et al., 1963). Rather similar results have been obtained from surveys in Thailand.

It has also been suggested that increased iron absorption in thalassemia might be an advantage in periods of iron depletion such as pregnancy. However, it has been shown that thalassemia heterozygotes do not, in fact, have an increased absorption of iron, or, if iron absorption is increased total body iron levels are not elevated above normal (Bannerman, 1964). Whether the higher mean fetal hemoglobin levels found in thalassemics between the ages of 6 months and 1 year have any protective value has not yet been worked out (Lehmann, 1959).

Clearly the factors which maintain the high thalassemia gene frequencies are not fully understood. One complicating factor is the tendency for the β-thalassemia gene frequency to be depressed in areas where there is a high frequency of β-chain hemoglobin variants, because of the severe clinical sequelae of the interaction of the 2 genes. This mechanism would be more important in Thailand with a high incidence of hemoglobin E, than in Greece. It appears that malaria is a more important selective agent for maintaining the high frequency of sickle-cell

and G-6-PD deficiency genes than thalassemia. Whether the proposed tendency for frequent gene reduplication and nonhomologous crossing on the β-δ-gene complex (Nance, 1963) is an important factor in maintaining the high frequency of thalassemia is uncertain, but even if this were so, strong selective environmental forces would still be required.

Nature of the Genetic Defect in Thalassemia

Numerous molecular models have been designed to explain the findings summarized in previous sections (Weatherall, 1965). It seems unlikely that any single hypothesis is sufficient to provide a basis for all these disorders. Furthermore, models based entirely on the levels of different hemoglobin fractions in red cell lysates and on estimations of mean corpuscular hemoglobin levels must be regarded with caution since there is increasing evidence that such material may give an erroneous picture of actual hemoglobin synthesis within the marrow.

The original hypothesis of Itano (1957) that defective globin synthesis might follow structural changes in the globin chains, unassociated with a change in the total charge of the molecule, has been partly explored. Hemoglobin A from 2 Negroes with interacting hemoglobin S or C thalassemia has been purified and the amino acid composition of the tryptic peptides determined. These compositions were identical to those of hemoglobin A (Guidotti, 1962). Similar studies have been performed on about two thirds of the tryptic peptides of an amino-ethylated β-chain obtained from an Italian child with homozygous high-hemoglobin A_2 β-thalassemia, again no abnormality being found (Weatherall and Naughton, 1966). The tryptic peptides have also been examined from hemoglobin A prepared from a person with hemoglobin H disease, and found to be normal (Jones and Schroeder, 1963). These important, if somewhat tedious, studies suggest that the "hemoglobin A" which is produced in α- and β-thalassemia is, at least in some cases, identical in *composition* to normal hemoglobin A, although do not rule out an alteration in amino acid *sequence*.

The structural studies on hemoglobin Lepore have already been summarized. It has been further suggested that all the thalassemias result from unequal crossing over (Nance, 1963). A whole series of theoretical fusion genes have been proposed which would interfere with hemoglobin synthesis by either causing defective transcription of messenger RNA, by competing at ribosomal level with normal messenger, or by "feedback" inhibition of protein synthesis by the production of an abnormal globin or peptide fragment. While it seems unlikely that all the conditions outlined in this review result from this type of mechanism, the growing

heterogeneity of the Lepore hemoglobins suggests that unequal crossing over may be fairly frequent at the δ-β-gene region. A whole series of δ-β-fusion products could result, some of which might have a similar charge to hemoglobins A or A_2. If the fusion gene contained the rate-limiting areas for δ-chain synthesis, the resulting "hemoglobin A" might have a markedly reduced rate of synthesis, yet appear to be identical to normal adult hemoglobin (Smithies, 1964). Very careful studies of hemoglobins A and A_2 from individuals with conditions such as β-δ-thalassemia and δ-thalassemia are clearly indicated.

In the absence of evidence for defective structure as a basis for many of the thalassemias an abnormality in the mechanisms controlling the rate of peptide chain synthesis must be evoked. To date there is limited experimental evidence in favor of this possibility. All this work has utilized the well-known observation that reticulocytes or nucleated red cells will continue to synthesize hemoglobin in the test-tube for several hours after removal of a blood or bone marrow sample. Thus by incubating red cells with a series of radioactive amino acids, hemoglobin synthesis can be followed. There are many difficulties involved in interpreting data obtained from *in vitro* biosynthetic studies and it is difficult to compare one experiment with another in terms of total amounts of isotope incorporated. Despite this restriction these studies represent the only real approach to the thalassemia problem which is currently available.

In vitro studies utilizing bone marrow have provided evidence that globin synthesis per red cell precursor is diminished in thalassemia (Necheles, Baldini and Damashek, 1964). Further, when ribosomes are prepared from peripheral blood of thalassemics the rate of incorporation of isotopes per weight of ribosome is markedly reduced, as is the incorporation into soluble hemoglobin (Burka and Marks, 1963). The incorporation of isoleucine into hemoglobin F is as efficient in thalassemic cells as in those from individuals with sickle-cell anemia and acquired hemolytic anemia (Burka and Marks, 1963). These studies suggest that there is a specific defect in hemoglobin A synthesis in the thalassemic cells.

More recently utilizing a sensitive chromatographic method for separating the globin chains, it has been possible to compare the incorporation of C^{14}-labeled amino acids into α- and β-chains in the cells of thalassemic and nonthalassemic persons (Heywood, Karon and Weissman, 1964; Weatherall, Clegg and Naughton, 1965). Furthermore, it has been possible to estimate the total radioactivity incorporated into each chain in the whole cell and also to examine the distribution of radioac-

tivity on newly made chains on ribosomes prepared from these cells (Weatherall, Clegg and Naughton, 1965). In high A_2 β-thalassemia homozygotes the total amount of radioactivity incorporated into α-chain was considerably more than that incorporated into β-chain. Furthermore, while in normal individuals the specific activity of the α- and β-chains was identical after 30 minute cell incubation, the specific activity of the α-chain exceeded that of the β-chain by ratios ranging from 1.5 to 3 to 1, (Fig. 11) in all β-thalassemia samples. In addition, unlike normal cells, it was not possible to label β-chains on the ribosomes in thalassemic cells. The lower specific activity of the β-chain suggests a relative "hold-up" in synthesis at ribosomal level, uniformly labeled α-chains combining with only partially labeled β-chains, the specific activity of the chains not equalizing until this block is overcome. These results suggest a "slow point" in ribosomal assembly of the chains. If, for example, the defect in thalassemia followed a simple reduction in the production of mRNA the rate of hemoglobin synthesis would be reduced but any β-chain synthesized would be uniformly labeled at the same rate as the α-chains. These studies, and those in which the capacity of thalassemic ribosomes to incorporate phenylalanine in response to poly U has been found to be unimpaired (Bank and Marks, 1966), all point to a qualitative abnormality in mRNA as a basis for at least some β-thalassemias.

The findings in hemoglobin H disease using this type of approach have been particularly interesting (Weatherall, Clegg and Naughton, 1965). It has been shown that the rate of β-chain synthesis is 2 to 3 times that of α-chain synthesis and that β-chains, as they are synthesized, enter a large pool which supplies β-chains for newly made α-chains as they become available. Presumably hemoglobin H exists in the form of this pool of β-chains. The reason for the reduced rate of α-chain synthesis is quite unknown.

The observations on hemoglobin biosynthesis in β-thalassemia point to a qualitative abnormality of messenger RNA as a basis for the disorder. The nature of this abnormality is completely obscure. It has been suggested recently that, since the genetic code is degenerate, the presence of a codon for an sRNA which was in short supply might markedly reduce the rate of peptide chain synthesis while leaving the structure unchanged (Itano, 1965; Weatherall, Clegg and Naughton, 1965). Although experiments to date have not shown a clear "slow spot" in synthesis along the chain (Weatherall, Clegg and Naughton, 1965), further work in this area is indicated. If indeed a "slow spot" can be found and if further chemical studies including sequencing reveal no structural abnormality in the affected region, such an abnormality of messenger RNA might

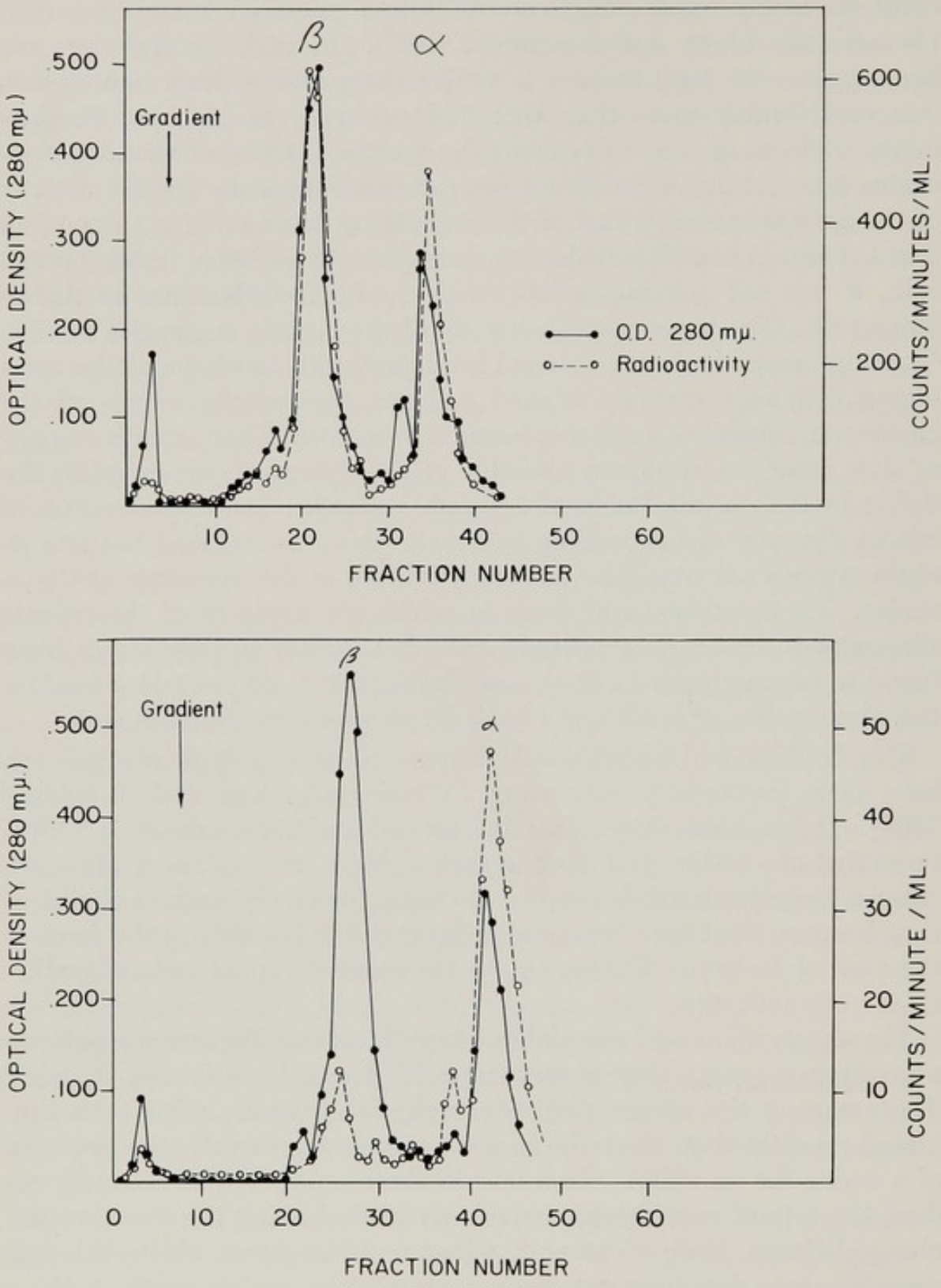

FIG. 11—Separation of α- and β-chains with incorporated radioactivity after incubation of reticulocytes with C^{14} leucine for 45 minutes from patients with (top:) β-thalassemia; and (*bottom*:) hereditary spherocytosis (for details see text).

well be the underlying abnormality. At least this possibility is in part open to experimental verification. Indeed as methods for isolation and purification of mRNA become available it may be possible definitely to confirm or refute this hypothesis.

Several models of hemoglobin synthesis have been proposed which utilize the "operon" system of Jacob and Monod. The closely linked β- and δ-loci are thought to be under the control of a closely linked operator gene. It is certainly possible that hereditary persistence of fetal hemoglobin, in which there is a failure of neonatal activation of β- and δ-chain synthesis, has a similar basis as the 0^- mutation which also acts only in the cis position. A further extension of this model to provide a basis for all the thalassemias has been worked out (Zuckerkandl, 1964) but, although this is an interesting approach, it is not at the moment open to any experimental verification.

It is clear from this short summary that little is really known about the etiology of most of the thalassemias. The most recent biosynthetic work has at least provided a guide as to where future efforts must be directed. Such work is clearly worthwhile, for the thalassemias offer a model system for the study of diseases due to inherited defects in the rate of synthesis of normal protein.

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Chapter 3

The Porphyrias: A Genetic Problem

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Nomenclature

As an introduction to a discussion regarding the heredity of the porphyrias some critical comments on the nosologic, diagnostic terminology seems to be appropriate. One of the authors (J. W.) has maintained that we must draw a very sharp limit between constitutional, inherited porphyria and purely symptomatic porphyrinuria (1937). In the latter group we find all the conditions, where porphyrins are secondarily increased in the body fluids as a result of another, primary, malady or an intoxication.

The most classical example is lead poisoning, where interesting disturbances of porphyrin metabolism have been known to exist for a long time. The coproporphyrin content in the urine of these patients was studied already at the end of the last century under the name of hematoporphyrinuria. Later it was found that the red corpuscles contain increased amounts of protoporphyrin in plumbism.

During the last decade another metabolite belonging to the chain of porphyrin synthesis has gained great practical and theoretical importance for the study of plumbism. This is δ-aminolevulic acid (ALA), a precursor of the porphyrin synthesis that is also present in the urine from carriers of the acute porphyria trait. This fact seems to show that a toxic substance (Pb) may cause great disturbance in the synthetic porphyrin chain (erythrocytic protoporphyrin, urinary ALA and coproporphyrin). It is important theoretically, however, that experimental lead poisoning in animals *always* gives such disturbances if it is severe enough. We do

not seem to have any signs of constitutional sensitivity toward lead. Its action is toxic, probably on some special enzyme system(s). The same holds true of a number of other toxic chemicals that may cause disturbances of pyrrhole metabolism.

Among this group one substance has received very great attention recently because of its action on man in one certain geographical region. In the year 1956 there was an outbreak in several provinces in Turkey of something that seemed to be porphyria. It could be demonstrated that this "epidemic" was caused by the use of adulterated grain for baking bread. The chemical substance responsible for this was hexachlorobenzene (C_6Cl_6) that is used as a fungicide. Animal experiments soon proved that this agent very markedly influences the porphyrin cycle in all poisoned animals. It has become a habit to talk about Turkish porphyria, and it is, of course, much easier to use that word instead of the more tongue-breaking porphyrinuria. On the other hand, the correct term should really be the latter, as this is a toxic effect developing in all severly exposed individuals. Many authors have maintained, however, that the mechanism of the disease is explained by the action of the porphyrins. If we use this definition for porphyria, we will see that many porphyrias should not be included. It appears, however, that it will be impossible to eradicate the term Turkish porphyria, and we guess that it has come to stay (Schmid, 1960).

There are a number of other substances that may cause disturbances of porphyrin metabolism. Some of them have been used experimentally to produce conditions similar to the clinical porphyrias, but, so far it may be said that we do not know for certain their real mode of action, and such important clinical conditions as the acute porphyrias have not been produced experimentally.

Clinical Pictures and Nomenclature of the Different Porphyrias

The different systems used for the classification of porphyrias all have both advantages and disadvantages. Porphyrins were detected to be a cause of light sensitivity, and a corresponding congenital disease in man was described as early as 1911. This disease was studied in detail by Günther (1912) and the name Günther's disease is often used. It has the great advantage of being noncommitting. Later Watson and his group in Minneapolis investigated the disease in great detail and found that the normoblasts but not the erythrocytes contained uroporphyrin (Schmid et al., 1954). Some patients also had hemolytic anemia with splenomegaly, and isolated cases have been published, where the anemia was improved by splenectomy. Watson has named the group of porphyrias with

porphyrins in the red cells *erythropoietic*. We must not forget, however, that these patients may have enormous deposits of porphyrins in many organs: spleen, kidneys and liver (Borst and Königsdörffer, 1929). The most dramatic clinical symptoms are seen on the skin, where blisters and ulcers develop, that may lead to terrible mutilations of fingers, nose, ears, etc., and to severe scarring. Clinically this is therefore a *cutaneous porphyria*.

We prefer to use the old term porphyria congenita (Günther, 1912) with the addition, erythropoietica.

Recently another cutaneous porphyria with porphyrin content in immature and also in mature red cells has been described. It has been called protoporphyria erythropoietica (PPE). Light sensitivity is much less marked than in the previous condition. The patients notice itching, erythema, swelling and very seldom superficial blisters (but never deep ulcers with scarring on the parts of the skin that are exposed to light). The symptoms start in childhood. One of us (Haeger-Aronsen, 1963, 1966) has described latent cases in the family.

Continuing with the porphyrias that cause light sensitivity (cutaneous) we find another type that does not develop until later in life (Waldenström, 1937). It is called porphyria cutanea tarda (PCT). This disturbance of porphyrin metabolism is accompanied by some signs of liver damage (fibrosis morphologically; very seldom a slight jaundice but retention of bromsulphalein probably constant in active stages). Severe liver failure is rare. Alcoholism is very common, and there is great improvement if alcoholic intake is stopped. Porphyrin is present in liver biopsies and Watson places this among the *hepatic porphyrias*. We prefer the old designation, porphyria cutanea tarda, placing stress on the 2 most important clinical findings: skin symptoms, developing late in life. There are never seen the same mutilations as in porphyria congenita erythropoietica. Ulcers may arise on the parts of the skin that are exposed to light. This means that people working in the sunshine naked to the waist may have severe symptoms on the chest and back. It is evident that the skin on the parts that are exposed to light is fragile against mechanical trauma. Knocking against a hard object or scraping with the finger nail may cause a small blister, and secondarily an ulcer. It seems to us that this mechanical fragility on the irradiated parts of the body is the most characteristic finding (Nikolsky's sign).

In all the 3 conditions discussed so far we have reason to believe that porphyrins circulate in the body fluids and cause light sensitivity. The fourth condition, where light sensitivity is important, does not show much porphyrinuria, but, preformed porphyrins are present in the stools, and it is therefore probable that they are formed in the liver and excreted by

the bile. Porphyrinemia has not been established as a clinical finding. There has been much discussion regarding the best name for this disease. Watson (1954) regards it as a hepatic porphyria. It seems to us that light sensitivity and mechanical fragility of the skin must be the most constant symptoms clinically. It is remarkable, however, that some patients may develop very severe, even lethal, symptoms which are otherwise only found in acute intermittent porphyria (see below). Colic, constipation, nervous symptoms of different kinds and death in respiratory paralysis may occur, probably often caused by the administration of barbiturates as in acute intermittent porphyria. Because of the very variable clinical picture, with cutaneous, abdominal and neurological symptoms in the same patient, this type has been called *porphyria variegata* (PV) (Dean, 1963). This seems to us to be a purely descriptive name that should be accepted. It is synonymous with South African porphyria. In 1957 one of us (J. W.) introduced a new name protocoproporphyria for some patients observed in different European countries. At present it seems clear that their clinical and biochemical picture is compatible with what we now know regarding South African porphyria. They should therefore have the diagnosis porphyria variegata.

In Sweden we have seen a great many cases of porphyria, never with cutaneous symptoms, but with acute attacks of abdominal colic and constipation, sometimes complicated with very dramatic and severe nervous disturbances. The prognosis was previously regarded as very serious with a mortality of 50–60 per cent, until it was realized that barbiturates were the lethal factor. These patients do not have much or any primary excretion of preformed porphyrins. Precursors such as ALA and PBG are present in great quantities and Waldenström (1937) used PBG as an indicator that a person was a carrier of the trait even if he has no symptoms (latent porphyria) of clinical disease. In order to stress the fact that porphyrins—and light sensitivity—are absent from the bodies of these patients Waldenström suggested the name pyrrholia, but suspected that this would never be accepted! Eales (1961)has recently coined the term *pyrroloporphyria,* and we think this has many advantages.

The usual name has been acute porphyria, but this is a somewhat ambiguous term as South African porphyria variegata is also acute. Porphyria acuta intermittens (PAI) has been an accepted name and will therefore be used by us.

Biochemical Patterns in the Porphyrias

Three types of patterns are important for the differential diagnosis among the porphyrias. One is the *clinical* pattern; another is the

hereditary pattern; and the third is the *biochemical* pattern. An important but difficult problem has to do with the question of whether people in all the previously described conditions may be carriers of the trait without showing any biochemical abnormality. We were able to show that family members, who had never had any clinical symptoms of disease still showed biochemical aberrations marking them as latent acute porphyrics. Another problem is related to the question if the biochemical pattern always remains true to type (Table 1).

Before we tried to decide about the most important biochemical findings in the 5 different porphyrias, we had to choose the parameters that could give reliable answers. It is seen from Figure 1 that a number of different metabolic steps lead to the final synthesis of heme in hemoglobin. This means that many metabolites could be suspected as being of importance. Waldenström in his monograph (1937) studied the colorless chromogen that gives a strong Ehrlich aldehyde reaction. Later, (1939) he and Vahlquist tried to isolate this substance and named it porphobilinogen (PBG), because it was a chromogen and could be transformed both to uroporphyrin and to another red pigment that was called porphobilin. Waldenström (1937) could show that there are persons, who excrete PBG without being ill but later develop manifest porphyria. PBG was crystallized by Westall (1952) and its chemical composition determined. This substance has a central position in porphyrin chemistry, even if it is not itself a porphyrin but rather a quarter of a porphyrin—a substituted pyrrhole. Vahlquist and Waldenström (1939) had supposed it to be a dipyrrhole.

When Shemin and his collaborators (1953, 1955) had succeeded in showing that ALA was the precursor of PBG, this substance was also looked for in the urines of patients. It was found in increased quantities, e.g., in acute porphyria, as well as later in acute stages of porphyria variegata (Mauzerall and Granick, 1956).

Uroporphyrin with 8 carboxyl groups is regarded as the first porphyrin in the cycle and the others are formed by successive decarboxylations of the reduced porphyrins (uroporphyrinogen, etc.). Uroporphyrin may arise in the urine from PBG without any influence of enzymes, and this led Waldenström and Vahlquist to the assumption that there was a spontaneous condensation of porphobilinogen molecules to uroporphyrin in the urine. The fact that only 2 types of porphyrin isomers are seen in nature, the symmetrical type I and type III, made us believe that there were 2 kinds of dipyrrhole methanes that could condense in two ways to form the I or the III porphyrins. This is a simple explanation but has not been confirmed biochemically.

It is now possible to make quantitative determinations of uroporphyrin, coproporphyrin and protoporphyrin in excreta, and also to determine ALA and PBG in the urine. Figures 2, 3 and 4 show the patterns of the different types of porphyria. It is important to stress the fact that quantitative determinations of porphyrins in the stools are of great importance for the diagnosis of porphyria variegata. The presence of this

TABLE 1

Porphyrinmetabolism Deranged by:		Urine			Feces			Serum	RBC
		ALA	PBG	UP	CP	CP	PP	ALA	PP
Porphyria acuta	Latent	(+)	++	++*	+	+	+	(+)	N
intermittens	Manifest	++	+++	+++*	++	+	+	+	N
P. variegata	Latent	(+)	(+)	N	N	+++	++	N	
	Manifest	++	+++	+++*	++	+++	+++	+	
P. cutanea tarda	Latent	N	N	++	(+)	+++	++	N	N
	Manifest	(+)	N	+++	++	++	+	N	N
P. congenita		N	N	+++	+++	+++	(+)		+++
Protoporphyria erythropoietica		N	N	N	N	N	(+)	N	+++
Apronal rabbits, rats		+	+++	++*	+	+	++		N
2-Allyloxy-3-methyl-benzamine Man, rabbits		+++	++	+	+++	+++	++		N
Sulphonal rabbits		N	N	N	(+)	N	N		?
Hexachlorobenzene (C_6Cl_6)									
Man		N	(+)	++	++	N	N		N
Rabbits, rats		+†	+†	++	++	+	++		N
Guinea pigs, mice		(+)	N	N	+	N	N		N
Diethyl 1,4-dihydro 2,4,6-trimethylpyridine -3,5-dicarboxylate (DDTD; 2,4,6)									
Rabbits		+	+++	+++*	+++	+	+++		N
Rats		++	+++	+++*	++	?	?		?
Griseofulvin									
Rats		++	++	(+)	+	++	+++		+++
Lead									
Man		+++	N	(+)	++	N	N		+++
Rabbits, rats, Guinea pigs		+++	+	+	++	N	N		+++

*Chiefly formed in vitro from PBG.

†Terminal rise in the rats.

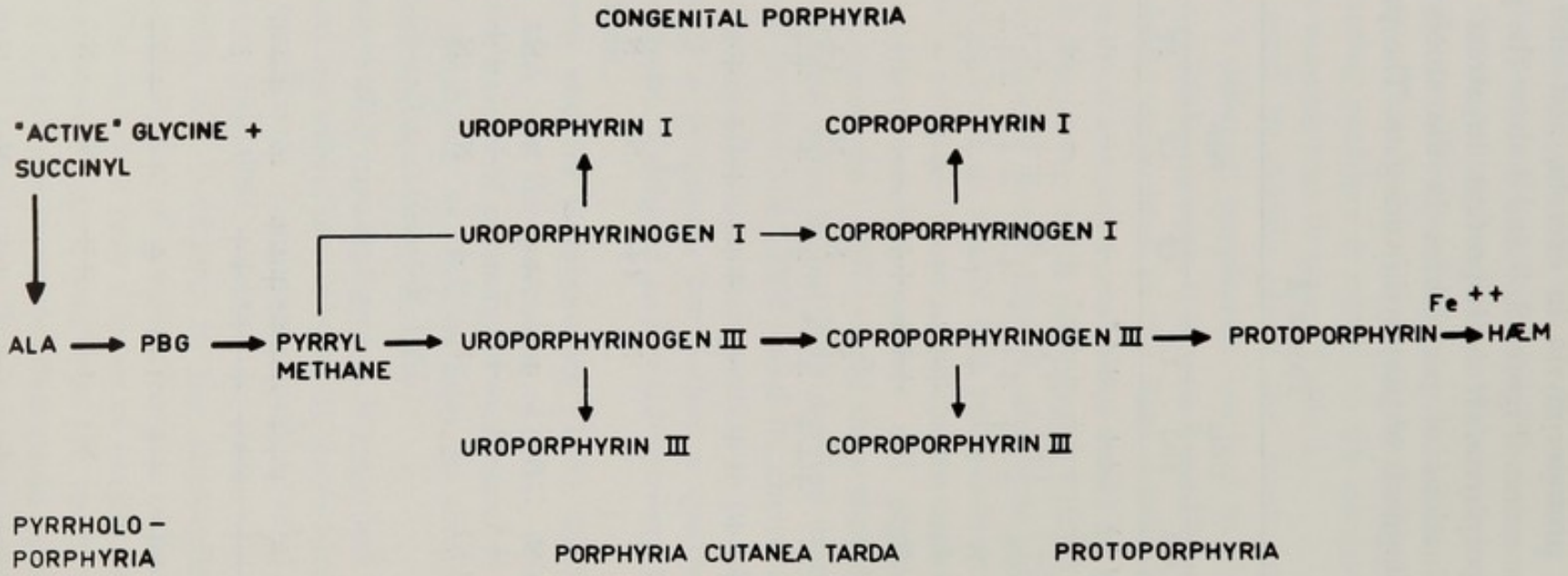
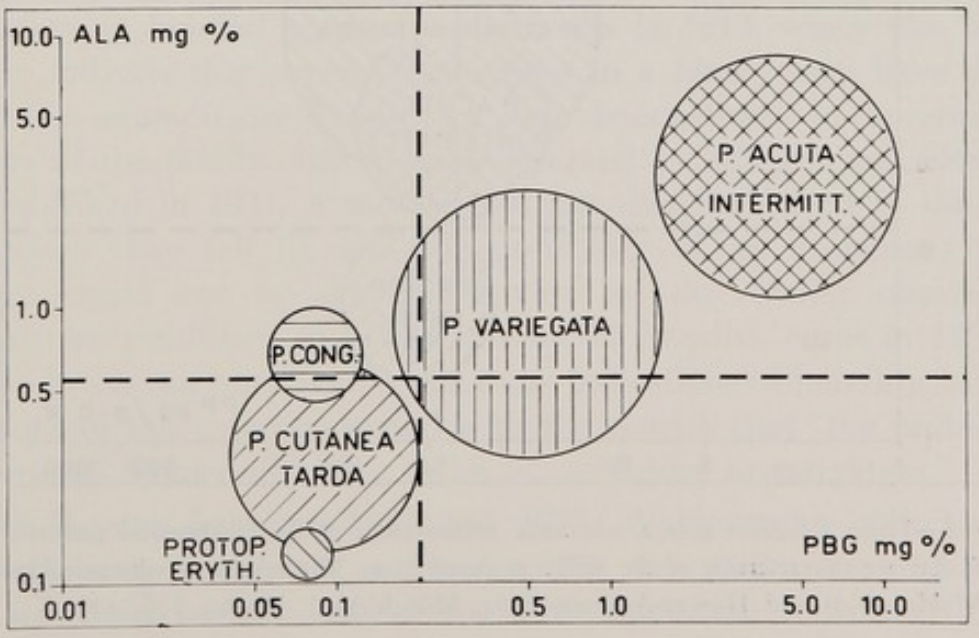


FIG. 1—Schematic drawing indicating the different biochemical steps in heme synthesis. Nothing is known regarding the basic biochemical defect responsible for the different clinical pictures but the situation of the different porphyrias in the chain of biochemical events is given.

condition may be missed, if only urine is examined, even if coproporphyrin is usually present. This is a symptom, however, that may be found in a number of conditions and may be difficult to evaluate. It is seen that protoporphyria erythropoietica occupies normal positions in the 3 diagrams even if increased fecal protoporphyrin gives a good lead in most cases. The most important sign for this diagnosis is increased protoporphyrin in the red cells; a sign that is almost never absent. Two possibly latent cases have been described, where increased protoporphyrin was found in the stools, when the red cells were normal and the patients belonged to families with clear-cut porphyria (Redeker and Bronow, 1964). The diagnosis of porphyria congenita hardly causes any trouble as the copro- and uroporphyrin content of the urine and the coproporphyrin in the stools is very high. The precursors are hardly altered, even if slight increase in urinary ALA has been noted. This might also be true of porphyria cutanea tarda. Otherwise this disease is characterized by increases in urinary uroporphyrin, that are usually marked and accompanied by increase in coproporphyrin. Stool examinations are not of much importance except for the "reciprocal" excretion described by Rimington (1952) and also seen by us.

The diagnosis of acute intermittent porphyria (pyrholoporphyria) is compatible with a completely normal color of the freshly voided urine. On standing, especially after acidification and warming, these urines develop a red or red-brown color caused by uroporphyrin but chiefly by other as yet unidentified pigments. If the patient is put on therapy with alkali, the urine becomes regularly light yellow and only chromogens are seen even on standing. It is therefore clear that the amount of



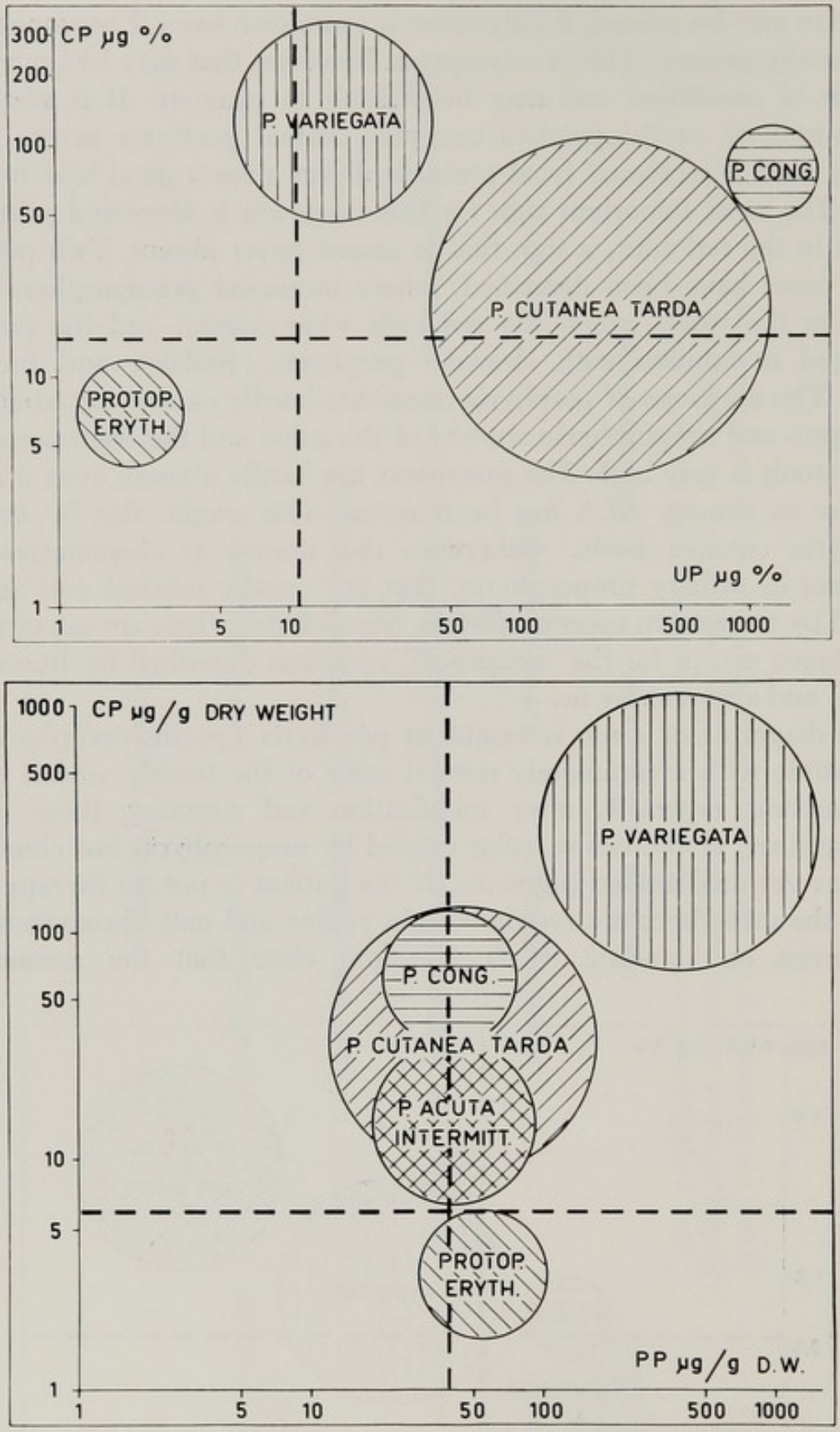


FIG. 2–4—These 3 figures give a schematic presentation of the biochemical patterns in urine and stool that are characteristic of the different porphyrias. The results are discussed in the text. (From Waldenström and Haeger-Aronsen 1964. Münch Med. Wschr. 106, 1339.)

uroporphyrin in the urine depends upon the conditions of porphobilinogen *in vitro*. In an acid urine there is much transformation into pigments; in an alkaline medium the colorless chromogen is much better preserved. Different factors also influence the relation between uroporphyrin and porphobilin formation. Clearly this must mean that determinations of uroporphyrin in acute porphyria do not mean anything. Both ALA and PBG are much increased, however, and their levels are important parameters. Even this general rule may have some exceptions. We have seen clear-cut carriers, who have had normal values for ALA with increased PBG in the urine. This is remarkable in so far as PBG is quite unstable, whereas the content of ALA does not decrease appreciably with time in nonalkaline urine.

The examination of stools has great value for the differential diagnosis between porphyria variegata and porphyria acuta intermittens. In the latter condition, slight increase in coproporphyrin is not uncommon, whereas protoporphyrin may be borderline. In porphyria variegata, however, both porphyrins often are much increased. The stools may show a bright red fluorescence on direct examination in Wood's light. This is never the case in acute porphyria (Barnes, 1958; Dean and Barnes, 1958, 1959; Waldenström and Haeger-Aronsen, 1963).

Porphyria Acuta Intermittens (PAI) or Pyrroloporphyria Heredity

As early as 1890, Ranking and Pardington in England had noticed that 2 unrelated persons living in the same house had developed symptoms of acute porphyria at about the same time. Barker and Estes published an observation on familial hematoporphyrinuria in 1912, where the history seems to indicate that several generations in a family may have shown signs of the same disease. Porphyrins were demonstrated in the urine of 2 members of the family. The 2 cases observed by Micheli and Dominici were published in 1931. A mother and her daughter lived in the same house when they fell ill, and the possibility of an exogenous factor therefore could not be denied in view of the earlier observation. Waldenström published investigations on 11 Swedish cases in 1934. At this time it was not possible to detect any indications of heredity, and the discussions of this question ended with the remark that "the problem of constitutional factors will have to be left to future investigation."

In the 3 years between 1934 and 1937, Waldenström collected 100 cases with acute porphyria in Sweden. For this study, investigation of family members became one of the most important tools and it soon

became clear that heredity must play a very large part for the development of this disease. We have continued these investigations on a large scale and the discussion of these problems is chiefly based on our experience from Sweden. Our present material of Swedish acute intermittent porphyria now comprises some 600 persons, and the number is increasing steadily.

Concordant manifestation in identical twins was observed by Kehoe, Rudensky and Reynolds (1957). We have also observed one pair of twins with porphyria acuta intermittens, who were certainly concordant even if this was never definitely proved.

Age and Sex

There are several clinical observations regarding this condition that are also of great interest from the geneticist's point of view. One is the fact that the disease rarely if ever occurs in children, at least not in small children. In our very extensive material we have not had more than 5 observations of persons in the prepubertal age group (<15 years), who had manifest symptoms. With present-day technics we believe that we can make the chemical diagnosis of a carrier, even when he has no clinical symptoms during a long life. This problem will be discussed later.

If it is true that the trait occurs as a dominant character, we must expect that 50 per cent of all children born with one porphyric parent should be latent porphyrics. We have systematically collected urinary samples from children in porphyria families and have obtained a large number of such observations. The fact that we are now able to detect the presence of 2 metabolites (both PBG and ALA) in abnormal quantities gives us a good opportunity to find out if one or the other is a better indicator of latent porphyria. We have arranged our results according to age groups and would be inclined to assume that there is an increasing percentage of positive test results with increasing age. On the other hand, such conclusions should be confirmed by statistical treatment, and the number of children in each age group is probably not large enough to give significant differences. This does not mean, however, that there could not be such real differences with age. If we place all the 54 persons investigated below the age of 12 together, we shall find that 39 show pathological excretion of PBG but only one person of ALA. It should perhaps be noted that only 6 had a marked increase in PBG. We do not know if the fact that PBG is a more sensitive indicator than ALA means that it has a more central position in the biochemical disturbance because of the localization of a hypothetical metabolic block. This is probably worth investigating (Fig. 5).

It might well be that the development of puberty is an important biochemical event with widespread metabolic repercussions. Could it be that the enzyme concentration necessary to keep practically normal metabolic homeostasis is sufficient in a smaller organism, whereas a bigger person becomes decompensated? It is difficult to find parallels, because most of the other inborn errors of metabolism cause symptoms that lead the carriers to the pediatrician (galactosemia, Fölling's disease, orotic aciduria, etc.). Another explanation could be that the formation of hormones from the gonads tends to upset the metabolism of the pyrrhole substances. There are several observations that could be quoted as proof of this as will be discussed later.

There are, of course, examples in medicine of inherited diseases that do not give any symptoms at an early age, even including the first 4 decades. Only later do these patients develop disturbances which could possibly be caused by an inborn error of metabolism. A classical example of this is Huntington's chorea, where we do not know anything about the metabolic disorder, however. It is therefore difficult to tell if this is a metabolic defect that might be clearly demonstrable already in children, if we only knew what we should look for. In recent years, Refsum's disease has been described as another inherited, definitely metabolic

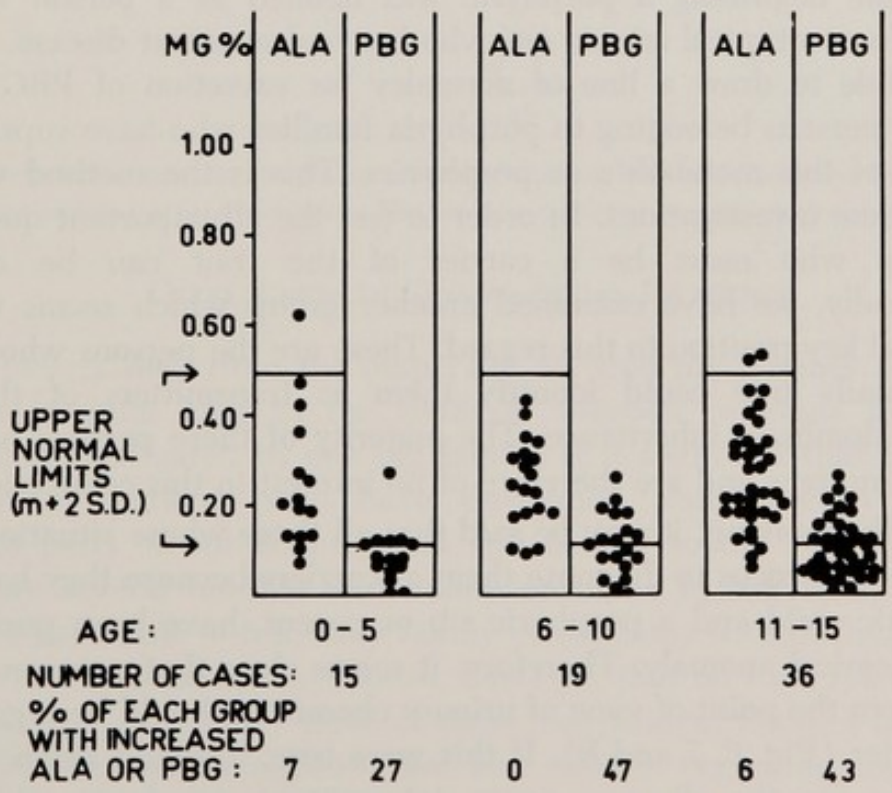


FIG. 5—It is clearly seen that ALA determinations are of little value for the detection of carriers among children.

disorder that does not give symptoms until later in life. Here it is possible, however, that several decades are necessary before the (possibly exogenous) pathological metabolites accumulate in such quantities that they become harmful (Klenk and Kahlke, 1963).

Already the fact that the manifestly ill person, who carries the acute porphyria trait is almost twice as often female as male, shows that clinical manifestation is more common in the female sex. We may ask ourselves if this means that there is a prevalence of latent cases among male carriers of the trait. The simplest explanation would be that 50 per cent of all women in affected sibships (i.e., 100 per cent of female carriers) develop manifest disease; whereas, only one half of the males become manifestly ill, and the other half is latent but shows biochemical abnormalities. We have arranged our material in order to find out if this is true, but it is clear that the conditions are not so simple; we have found that latent porphyria is clinically latent in 62 per cent of the women as well as in 43 per cent of the males, if calculated from sibships where we have examined every member. We define a carrier of the trait as a person who has the biochemical defect. Are they always recognizable if their urine is examined by a competent investigator?

Latent porphyria

From the beginning a porphyric was defined as a person who has suffered from a typical attack, i.e., who has had manifest disease. Later it was possible to draw a line of normalcy for excretion of PBG and to define all persons belonging to porphyria families who have supranormal excretion of this metabolite as porphyrics. This is the method we have used in these investigations. In order to test the all-important question if everybody who must be a carrier of the trait can be detected biochemically, we have examined another group which seems to us to have a real key position in this regard. These are the persons whose place in the family tree could identify them as transmitters of the trait, assuming dominant inheritance. The majority of these persons have had typical symptoms and are therefore of no interest in this connection.

Generally speaking, it may be said that all those whose situation in the family tree allows us to diagnose them as carriers because they have both a porphyric child and a porphyric sib or parent, have been positive for the biochemical anomaly. Therefore, it seems clear that a person who is normal from the point of view of urinary chemistry should be regarded as a noncarrier (Fig. 6, 7 and 8). If this were true, it would mean that the trait basic for the disease, acute intermittent porphyria, should be regarded as genetically monofactorial and dominant, i.e., active in a

single dose. Another question is, do modifying genes facilitate the development of the porphyric catastrophe?

From the foregoing we might assume that all carriers of the trait may be detected biochemically.

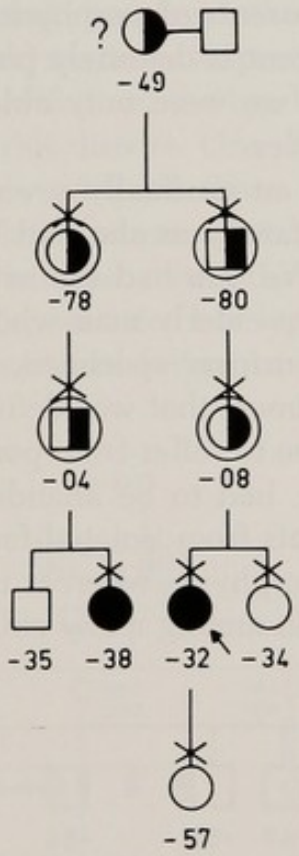


FIG. 6—Indicates that the porphyria acuta intermittens trait may be latent for generations and become manifest in second cousins. The biochemical defect was present however.

DIFFERENCES IN GEOGRAPHICAL INCIDENCE

It is clearly seen from the map (Fig. 9) that the number of different families is quite high in the south of Sweden, whereas Norrland is completely dominated by one big family. It is also clear that there are a great many patients who have developed acute porphyria in the southern parts of our country and still belong to the Norrland family. There are several families with many members in Norrland, where we have not been able to trace any family connections with the big porphyria family. This does not always mean, however, that no such relations exist, as at least two or possibly three of these independent families are of Lapp ancestry. The church registers are less reliable regarding the nomadic Lapps, who have had no farms. The connection between farm and family is very strong in the church registries. The forefathers of these Lapps have

therefore been lost around 1780, and it might well be possible that they are related to some persons of Lapp origin, known to be members of the big family where Lapp ancestors are known.

In order to find out whether we could assume *first mutations* as an explanation for some of our southern cases, we have tried to investigate the urines from as many parents of porphyrics as possible. We now have 82 sibships, where one parent is definitely porphyric. For many years we had some families, where we were only able to investigate one parent. We shall give a few examples.

One woman developed an unusually severe acute porphyria, and we became interested in the family as she had 8 sibs. They all very kindly sent us urine specimens. We also had the urine from the mother. It was negative, but the father, an elderly man who had always been perfectly well, did not send us any urinary specimen. After many years, however, we finally obtained a specimen, that was definitely pathological. The idea that this girl, who was alone to suffer from porphyria in a sibship of 9, was a first mutation, therefore, had to be abandoned.

Regarding pairs of parents from isolated families, we have seen 8. In 5, one of the parents was a porphyric, whereas in 3 both were negative. It is interesting that only one sib among many children (3/21) is a porphyric.

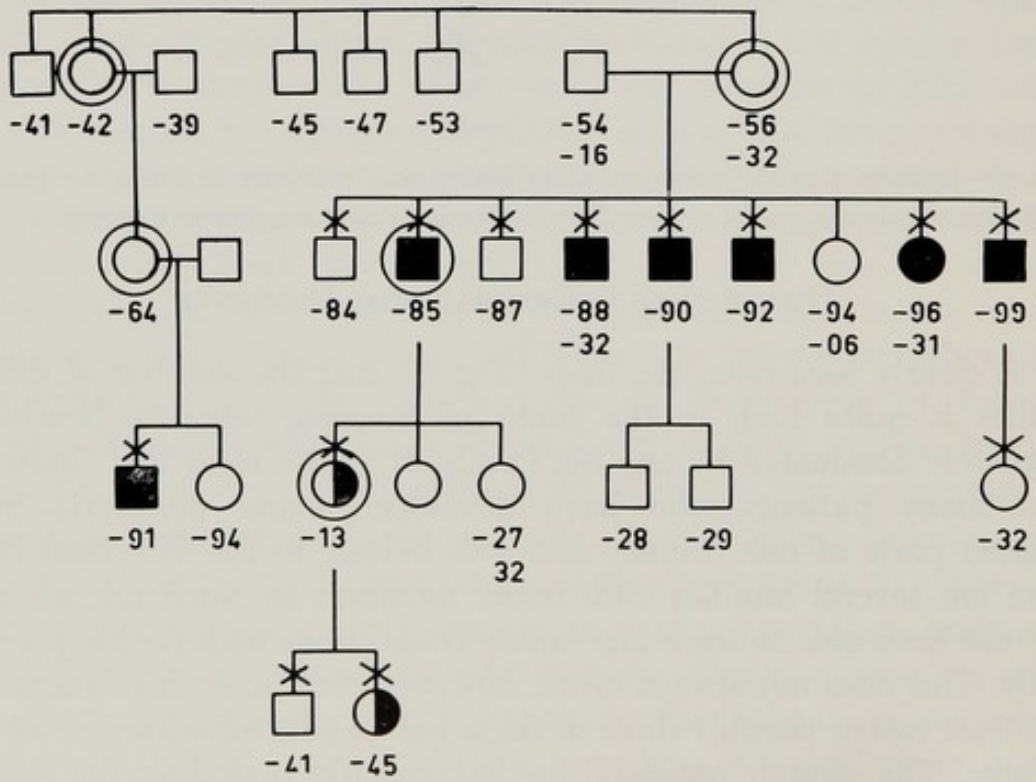


FIG. 7—The pedigree demonstrates that a succession of probably latent cases may hide the genetic links between cases of porphyria in the same family.

Could this mean that the 3 are first mutations, or does it simply mean illegitimacy? (Fig. 10).

An interesting example of the difficulties encountered in clinical genetics is the following that has been mentioned by the authors before. We know of one girl with severe acute porphyria, who had 2 healthy sibs. The mother could be examined, but the father had died without ever having had any signs that could make us suspect porphyria. We could not in any way bring him, nor his wife, into the big Lapland family in spite of the fact that they all lived in this district. One of us (B. H.-A.) has had a very extensive correspondence with all these cases and also with the mother. After several years a letter came starting with the phrase: "I have a confession to make," and then she stated that the porphyric girl, who was the eldest among the sibs, was not her legal father's daughter. She also gave the name of the biological father, and he fitted very well into

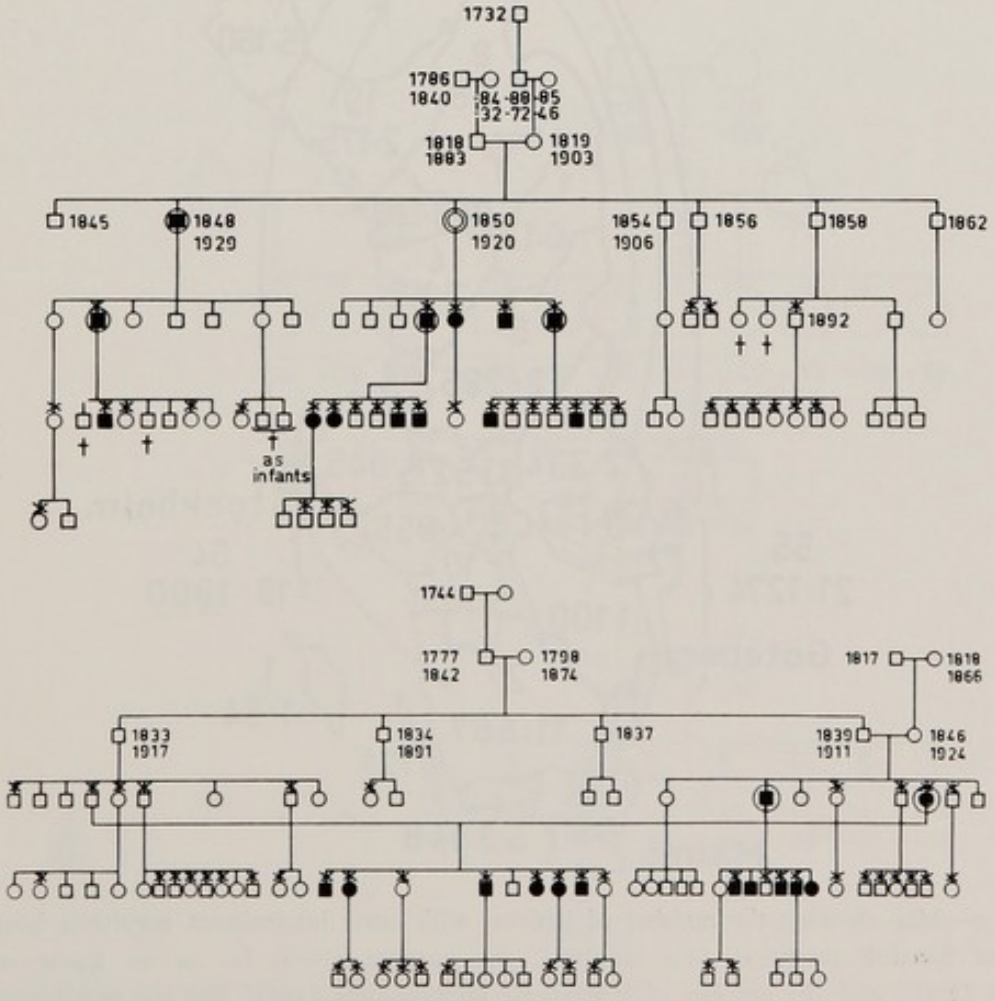


FIG. 8—Both these families are of Lap origin. It is clearly seen that the rule "once free, always free" seems to hold true in several branches of these trees. The dominant pattern is obvious.

the Lapland family and had some other porphyric children in his marriage. This is therefore a good example of the fact that we should never draw too far-reaching conclusions from single exceptions that may have a natural, although illegal, explanation.

One of the most remarkable facts regarding the inheritance of

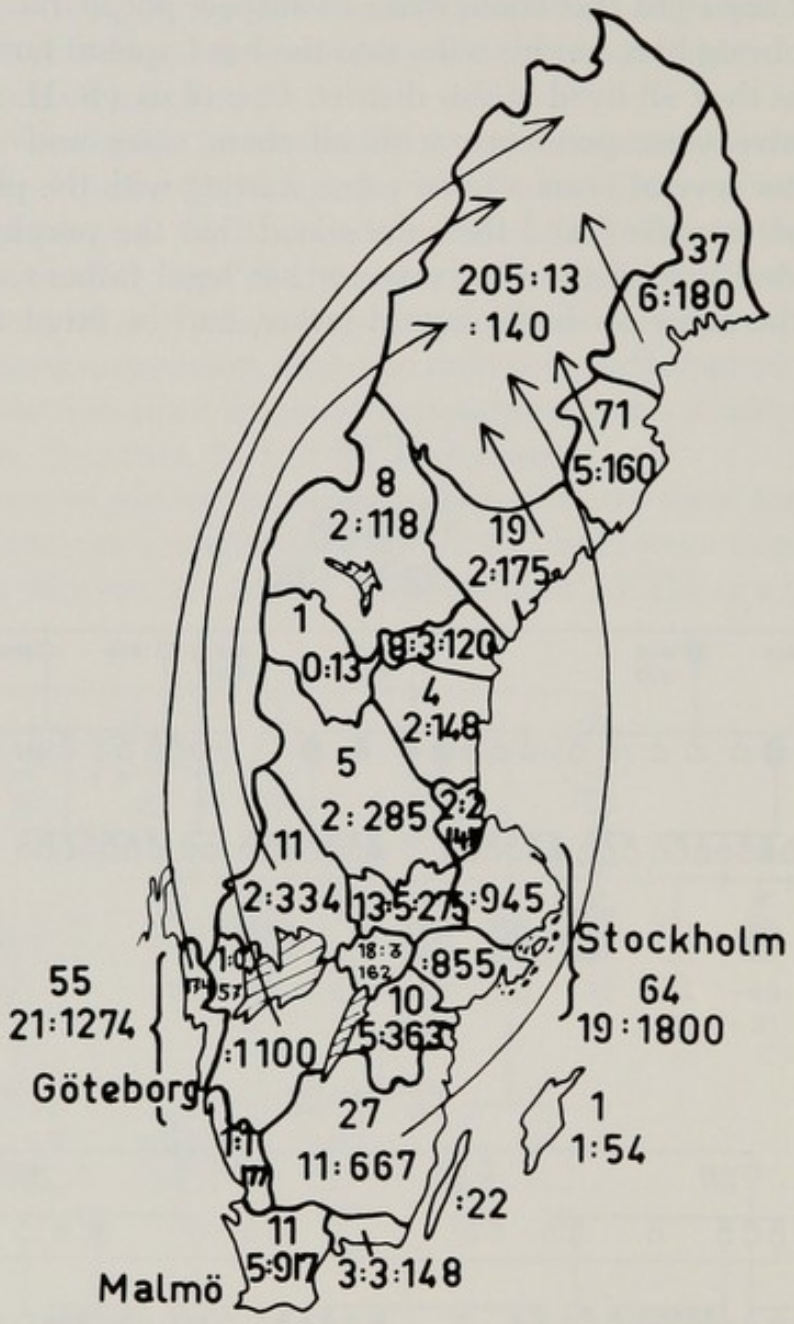


Fig. 9—Map showing the number of patients with acute intermittent porphyria born in the different Swedish provinces (1st number), the number of—as far as we know—unrelated families (2nd), and the number of inhabitants in thousands (3rd). For the two biggest cities, Stockholm and Göteborg, the city and the adjacent provinces have been taken together. The results are discussed in the text. In many provinces patients were born who belonged to one of the big families from the far north. Arrows indicate such connections.

porphyria acuta intermittens and porphyria variegata seems to be the great accumulation of patients with porphyria acuta intermittens in the far north of Sweden and of porphyria variegata in South Africa. It could, of course, be maintained that unusually careful family investigations have been performed in these 2 parts of the world. We have clearly spent very much time and interest on establishing the family connections between the Laplandic cases of porphyria (Fig. 11). It is rather remarkable that this family should contain 227 affected members, practically all of them living in the valley of the Skellefte and Pite rivers. We must not forget, however, that there are also other families in the north of Sweden containing several affected members, where we cannot trace any connection with what we call the "big family." In southern Sweden, on the other hand, we usually find sibships with 5 members at the most. One possibility would be that, even in unrelated families, carriers of the trait living in Lapland get the disease in a more severe form than people in other parts of the country. This would then mean that there should

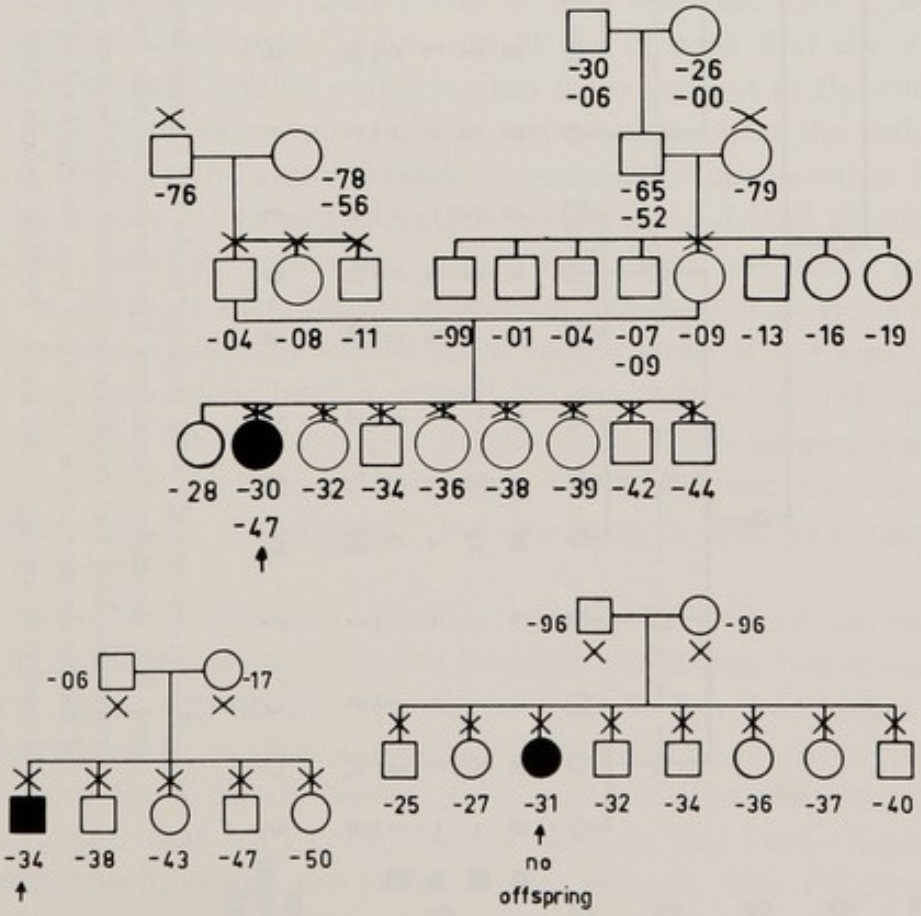


FIG. 10—These 3 pedigrees present the data discussed on p. 72. It seems interesting that the only 3 families where both parents show normal biochemical conditions have only one sib with porphyria acuta intermittens among a large number of examined brothers and sisters (3/21). × = examined.

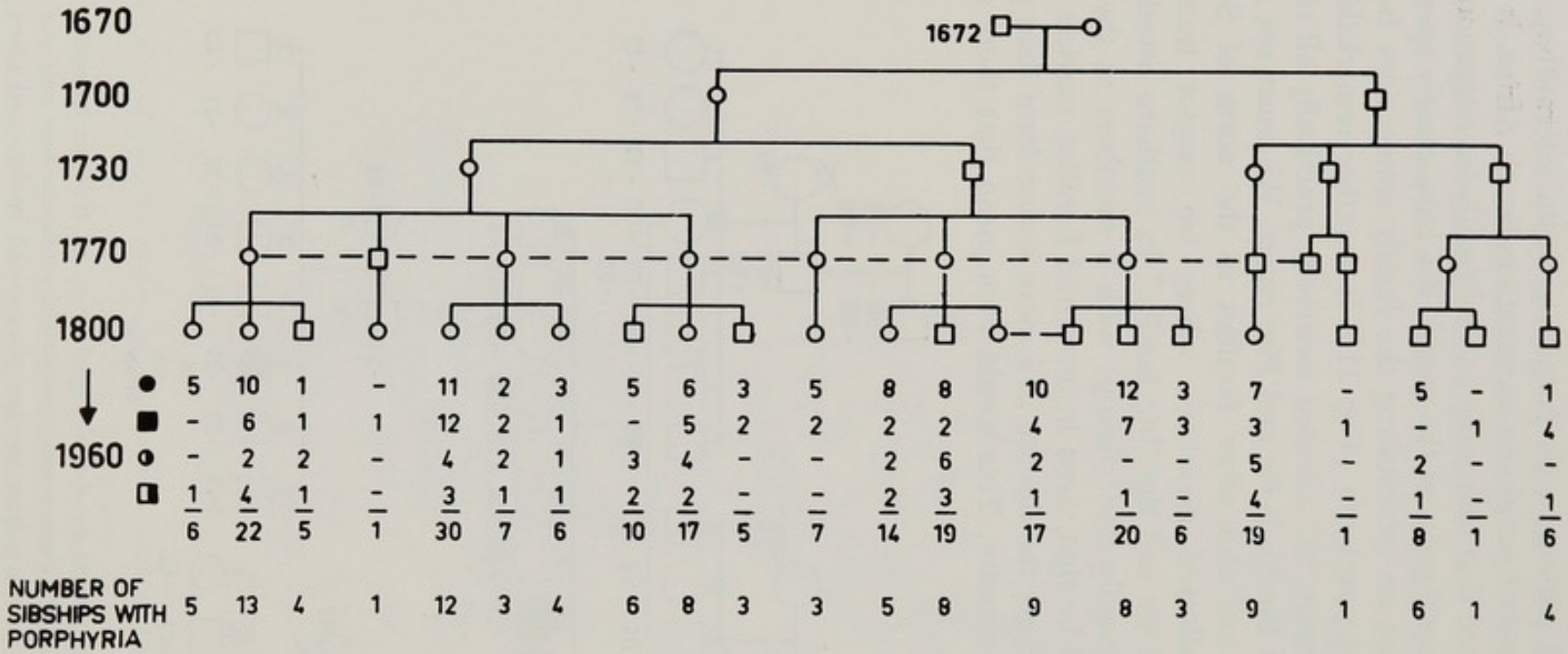


FIG. 11—Shows the basic pedigree in the big Lapland family. It is, of course, impossible to state that the earlier generations have carried the trait but the fact that 2-3-4 sibs in early generations have had porphyric descendants could be used as an argument for the correctness of the family connections represented here. At this level only 2 intermarriages between possible heterozygotic carriers were noted. Nothing indicates that homozygosity resulted from these marriages.

be some exogenous factor in this part of the country either climatic, geological or nutritional. It is clear that this is the coldest part of Sweden, and we may always discuss the theoretical possibility that this could have some influence on porphyria just as we may believe that the strong sunshine in South Africa favors manifestation of porphyria in that country. Such speculations are only worth discussing if it could be found that, e.g., Canada or the northern parts of the USSR have a higher incidence of acute porphyria than the rest of these countries. Another could simply be that the number of children in this part of the country was especially big. A third could be that porphyrics did not die to the same extent from their disease in the north, and that the negative selection therefore did not work to the same degree as in the south.

Waldenström (1937) has already treated the possibility that these patients might be exposed to some exogenous factor that could be toxic, if they carry the gene for porphyria. The reason why this problem was taken up was the fact that some of the biggest lead mines in the world are situated in these river valleys. This subject was discussed after some conferences with geologists. They were of the opinion that the different ores containing lead, arsenic and selenium were present in the rocks and that very little of these metals could be expected to enter the soil, where plants and, secondarily, animals could be poisoned. On the other hand, it seems to be a peculiar coincidence that porphyria and lead should occur in the same districts, when we know that lead has such powerful influence on pyrrhole metabolism generally. The best way to investigate this problem more critically would be to follow some of the families that carry the Laplandic gene but have lived for many decades in the south of Sweden and see if the incidence of porphyria in these familes was lower than among their relatives in Lapland. There are not so many such families that may be investigated; but we have 3, and they have been carefully analyzed.

Quite recently Wetterberg (1966), who is at present investigating psychiatric problems connected with Swedish porphyria, has re-examined these ideas by analyzing the content of lead in fish from lakes in the porphyria district. It was found that they contained many times more lead than fish from the Gulf of Botnia. It is possible that this could be an explanation, and that lead could be enriched in the food chain before passing with the food into the bodies of the carriers. The validity of this suggestion remains to be tested.

We would be inclined to think that continuous small doses of lead could favor the formation of ALA in persons who have the genetically determined defect. If this is the explanation for the accumulation of cases

in north Sweden, it would certainly constitute an interesting example of the interplay between exogenous and endogenous (genetic) factors.

We must still ask ourselves about the reasons for the great prevalence of South African porphyria in that country. We have already mentioned the fact that many authors believed that Swedish porphyrics would get light sensitivity if they were transferred to the radiant sunshine of South Africa. We have always opposed this assumption, and we know now that South African porphyria may be found in Sweden, and, therefore, that even our sunshine is sufficient to cause photosensitivity in such patients. This is a recent discovery. There are, however, some peculiar experiments by Pimenta de Mello (1951), who exposed one group of animals to strong light and found that this group formed more porphyrins than the nonirradiated. He therefore assumed that irradiation with visible light might cause disturbances of porphyrin formation. If this were true, it might perhaps be assumed that South African porphyria shows such a high penetrance in Africa because of the sunlight and Swedish porphyria in Lapland because of the influence of heavy metals.

Regarding both these diseases, the number of children in each sibship must, of course, be decisive for the continued transmission of the gene. We have investigated this problem quite closely in the Laplandic family and know the number of children in the different generations quite well. For many reasons this was difficult to ascertain for the other families, and we have, therfore, no really good material for a comparison. Dean (1963) stresses the point that his big family is very rich in children, and, as a matter of fact, the first couple, of which one was probably the first carrier of the disease in South Africa, married in 1688 and had 9 children. The next generation, where the presence of carriers is assumed, also had a 9 child sibship. This means that the gene had a great initial chance of being perpetuated through the generations.

If we look at the data regarding acute porphyria in other countries, we shall find that With (1960) gives an incidence of about 2/100,000 for Denmark. Western Australia has 750,000 inhabitants with 16 manifest and 7 latent acute porphyrics. This would give 2.4/100,000, but the authors state that probably many more latent cases could be found if more relatives were examined (Curnow et al., 1959).

Porphyria Variegata

Most of the families with porphyria variegata come from South Africa and were investigated by Dean, Barnes and Eales. These authors have observed large numbers of cases. The combination of a distinct biochemi-

cal pattern with a high content of proto- and coproporphyrin in the stool, and light sensitive skin, with neurological and abdominal symptoms, is obviously present in very many cases. Outside South Africa only relatively few sibships have been found, for instance, by Tio (1956) in Holland and by Holte et al. (1958) in England.

We have recently observed a family, in which 7 members seem to be carriers of the gene for porphyria variegata. The diagnosis was made by Hamnström, and a paper regarding the results is being prepared in collaboration with him. The propositus is a man born in 1937. He has had considerable light sensitivity, as well as abdominal and neurological symptoms, and has for a long time been an alcoholic. In our large experience with acute intermittent porphyria of the Swedish type, we have never seen any indications that chronic alcohol intoxication might play a part for the manifestation of that disease. This is different from porphyria cutanea tarda.

The investigation of this family for porphyrin metabolites definitely proves that this disease, as it occurs in Sweden, is not related to porphyria cutanea tarda, nor does it resemble porphyria acuta intermittens. Urine and stool from 41 members in 3 generations have been examined. The results have been that the propositus has the most prominent biochemical symptoms. His father, 2 paternal aunts and one paternal uncle are definitely pathological biochemically. This is also true of 2 cousins, who are daughters of one of the affected aunts. The heredity here seems without any question to be dominant but perhaps with incomplete penetrance in some sibships.

In another family we know of one woman, who has 2 affected daughters and one affected son out of 4 children. In this family, we have not been able to examine very many members of the previous generation. The 2 affected sisters have had classical abdominal and neurological symptoms of acute porphyria but are at the same time highly light sensitive. Their mother has never had any cutaneous symptoms (Fig. 12).

We know of another patient with typical porphyria variegata, who has been admitted to a Swedish hospital. She belongs to a Danish family, however, and her mother died in a hospital in Denmark with the diagnosis porphyria. There are no other relatives that could be examined in this family.

It is remarkable that the 2 Swedish families with a disease that seems to be very rare in our country come from the same part of one sparsely populated province—Småland. In spite of very careful search in the church registers that has led to complete family trees for both families

back to the time around 1860, we have not been able to trace any link that could explain that porphyria in these 2 families is the result of the same mutation.

The fact that these patients have not shown very remarkable symptoms of skin disease might well suggest that they could have been regarded as ordinary acute porphyrics, when they have had colic. On the other hand, Dean's experience with South African porphyria definitely indicates that it is very rare for patients to have only abdominal and neurological symptoms without cutaneous symptoms. It is difficult to know if this only means that the scorching sun of South Africa always sensitizes the skin of carriers if they are not unusually protected.

It is hard to find out from the literature if the values for PBG and ALA are always normal between attacks in patients with variegate porphyria. This problem must be settled in South Africa as it is obviously important for the differential diagnosis. The work of Dean and of Barnes seems to indicate that a large number of South African porphyrics, who have distinctly increased values for stool porphyrins, do not have increased values of PBG and ALA. This would lead to the illogical assumption that the family described by Watson (1962) really belonged to the Swedish type and was a "mixed" porphyria of a kind that we have never seen. On the other hand, it seems remarkable that the only 3 families in Sweden with a clinical picture of South African porphyria also had the typical South African biochemical pattern.

Dean has performed some very remarkable studies concerning the heredity of variegate porphyria. His numbers of affected sibs in different generations are partly based upon the family traditions that the skin was

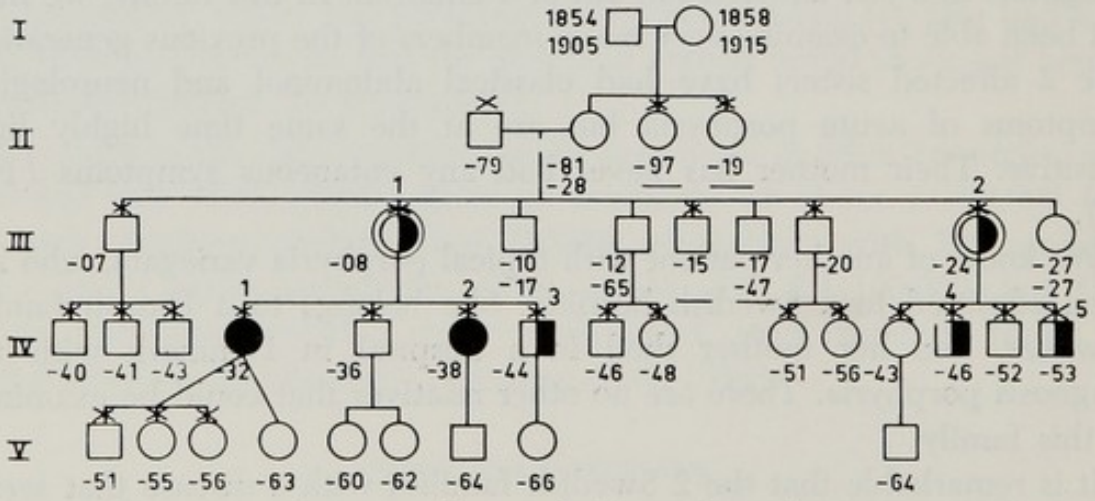


FIG. 12—This pedigree shows one of our 2 families with porphyria variegata. Two sisters have both cutaneous and abdominal symptoms as also the mother. The mother and one brother as well as one aunt and her 2 sons have always been latent and were only detected through biochemical studies in the family.

fragile. It is probable that this information is on the whole correct, but it is difficult to find out how many patients have been really investigated by a doctor. In generation 5 in the genealogical tree of G. R. van R., it may be supposed that a number of persons have been investigated, and it is found that 36.8 per cent of all the members of this generation were affected (Dean, 1963). It is clearly seen from the family trees that there are several instances of inheritance from parent to child in both sexes and the data presented by Dean definitely support his theory that the inheritance is dominant and autosomal. As is the case in Swedish porphyria, there are many latent patients, and it is interesting that there is only one instance of acute abdominal episodes without skin fragility. This is case 151 in the same family, who died at the age of 19. She had no blisters. She took large doses of analgesic and sedative drugs and then developed colic and severe constipation and was given Tiopentone anesthesia before laparotomy. Later delirium, general paralysis and tachycardia developed. Porphyrins and PBG were present in the urine. She died after a week.

Dean has devoted an enormous amount of work, skill and ingenuity to prove that practically all persons among the white population in South Africa in whom he was able to make the diagnosis of the disease can be traced back to one couple, who were married in 1688.

Dean makes the calculation that there are now some 5,000 porphyrics in South Africa. Demographic studies in South African Boer families seem to have many advantages: (1) The white settlers came at a comparatively late period and white girls did not arrive in the country until 1688. This means that all the old Boer families represent no more than a limited number of lines; (2) The couples had very many children and interest in genealogy has been great; (3) Practically all families from the early times are well mapped, and it is therefore easy to bring related persons together.

It is important to know the frequency of porphyria variegata in other countries. In view of the fact that the gene for South African porphyria was imported from Holland, this country is especially interesting. It is well known that we see this type of porphyria in Holland. One of the present authors (J. W.) has often been asked to discuss cases of acute porphyria in England and the United States. Having until recently only seen the Swedish type porphyria in my own country, I was quite astonished to see how many of these cases were of the South African type. I am therefore convinced from personal experience that South African porphyria is not uncommon in these countries. A small number of publications also prove that this is correct. Dean made an attempt to find out if the

girl, who came from Holland to South Africa in 1688 had some porphyric relatives in Holland. This interesting idea could not be investigated because the girl came from an orphanage and her parents were unknown.

Eales (1963) has made a calculation regarding the prevalence of the disease among the white inhabitants of Boer descent. He gives the figure of 3/500. Dean points out that the disease is more common in certain parts of South Africa than in others, and this, of course, makes all estimates quite difficult. A very interesting program was performed by Dean in 1957–1959. Six hundred forty-five nurses from the neighborhood of Port Elisabeth were examined and 8 had porphyria variegata. Five thousand six hundred adults were tested as patients in a hospital, and 23 were found to have porphyria variegata. In another routine examination, 23 of 5,000 patients were positive. Dean estimates that this population should have an incidence of 1/250, but points out that in the hospital material porphyrics may be overrepresented. This means that porphyria variegata is a common disease in certain strata of the white South African population.

If it may be difficult to recognize the presence of porphyria variegata among all the many patients with acute porphyria in Sweden, *mutatis mutandis*, the same must hold true of South Africa. Both Dean and Eales have seen cases that have had typical acute intermittent Swedish porphyria in South Africa. In South Africa the situation is further complicated by the fact that the Bantu population has suffered from symptomatic disturbances of porphyrin metabolism, that are obviously acquired and probably have to do with large iron intake and the consumption of home-brewed alcoholic beverages. There is also genetical admixture, and the hereditary situation in the colored population must be very difficult to analyze owing to the strongly matriarchal family organization.

The presence of porphyria variegata in other countries has not been clearly stated. It seems as if the large material from Western Australia that has been quoted contained only acute porphyria (Curnow et al., 1959). From Eire, on the other hand, 13 families with acute porphyria and one with porphyria variegata have been published (Fennelly, Fitzgerald and Hingerty, 1960). This would thus be more similar to the Swedish pattern.

It is an interesting philosophical problem that the difference between the administrator's and the scientist's outlook will never be reconciled. The law tells us that every patient should be pigeonholed in order to fit into medical statistics. The scientific outlook should always lead to a search for new explanations, when something does not fit into our

diagnostic frames. All these poor people had firm diagnoses and were duly registered. The tragedy in their situation was that these diagnoses often were wrong and therefore treatment disastrous. It may do a lot of good if we all have this in mind, when we see new and enigmatic syndromes.

Would we know anything at the present date about porphyria if the urine did not show a dramatic color? There are certainly still many "white" metabolic disturbances with colorless urinary metabolites to be detected.

Porphyria Cutanea Tarda

The question regarding the possible heredity of porphyria cutanea tarda, which belongs to the hepatic porphyrias according to the nomenclature of Watson, is very intriguing. We know that this is a rare condition induced chiefly in elderly males by alcohol and disappearing when, or if, the patient becomes abstinent. Some authors have regarded it as a typical exogenous hepatic malady of a toxic nature. On the other hand, it is clear that this complication must be rare among alcoholics. In a very large experience with alcoholic cirrhosis from the city of Malmö comprising 360 well-investigated cases (Hällén and Krook, 1963), we have only seen 7 patients with cutaneous porphyria. It is also interesting that these persons never have shown very severe signs of hepatic dysfunction or portal hypertension, even when their alcoholism was quite pronounced. This means that alcohol must have a different influence on different livers. The hypothesis that also this type of porphyria must have a constitutional, probably hereditary, basis seems reasonable.

Among the 44 patients from different parts of the country suffering from this disease, we have seen signs of disturbed porphyrin chemistry in more than one member of the same family only thrice. In one family the propositus (H. B.) was a woman, who had been consuming moderate amounts of liquor for many years. She had all the typical symptoms including hypertrichosis of the face. After stopping her alcoholic intake all symptoms disappeared, including the pathological biochemistry. If she were a carrier of a genetic biochemical trait, this finding must mean that the porphyrins in urine and stool are not a good yardstick to demonstrate the disturbance. It is possible that an alcohol load test may help us to detect such hidden defects. For psychological reasons, we have not used this method, however. We have seen that the BSP test also becomes much more normal, together with the normalization of the porphyrin metabolism and the condition of the skin (Waldenström and Haeger-Aronsen, 1960), when the patient has avoided alcohol for some time.

The lady in question has 2 brothers (I and II). We have not been able to observe them personally, but we have had urine and feces sent to us for examination. The values were decidedly pathological indicating the same type of disturbance as we see in clear-cut porphyria cutanea tarda. The data are seen on Table 2 and Figure 13.

In another family the propositus (S. L. IV) had typical cutaneous symptoms and biochemical findings. He is a dentist, and his brother is a doctor (VI). Very careful discussion of the alcoholic problems with both brothers did not reveal any sign of increased alcoholic intake. It might be possible that the patient's liver is unduly sensitive even to small amounts of alcohol or that he and his brother may give a somewhat embellished picture of his drinking habits. The third possibility would be that this is not an alcoholic case. The teetotaller with porphyria cutanea tarda is still to be discovered, however, even if such possibilities should never be denied *a priori.* We have been able to investigate this family in some detail. The brother and 2 sons (V and VII) of these 2 men have shown values for urinary porphyrin that are not normal. It could well be that these persons have a special type of porphyrin disturbance that is not alcohol dependent and manifests itself spontaneously biochemically at an early age. If this is true, this could be a new type of inborn error of metabolism. Only the propositus has so far shown clinical dermal signs of disease.

The third observation in this field is by far the most interesting. We have observed 2 brothers, who are twins, probably identical. The case histories are given briefly below.

S. G. and A. G., born in 1917 are twins and look very much alike. They have always been regarded as identical, and we have not detected any difference. Their blood groups have been determined, and they are both identical, A2, MN, P—, K+, Rh—, Hp 2-2. We have not been able to group the parents as they are both dead, but nothing speaks against identity. The 2 brothers have had about the same habits and are both commercial travellers. Both have a high alcoholic intake and probably consume 300–400 ml. strong alcoholic beverages several times a week. We saw them for the first time in 1959, when they seemed healthy except for typical changes of porphyria cutanea tarda on the hands. One of them (S. G.) had a lot of abdominal troubles, but these later disappeared after appendectomy. Otherwise no abdominal symptoms. He has noticed that hair has started growing on the temples meeting the eyebrows, as is commonly seen in these types of cutaneous porphyria. A. G. does not show this symptom. Both brothers were examined hematologically (white cells, Hb, red cells, sedimentation rate); all was normal; no

proteinuria or glycosuria was detected: NPN 29 and 36 mg./100 cc.; Bilirubin 1.2 and 1.3 mg./100 cc.; alkaline phosphatase normal; no urobilinuria; BSP retention was tested on both and was found to be 15 and

TABLE 2

Family	Case No.		Date	Urine U.P. μg./100 ml.	Urine C.P. μg./100 ml.	Feces C.P. μg./gm. (dry weight)	Feces P.P. μg./gm. (dry weight)
		I	Sept., 1958	9	14	—	—
	A.B.	♂	Mar., 1960	7	12	16	54
		II	Sept., 1958	22	14	—	—
B	J.B.	♂	Mar., 1960	24	17	10	52
			May, 1966	1	3	13	24
		III	Oct., 1957	4,913	1,087	—	—
			Mar., 1958	49	18	—	—
			Oct., 1959	13	7	—	—
	H.B.	♀	June, 1960	31	4	3	0
			Feb., 1962	6	3	5	24
			May, 1966	5	6	8	11
		IV	Sept., 1957	1,750	213	—	—
	S.L.	♂	Apr., 1958	106	12	—	—
			Sept., 1958	178	8	—	—
			June, 1960	133	5	19	0
			Sept., 1960	101	8	8	23
			Mar., 1961	67	9	5	16
		V	Oct., 1957	0	94	—	—
	B.L.	♂	Apr., 1958	10	8	—	—
L			Mar., 1961	22	34	—	—
		VI	Oct., 1957	12	17	—	—
	G.L.	♂	Apr., 1958	10	26	—	—
			Sept., 1958	16	20	—	—
			Mar., 1961	13	17	—	—
			Feb., 1962	—	—	1	11
		VII	Oct., 1957	10	35	—	—
	C.L.	♂	Apr., 1958	10	22	—	—
			Mar., 1961	12	19	—	—
			Feb., 1962	—	—	14	40
		VIII	Dec., 1959	167	31	68	27
G	Twins S.G.	♂	May, 1966	270	50	129	129
		IX	Dec., 1959	77	10	21	20
	s A.G.	♂	Normal	≤10	≤15	≤6	≤40

16 per cent. (Upper limit of normal 10 per cent.) The porphyrin data are seen on the Table 2. One brother was re-examined in 1966.

Evidently, the cutaneous symptoms of porphyria cutanea tarda were concordant among these twin brothers. They also have the same drinking

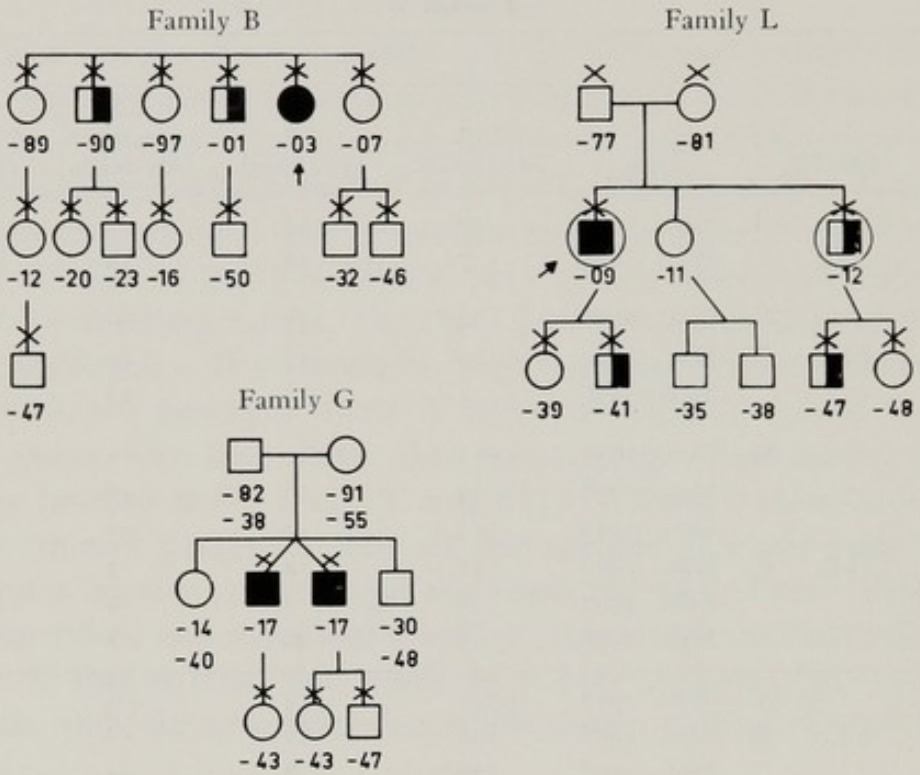


FIG. 13—Shows our positive findings that may indicate a constitutional basis also for porphyria cutanea tarda. The pedigrees are discussed in the text.

habits. Considering the rarity of this disease and the common occurrence of alcoholism, this seems to prove a constitutional basis.

We have made thorough investigations of 38 members belonging to the families of our other porphyria cutanea tarda patients with very little success. A large number of our patients are elderly with no or few living sibs and no parents alive. Many of them are also more or less to be regarded as bums, not married or without children, so that following generations cannot be traced. The problem of the genetic base for porphyria cutanea tarda will therefore have to be left to the future.

The fact that Berman and Braun (1962) have reported a high incidence of hepatoma in porphyria cutanea tarda in Czechoslovakia should also be noted.

HEREDITARY COPROPORPHYRIA

An enigmatic mechanism seems to be the basis of the syndromes that have been called hereditary coproporphyria. Isolated cases were described in the European literature as early as 1933 by Hijmans v.d. Bergh

and Grotepass. (See also Watson et al., 1949.) Another observation was published by Watson (1954), who saw 2 men with large amounts of coproporphyrin III in the stool and urine without any clinical symptoms. It is probable that the patient investigated by Dobriner in 1936 belonged to the same group. She had psychiatric symptoms, but they seem to be unrelated to her metabolic disorder. Details about these patients and the further development are not known.

Berger and Goldberg (1955) published a study on a very interesting patient under the title of hereditary coproporphyria. The propositus, an 11 year old boy, had a very complicated clinical history, but symptoms of photosensitivity, colic or neurological disturbances were not noted. It was thought that the patient also suffered from rickets and riboflavin deficiency. His stools contained large amounts of coproporphyrin III. Coproporphyrin III could be isolated from the urine. The uroporphyrin was regarded as being mainly uroporphyrin I. Both the mother and the father had increased amounts of coproporphyrin III in urine and stools but were clinically normal. It is interesting that the mother and the father were cousins and descended from the same grandparents, who must have been carriers of the gene. The authors interpret the condition as being caused by heterozygosity in the father and mother and homozygosity in the affected child. It is evident that the amounts of coproporphyrin found both in stools and urine were much higher in the child than in the parents.

In 1965 Barnes and Whittaker reported a patient, who suffered from severe colic and constipation and whose urine became dark red on standing. It contained large amounts of porphobilinogen and coproporphyrin. Colic and constipation subsided spontaneously, and he has remained well. Several sibs were investigated, and it was found that 4 members in a sibship of 5 had constant, excessive excretion of coproporphyrin. Two sibs beside the propositus had had unexplained abdominal colic. In their paper, the authors state that Ziegler from Switzerland later found considerable increase in δ-aminolevulic acid, porphobilinogen and uroporphyrin in the urine from Berger and Goldberg's patient. This, of course, makes the interpretation still more complicated. There has also been a recent publication from Portland, Oregon, where Cowger and Labbe (1965) described a patient, aged 7, with a family history of hereditary coproporphyria.

The fact that coproporphyrin was the predominant porphyrin metabolite in Berger and Goldberg's family must indicate that the biochemical process is different from acute intermittent porphyria and porphyria variegata, where porphobilinogen forms uroporphyrin and where very

little coproporphyrin is found. A thorough biochemical follow-up in these juvenile cases seems very important. The fact that "porphobilinogen" occurs together with coproporphyrin is hard to explain, if we do not assume that this is a porphobilinogen different from the porphobilinogen that forms uroporphyrin. These matters need further biochemical clarification.

Protoporphyria Erythropoietica

It has long been known that there are persons, who suffer from a typical light sensitivity syndrome without having any obvious disturbance of urinary porphyrins. This disease has usually then been called hydroa aestivale, and it has been regarded as not connected with porphyrin metabolism. We have seen urinary samples from a number of such cases, where nothing of interest was found.

Kosenow (1954) and Kosenow and Treibs (1953) have reported a patient, who showed relapsing photosensitivity, manifesting itself clinically as hydroa aestivale. It was established that she had a large number of fluorescent erythrocytes in the blood and a curious porphyrin metal complex in the serum. No porphyrinuria was found. The literature was discussed in detail and it was established that no similar diseases had been described. In the second paper, Kosenow studied the fluorocytes

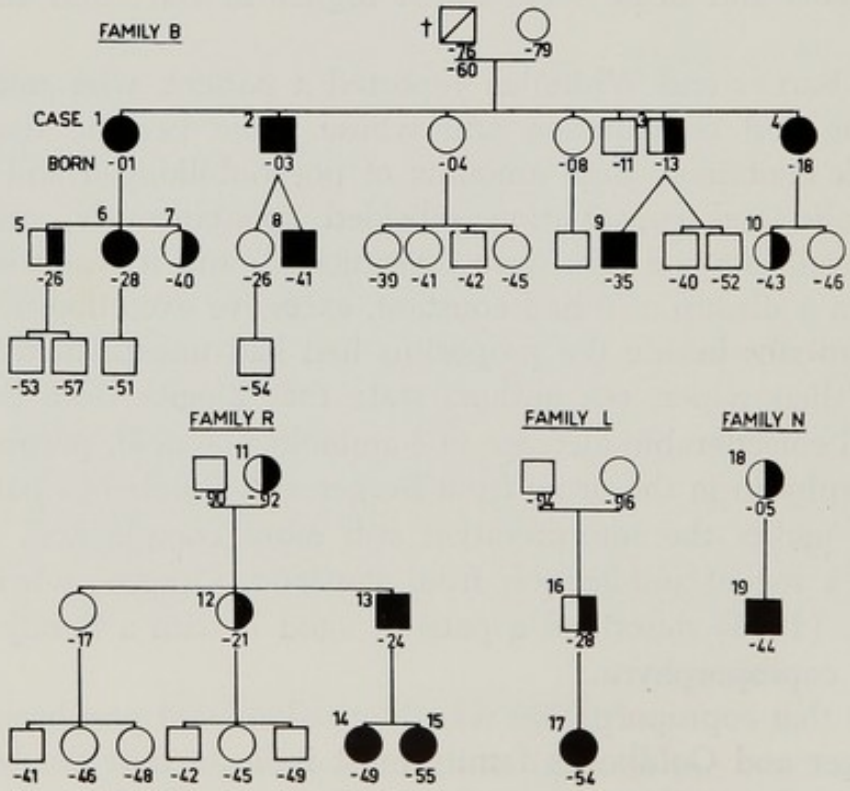


Fig. 14—Families with protoporphyria erythropoietica (Haeger-Aronsen and Krook, 1966. Acta Med. Scand. Suppl. 445).

in the fluorescence microscope, and it was found that these cells represented about 10 per cent of all erythrocytes. The stools contained protoporphyrin and very little coproporphyrin.

In 1960 and 1961, Langhof and collaborators published instances of familial "light urticaria." In the second paper, they were able also to establish the same biochemical picture as had been described by the other German authors. It was found that 2 brothers had increased amounts of protoporphyrin in the red cells. There was no protoporphyrin in the urine, but the stools contained some increase of this substance. In the same year, Magnus et al. (1961) reported a case of the same type and showed that the skin was especially sensitive to ultraviolet light with a wavelength of 400 mμ. Their patient was a man, 35 years old, with no other signs of disease. They coined the excellent name for the disease: Protoporphyria erythropoietica.

Since that time this diagnosis has been made in some 50 cases from different countries.

One of us (B. H.-A) has published 24 Swedish cases together with Krook (1966). Fifteen have symptoms of light sensitivity and 9 are latent. One of the families (R) was described in 1963 by Haeger-Aronsen. In this family the father and both his daughters were affected. The family has been studied further, and the data are presented on Figure 14. It is clearly seen that the 5 families studied all show a pattern well compatible with a dominant inheritance. In the same families there occur both clinically manifest and latent cases, and there are clearly no indications that the latent cases should be heterozygotes and the manifest homozygotes. There is also no indication of sex linkage, and we think that it is correct to assume that the disease is inherited as an autosomal dominant gene with incomplete penetrance. Only further work will show if there might be different biochemical or clinical patterns in this disease, but so far it seems as if it would be homogeneous, both clinically and biochemically.

There are, however, certain difficulties that should be discussed. In the first family the father has had quite severe symptoms since early adolescence. His mother, on the other hand, is an elderly lady, who has never had any troubles with her skin. Biochemically, however, she must be a carrier as her porphyrin metabolism is pathological. It is therefore evident that penetrance must be different in different generations and individuals. In the generation after the father, one girl has quite severe skin symptoms, whereas the other only suffers a little discomfort, for no obvious reason. The porphyrin levels are lower, however. Redeker and Bryan (1964) has observed concordance in identical twins.

We do not know anything about the mechanism at work in this disease. Ever since the heroic experiment of Meyer-Betz (1913), it is clear that severe photosensitization in man may be provoked by the injection of free porphyrin into the blood stream. This porphyrin is possibly attached to the red cells, and it could well be that the protoporphyrin in the red cells is not present inside the membrane, but is just bound to the surface and transported on the erythrocytes. If this were true, it may be that it could be liberated in the skin and there cause fluorescence. We know of conditions, where the red cells contain quite as much protoporphyrin without the development of light sensitivity, i.e., in lead poisoning. This means that the protoporphyrin in the red cells could hardly alone be responsible for the light sensitivity.

Regarding the incidence of this disease, we do not know very much. The fact that one of us has been able to collect more than 20 cases in a few years must mean that it cannot be rare. Probably a considerable percentage of the light dermatoses that were previously thought not to be porphyric will be classed in this group, when subtle methods of analysis as porphyrins in the red corpuscles and in the stool will be more commonly used.

It is very difficult to get a clear picture of light sensitization in this condition. It seems undeniable that the porphyrins must have a central role in this development, especially after the very careful studies by Magnus et al. (1961) who could prove that ultraviolet rays of a wavelength corresponding to the Soret band of porphyrins were the most active.

The early German workers noted that skin samples taken from patients with protoporphyria erythropoietica showed a very rapidly waning fluorescence. The biochemical basis was never investigated, but it could well be that these patients have porphyrins in their skin that act as sensitizers. In the excellent monograph, reporting studies of the organs from a patient with congenital porphyria, Borst and Königsdörffer (1929) noted very large amounts of porphyrins in the skin, and it is probable that these contributed substantially to the light sensitivity. In our patients with protoporphyria erythropoietica, there is no visible fluorescence seen on the surface of the skin. It seems as if many enigmas are still waiting for solution (Fig. 15).

Heilmeyer and Clotten (1964) have re-examined the patient, who was first described by Kosenow and Treibs (1953) 11 years earlier. The urine was normal but the red cells are now said to contain coproporphyrin and to show a moderate increase in proto- and uroporphyrin. The feces contained normal amounts of copro- and some dicarboxylic porphyrin. It

is evident that it is still necessary to study a great many cases with this type of disease before we can decide, if it is homogeneous or if coproporphyrinuria may in some way be related. So far the many

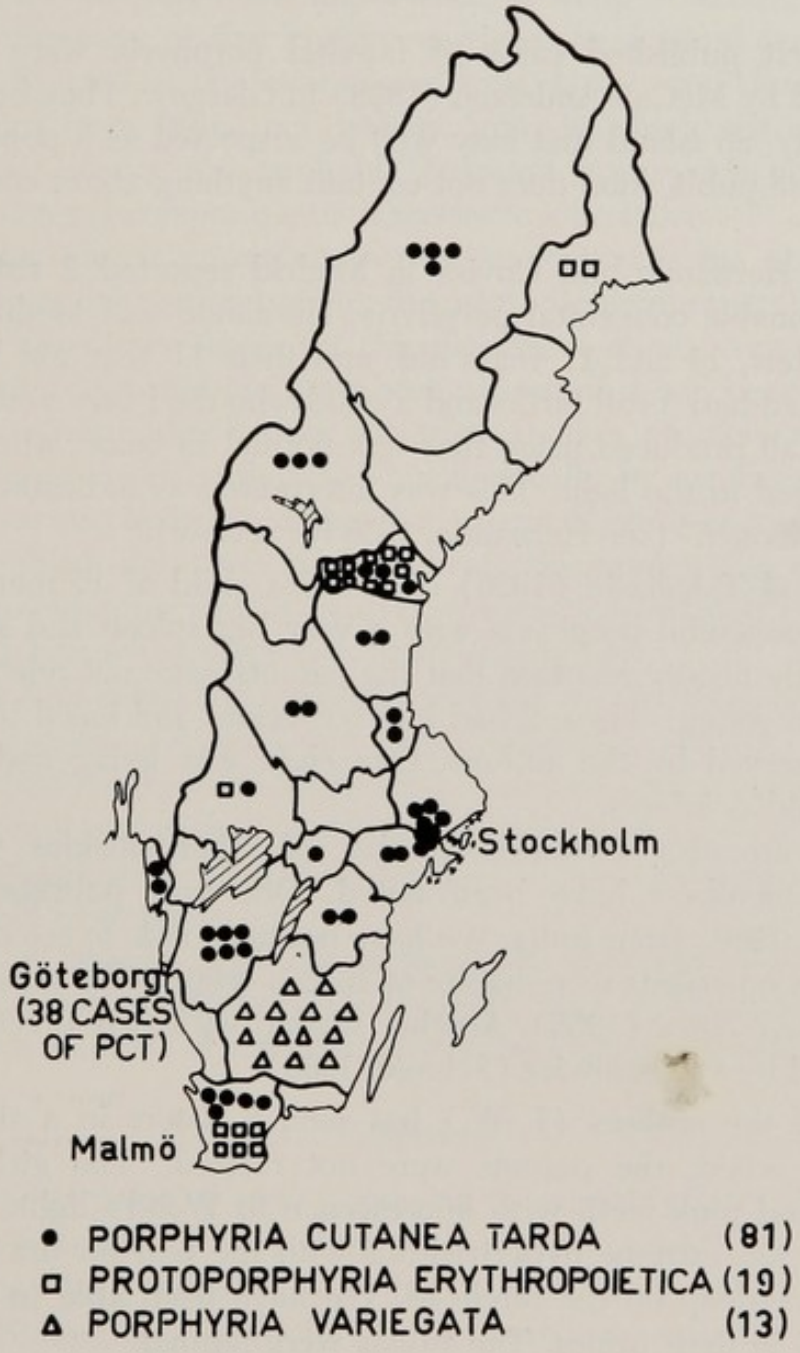


FIG. 15—The map shows all the patients with porphyria cutanea tarda, porphyria erythropoietica and porphyria variegata known to us from different parts of Sweden. The 38 cases from Göteborg have been especially studied in the Department of Dermatology in this City. It is clear that porphyria cutanea tarda occurs all over the country, whereas the 2 families with porphyria variegata both come from the same province even if the diagnosis was made in several places. The accumulation of porphyria erythropoietica in 3 provinces is explained by the presence of a small number of affected families. No predilection for Lapland as in porphyria acuta intermittens is found.

different cases from Sweden have shown the same biochemical pattern as the first German and English patients.

Congenital Porphyria

The first published cases of familial porphyria were two brothers described by McCall Anderson (1898) in Glasgow. They both came from Stornoway, an island that may well be suspected as a population isolate even if the publication does not contain anything about consanguinity of parents.

Later, Hernando and Covisa in Madrid reported 2 sisters, who had unquestionable congenital porphyria. Hernando and Medina observed 2 other sisters, 14 and 17 years old, and their 11 year old brother. Their mother had had 3 still births and a child who died very young. The living children all produced urine that was normal in color, when voided, but became red in the light. This was interpreted as indicating excretion of "porphyrinogen" (see Hernando, 1938 for review).

Sato and Takahashi (1926) observed a child of 15 months, who had typical congenital porphyria with a very big spleen and severe anemia. The family history revealed that the parents were not related. Two children died young. These 2 had had red urines and facial blisters like the child observed by the authors. One child was living and normal. The others died as infants.

There are also a limited number of other sibships where several affected members have been noted. One was published by Taneja (Schmid, 1966) from India. We have not been able to see this in original, but the two patients were babies of Indian origin. The same is true of the Chaudhuris' cases (1958). Another sibship with two affected sisters was published by W[illegible]mowicz (Schmid, 1966).

One of the authors (J. W.) has seen 2 sisters in a sibship of 6 in Holland, where the parents were not related. The girls had typical blisters and pink teeth with fluorescence in Wood's light. Most remarkable was the extreme hypertrichosis with the hair on the scalp growing down very deep on the forehead. We have been able to examine ALA and PBG in their urines. The values were normal.

The fact that there are several sibs affected by the disease even when the parents were found to be normal on careful biochemical examination decidedly speaks for a recessive mode of inheritance. The number of sibships with several affected members is small however.

It has been postulated that the disease is more common in the male. As Schmid (1966) points out this is certainly explained by the fact

that many cases with porphyria cutanea tarda had a wrong diagnosis. Congenital porphyria is equally frequent among the two sexes.

Consanguinity among parents was noted several times. It is, of course, always impossible to judge the importance of such a finding without knowing the frequency of first cousin marriages in a given population. This is especially true as one case comes from Japan, one sibship from Spain, 2 sibships from India, one patient from England and one from Germany. At all events, this probably frequent occurrence of consanguineous marriages is compatible with recessive inheritance.

Another factor that is important in this connection is the absence of transmission from one generation to the next. Remembering the great interest in the hereditary factor for this disease, it seems very probable that such inheritance would not have been missed, if it had been present. It seems safe to assume that it does not exist. Another problem would be if the heterozygote could be detected biochemically by careful examination. Nobody has succeeded in doing that except Heilmeyer and Clotten (1964), who publish data that might indicate the presence of heterozygotes. These investigators are, of course, of great interest and should be repeated by workers who have access to other families.

The primary data regarding complete sibships are very scanty and not always very reliable. Therefore, it seems premature to try to make calculations regarding the ratio of affected to normal sibs, especially as it does not seem improbable that an affected fetus may be aborted. Several reports regarding abortion have been published, even if the diagnosis of porphyria has not been made.

In summary, it may be said that all available facts indicate recessive inheritance. The possibility that heterozygotes may be found by using modern sensitive methods of screening should be tested in more families.

Animal "Porphyria"

From the genetic point of view porphyria in animals might, of course, be of special interest as breeding experiments could solve many problems. It has long been known that porphyria in cattle may be inherited and Rimington (1936) has studied such animals first together with veterinarians (Fourie, 1936) in South Africa and later also in England (Amoroso et al., 1957). The clinical picture with light sensitivity and brownish discoloration of the skeleton by uroporphyrins is most like congenital porphyria described by Günther (1912). Rimington (1936) published results proving that a father-daughter mating produced an affected calf, when neither of the parents showed any symptoms in spite of the fact that they belonged to a porphyric herd.

With (1960) has also studied porphyria in cattle and in pigs. The clinical histories given in his paper are not very detailed, and this author makes the clinical diagnosis in pigs from brown coloration of the teeth that is obviously caused by uroporphyrin (Jörgensen and With, 1955). Analysis of urine and stools speak in favor of the assumption that we have a biochemical pattern similar to Günther's disease. It is interesting, however, that uroporphyrin is found in larger quantities than is coproporphyrin in porcine porphyria, whereas the urine from porphyric cattle contains about the same quantities of both porphyrins. It must be regretted that With's experiments with breeding were not very successful owing to the low vitality of the affected animals, but it seems probable that there is a dominant inheritance in pigs. Any detailed investigations of porcine porphyria from other places are not known, but a sibship of such pigs has been observed in New Zealand. These animals were black and did not show any light sensitivity. The urine contained uroporphyrin I and feces contained coproporphyrin I.

The latest publication regarding porphyric cattle was published in 1965 and comes from Minnesota. Breeding experiments were performed and confirmed the assumption already made by Fourie and later also by Rimington on a family of English cattle that the inheritance was recessive. In animals with white and black hair, light sensitivity was only seen on the white parts (Wass and Hoyt, 1965).

We may therefore assume that a disease clinically identical with congenital erythropoietic porphyria (Günther) in man exists in cattle and has the same inheritance. Whether porcine porphyria has the same biochemical mechanism or not cannot be decided yet, but there is evidence that this trait is inherited as a dominant.

Cattle with porphyria have now been observed in many countries: South Africa, Britain, Denmark, Jamaica, Canada and the United States. Fink and Hoerburger (1931) published some uroporphyrin I isolations from so-called "ochronotic" bones observed in a German slaughter house. It is quite clear that the bovine disease occurs in different parts of the world, whereas porphyric pigs only seem to have been observed in Denmark and New Zealand so far.

The fact that mutations occur among animals leading to inborn errors of metabolism similar to porphyria in man is easy to understand. Zoopathology gives us another much more enigmatic observation regarding the common occurrence of "porphyria" as a normal phenomenon among the ground squirrels (Sciurus niger) in North America. The urines contain uroporphyrin normally, and the bones are brownish showing bright red fluorescence in Wood's light (Turner, 1937). To us this is a

most remarkable fact that could probably be compared to the well-known metabolic disturbance of purine metabolism in Dalmatian dogs, but as far as is known not in any other canine race. It is said that this metabolic defect is not only present in one special family of Dalmatians, but in all such dogs. This would mean that there is an absolute linkage between the gene that causes the metabolic defect and those responsible for the characteristics of a Dalmatian. In the squirrels, we would find something that could be interpreted as a metabolic defect similar to porphyria that is also a characteristic of one species of Sciurus. This seems to prove that hereditary metabolic disease in man may occur as "normal" conditions in certain animal species without becoming detrimental to their health. Perhaps the opposite is true; they may be advantageous.

The ground squirrel seems to be the only known example of "normal porphyria," genetically determined. There are, however, reports that an owl, Asio flammeus has a skeleton that contains large amounts of uroporphyrin (Derrien, 1926).

In the feathers of birds belonging to the genus Turacus and related genera, a copper complex of uroporphyrin III is normally present and gives the feathers a deep red color that is easily washed away by slightly alkaline solutions. (Fischer and Hilger, 1924).

Pteria radiata is a mussel from the Persian Gulf. Fischer and Haarer (1932), in Hans Fischer's institute, demonstrated the presence of uroporphyrin I in such shells. Waldenström had an opportunity to re-examine the residual porphyrins after crystallization of uroporphyrin I. Two porphyrins were found, one that was obviously uroporphyrin I and a second with a melting point of 238° C. Both were insoluble in ether and were therefore not coproporphyrins. Decarboxylation gave several fractions. One seemed to be identical with coproporphyrin III. It is interesting that the bulk of the uroporphyrin in this animal porphyria is also uroporphyrin I as is the case in congenital porphyria of man. Also, the bones and urine of Sciurus niger examined by Turner (1937) gave uroporphyrin I as the predominant porphyrin. Therefore, it seems correct to speak about physiological porphyria in these animals.

Some of the porphyrias represent excellent examples of what we now call pharmacogenetics. This group of diseases was first clearly recognized when the mechanism for hemolytic anemia after plasmoquine and the increased susceptibility to muscle relaxants of succinylcholin type had been found to depend on genetic traits. In both these conditions it seems clear that there is a defect in one enzyme that makes the patient susceptible to the influence of certain chemical compounds. The relation between plasmoquine and other quinine derivatives and the erythrocyte

enzymes is still not established as a biochemical mechanism. On the other hand, the relation between succinylcholin and the deficient cholin esterase seems to be clear-cut.

There are 2 types of drugs that seem to be especially dangerous for patients with porphyria. One is ethyl alcohol in porphyria cutanea tarda. This relationship is not proved experimentally, but there is indirect evidence in so far as alcoholism seems to be a prerequisite for PCT. We know how toxic alcohol may be for liver cells and liver enzymes generally. From an American study we know that liver cirrhosis as an anatomical finding was present in 30 per cent of 80 consecutive patients with alcoholism, where the liver was biopsied. From what we know about bromsulphalein loads in acute and subacute alcoholism, it seems as if one solid dose of alcohol may be very detrimental to the excretion of bromsulphalein even in persons with normal livers. Acute loads of alcohol also produce changes in coproporphyrinuria and the appearance of liver enzymes in the blood. These are therefore instances that do not belong to pharmacogenetics, but rather pure toxicology. It seems tempting to assume that porphyria cutanea tarda could present an analogous toxic condition regarding porphyrins. There are a few examples where normal persons have been given considerable doses of alcohol and their urinary porphyrin excretion has been determined. It was found to be slightly but definitely increased. This is, however, very different from the porphyria cutanea patients, who excrete large amounts of uroporphyrin in their urines even on quite moderate exposure to alcohol, whereas the majority of alcoholics do not show any increases in uroporphyrin. Therefore, it seems quite probable that a genetic factor is responsible for a latent porphyria that appears manifest only after chronic alcoholic intoxication. The same idea might explain why only a certain percentage of alcoholics develop liver fibrosis.

Lead poisoning is completely different. We know that every person, who is exposed to high amounts of lead, develops an increased level of ALA in the urine and blood, and also coproporphyrinuria. Opinions are different regarding the presence of uroporphyrin. We have worked much with experimental lead poisoning in rabbits and also with industrial human plumbism and have never been able to demonstrate any increased amounts of uroporphyrin. On the other hand, Watson and his group have reported slight increases in uroporphyrin from such urines. Waldenström has seen a patient, who died from acute porphyria after being exposed to lead. In spite of the fact that he lived in northern Sweden, we could not trace any other porphyrics in the family. It could, of course, also be maintained that his lead exposure had nothing to do

with his attack of porphyria. If this is true the combination might be a pure coincidence. We do not know anything about acute porphyria in animals, and it is therefore impossible to make experiments. Of course, it is impossible to expose latent porphyrics to lead as we do not know how severe an attack could become.

In the last century cases of porphyria acuta sulphonalica sive trionalica were seen as examples of so-called toxic porphyria. Attempts to produce porphyrin disturbances in rabbits by intoxication with sulphonal and trional have given no clear-cut results. It is also clear that only a small minority of the many patients treated with these 2 drugs developed severe, often lethal, symptoms. Waldenström has tried to locate some early patients from Sweden with known sulphonal porphyria, but without results. One person, however, who had 2 daughters with typical porphyria acuta intermittens but who had never himself suffered from an attack of porphyria, developed symptoms of this disease when he was given trional. This seems to be the only definite link between this classic type of toxic porphyria and "idiopathic" porphyria acuta intermittens.

We have analyzed the material from Sweden in great detail to examine the connection between the intake of barbiturates and the development of severe porphyric symptoms. We believe that abdominal colic may develop without any external cause, but we have never seen a patient with severe symptoms, such as localized or widespread paralysis, who has not previously been treated with barbiturates. Goldberg (1959), who has a large experience in Great Britain, is of the same opinion, and the concept that barbiturates and similar remedies should be absolutely forbidden if porphyria is suspected has gained widespread acceptance. It seems to us that the great decrease in mortality from porphyria could be explained chiefly by the fact that the diagnosis is made more rapidly and the patients are, therefore, not exposed to dangerous drugs.

Our knowledge regarding the biochemical mechanisms that are deranged in the different porphyrias is extremely meager. The easiest explanation would be to assume *a priori* that a metabolic block exists at some stage in the hemin cycle. The fact that ALA and PBG are formed in excess in porphyria acuta intermittens might, of course, also be explained by assuming that there could be another block on the way from the glycine pool to some other metabolite (serine has been suggested) and this would make more glycine available for synthesis of ALA. Such theories have been tested experimentally, but so far not successfully. From the work of Granick and Urata (1963), it becomes evident that increased synthesis of ALA may be induced by 3, 5-diethoxycarbonyl-1, 4-dihydro-2,4, 6-trimethyl pyridine that is strongly porphyrogenic and

cause stimulation of the corresponding synthetase. At present it seems as if induction of ALA synthetase is the most likely explanation for porphyria acuta intermittens. Watson (1966) has recently discussed the hypothesis that a similar ALA synthetase induction could also explain erythropoietic porphyria, if this enzyme increase happens in the normoblasts in the latter disease and in liver cells in the former. The reader who is interested in these problems should consult recent reviews by Rimington (1966) and by Watson (1966). While all these theories are very stimulating, it must be agreed that we still know too little about the basic mechanisms. The fact that spontaneous recovery is so common in porphyria acuta intermittens makes one believe that a correct picture of the biochemical mechanisms could lead to rapid restoration of health by correction of the basic biochemical disturbance.

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Chapter 4

Genetic Aspects of Liver Disease Associated with Jaundice

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In accordance with most other types of human disease, the genetic aspects of liver disease associated with jaundice have, with few exceptions, been poorly studied. This results from 3 factors, the longevity of the human race so that studies through several generations are impossible, the difficulty in separating environmental from genetic factors, and until recently, lack of interest of clinical investigators in genetics. The present paper summarizes the available information concerning the genetic aspects of the various types of disturbed bilirubin metabolism causing jaundice in man.

Bilirubin Metabolism

Jaundice results from a disturbance in bilirubin metabolism so that this pigment accumulates in the blood and the tissues become colored yellow. The normal total serum bilirubin level is 0.4–0.8 mg./100 ml. of which 0.1–0.5 is conjugated. Jaundice is observed when the level exceeds 3 mg./100 ml.

Bilirubin is produced in the reticuloendothelial system from the protoporphyrin portion of bilirubin released from effete erythrocytes. About 15 per cent so-called "shunt" bilirubin is not formed from destruction of mature erythrocytes and some of this may come from hemes in the liver (Robinson et al. 1965).

Glucuronide conjugation occurs in the liver so converting the lipid

soluble bilirubin into a water soluble conjugate for excretion into the bile. This process is mediated by one or more microsomal transferase enzymes. Unconjugated, and probably conjugated, bilirubin is transported in the plasma bound to albumin.

An enterohepatic circulation can be demonstrated for unconjugated bilirubin which is lipid soluble and bacterial action on conjugated pigment results in its deconjugation. This happens in the colon where absorption is limited.

Urobilinogen is formed from bilirubin glucuronide by bacterial action and since urobilinogen is absorbed poorly from the colon a large enterohepatic circulation of urobilinogen seems unlikely.

Mechanisms of Jaundice

Disturbances of bilirubin metabolism result at any stage from formation to excretion into the intestine (Sherlock, 1962, 1966). At each stage genetic influences must be concerned and these will not be considered in detail.

Hemolytic Jaundice

Excess hemolysis causes an unconjugated hyperbilirubinemia due to an overload of pigment on the liver cell. The scope of this article does not include genetic aspects of all hemolytic anemias which are well covered in standard textbooks. Two types must be discussed. Glucose-6-dehydrogenase deficiency in the erythrocytes is inherited in an X-linked fashion. It can cause neonatal jaundice. Instances have been reported from many parts of the world including Greece (Doxiadis and Valaes, 1964), Israel, (Freier et al., 1965), Nigeria (Gilles and Taylor, 1961) and Hong Kong (Yue and Strickland, 1965). The incidence of this defect among the population may be very high, but jaundice seems only to develop when there is some precipitating factor such as the administration of acetylsalicylic acid phenacetin, or sulphonamide transmitted in maternal milk, vitamin K analogues by injection or antiseptic preparations applied to the umbilicus. In some instance neonatal jaundice may be cholestatic even though the primary cause is hemolytic.

Shunt Hyperbilirubinemia

In this rare type of jaundice bilirubin is produced, probably in the bone marrow, in excessive amounts from sources other than the mature, effete erythrocytes. It probably represents an exaggeration of the early (4-5 days) bilirubin production from heme described by Yamamoto and co-

authors (1965). The original 4 cases included 3 in one family (Israels, Suderman and Ritzmann, 1959) so that the condition must, at least, be familial. These patients were of German origin. Two brothers of Italian descent have also been described with the condition (Arias, 1962). The mode of inheritance is not known.

Disturbances in Bilirubin Uptake

Gilbert's Disease

The best example of disturbed bilirubin metabolism due to this cause is the Gilbert's type of familial, unconjugated, nonhemolytic hyperbilirubinemia. Here bilirubin overload is excluded, conjugation is normal but there is good indirect evidence that the uptake of bilirubin into the liver cell is defective (Billing, Williams and Richards, 1964). This is a common condition usually diagnosed, by chance, at the time of a routine medical examination or when the blood is being examined for some other reason. It carries an excellent prognosis.

The number of cases involved in a family is difficult to determine for the serum bilirubin may be only minimally elevated and not constantly at that. Values greater than 3 to 4 mg./100 ml. are unusual. Foulk and co-workers (1959) have reviewed the genetic aspects. In one series, 8 of 58 patients gave a family history of jaundice and in 5 of these it had been present in successive generations. In another series of 15 patients hyperbilirubinemia was found in 26 per cent of the parents and 55 per cent of the siblings. Single families are also reported with evidence of transmission between 3 and 4 generations. It seems likely therefore that the mode of inheritance is dominant. Incomplete penetrance has been invoked to account for sporadic cases without familial involvement. Variable penetrance must be assumed to explain the absence of overt involvement in parents although latent disturbance can sometimes be seen in these people if a bilirubin loading test is done.

Disturbances of Bilirubin Conjugation

These may be due to deficiency of the amount of glucuronide conjugating enzyme present in the microsomes of the liver cell or to inhibition of its function.

Inadequate Enzyme

Prematurity. Premature infants provide the classic example of enzyme deficiency and this is the principle explanation of jaundice of immaturity

or so-called "physiological" jaundice. This has no obvious genetic implications except to act as an exacerbating factor to other genetically determined disorders of bilirubin metabolism manifest in the neonatal period. These include hemolytic disease of the newborn and enzyme deficiencies in the red cells.

Crigler-Najjar. This type of familial unconjugated hyperbilirubinemia is extremely rare but causes a very severe type of jaundice. The serum unconjugated bilirubin level is very high and, unless artificially reduced by exchange transfusion shortly after birth, kernicterus is a complication. In contrast to the Gilbert's syndrome, in this condition a definite deficiency in enzymatic conjugation in the liver can be shown.

In the original series of 7 patients 3 came from 3 related families (Crigler and Najjar, 1952). None of the parents was jaundiced and a recessive mode of inheritance seems likely. However, another family is described in which the father and one child suffered from severe unconjugated hyperbilirubinemia, a second child was unaffected, the mother was unrelated to the father and seemed unlikely to be a carrier (Sugar, 1961).

Inhibition of Enzyme

In 1958 Lathe and Walker demonstrated that serum from pregnant women and their newborn infants readily inhibited the formation of conjugated bilirubin by rat liver slices *in vitro.* In 1960 Lucey, Arias and McKay described a type of familial neonatal jaundice in which no other cause could be found and which was later attributed to the presence of excess inhibitory factor in the serum of the mothers and babies (Arias et al., 1965). This inhibitor was unidentified but was probably a progestational steroid able to inhibit glucuronyl transferase activity in the liver of neonates. Twenty infants of 8, unrelated, healthy, Caucasian mothers have been described. It is difficult to decide whether this is an inherited abnormality in the mother. Certainly every sibling was affected but the mothers were unrelated and there was no family history of severe or prolonged neonatal jaundice.

This condition must be distinguished from the prolonged neonatal unconjugated hyperbilirubinemia associated with breast milk. In this condition the sufferers are unrelated and the serum from mothers and infants does not contain any substance inhibiting bilirubin conjugation (Arias et al., 1964) although such a substance is present in the maternal milk.

Disturbances in Biliary Excretion

Dubin-Johnson and Rotor Syndromes

The Dubin-Johnson form of familial conjugated hyperbilirubinemia is of cholestatic type although some of the features of cholestasis such as pruritus and raised serum alkaline phosphatase and bile salt values are absent. The hepatic histological picture is characterized by the presence in the liver cells of large amounts of a brown lipofuscin-like pigment so that the condition has been termed "black liver jaundice."

The Rotor syndrome resembles the Dubin-Johnson except for the absence of brown pigment in the liver cell. Both carry an excellent prognosis.

A family history is obtained in about a third of patients with the Dubin-Johnson syndrome. Transmission of the disease is through successive generations, proved both biochemically and by study of hepatic histology through liver biopsy (Beker and Read, 1958; Wolf et al., 1960).

A large family of 242 living persons has been studied many of whom have some evidence of the Dubin-Johnson syndrome (Butt et al., 1966). The propositi were 2 sisters with the condition. The disease was seen in 3 generations and 29 of 38 family members had liver biopsies. There was no correlation between liver pigment and serum bilirubin. An interesting counterpart of the Dubin-Johnson syndrome exists in the animal world. A mutant strain of Corriedale sheep have a similarly inherited disorder which includes the presence of pigment granules in the liver cells (Arias et al., 1964).

The Dubin-Johnson and Rotor syndromes seem to be inherited as dominant characteristics with considerable variability of expression.

Familial Nonhemolytic Hyperbilirubinemias

It must be questioned whether the various forms of familial nonhemolytic hyperbilirubinemia—Gilbert's, Crigler-Najjar, Dubin-Johnson and Rotor—should be considered individually or, despite the different mechanisms of jaundice, as a group. It does indeed seem that there is much overlap between one type and another. The overlap between Dubin-Johnson and Rotor syndromes is shown by finding patients from the same family with conjugated hyperbilirubinemia but with or without pigment in the liver cells (Wolf et al., 1960; Butt et al., 1966; Arias, 1961; Sagild, Dalgaard and Tygstrup, 1962). Pigmented livers have been found in patients with unconjugated hyperbilirubinemia (Butt et al., 1966; Sagild et al., 1962). In the large family studied by Butt and his group

(1966) the propositi had the classic Dubin-Johnson picture but the commonest abnormality in the family was unconjugated hyperbilirubinemia. Moreover, patients with Dubin-Johnson syndrome were found to have abnormal handling of unconjugated pigment which would not be expected when the defect is solely in the excretion of bilirubin into the bile channels (Billing et al., 1964). Similar changes were found in 3 of 5 asymptomatic relatives, one of whom had a slightly raised basal serum unconjugated bilirubin level. None of the relatives showed the typical secondary rise in conjugated bilirubin level seen in the propositi.

Such observations add to the confusion in attempting to make a genetic explanation of the families involved or in deciding the mode of inheritance.

Idiopathic Recurrent Cholestasis

The patients suffer from multiple episodes of cholestatic jaundice of unknown etiology. Extrahepatic biliary obstruction is excluded by laparotomy or cholangiography. Permanent liver damage does not develop and recovery between attacks seems to be complete. One patient has had 22 attacks and 3 laparotomies to exclude a possible obstruction (Williams et al., 1964).

This condition might be of genetic or environmental origin or indeed both. In favor of the genetic possibility is the early age of onset, 50 per cent of reported cases starting before the age of 10 years. Kuhn (1963) has observed the condition in 2 brothers. Tygstrup (1960) described 2 unrelated cases from an isolated island in the Faroes. Since then he has observed 7 other cases, one of whom has a sister with long periods of pruritus and who has been jaundiced twice (Tygstup, 1966). She has never been examined while suffering an attack and it is uncertain whether the condition is really benign recurrent cholestasis. The condition appears to be inherited in an autosomal recessive fashion. Some of the patients have an allergic diathesis, rashes may be associated and the condition may recur at the same time of the year.

The Biliary Atresias

These defects may affect any level in the biliary tract even to the smallest interlobular bile duct. In 1932 Sweet reported 3 siblings with this condition but the evidence he gave was not sufficient to enable the accuracy of the diagnosis to be ascertained. Krauss (1964) described 2 male siblings with congenital extrahepatic biliary atresia. Hopkins (1941) described one patient of whom an aunt on the mother's side of the family had been icteric throughout her life. Of her 6 pregnancies, the 5 delivered

were icteric and in 3 postmortem examination showed occlusion of the common bile duct. Whitten and Adie (1952) described 2 cases in siblings. Rumler (1961) described 4 cases in 2 generations. In spite of these reports, other publications have failed to describe a familial incidence. Danks and Bodian (1963) made a genetic study of 58 cases of atresia of major bile ducts and 7 with intrahepatic atresia. There was no instance of another affected person among the first or second degree relatives. In the extrahepatic type there were 90 siblings, 207 aunts and uncles, and 410 first cousins, all unaffected. In 6 other reported patients with intrahepatic biliary atresia there was no familial incidence (Ahrens et al., 1950; Haas and Dobbs, 1958). The difficulty probably lies in distinguishing atresias from neonatal hepatitis in which condition a strong family tendency can be shown (see later).

Idiopathic Cholestatic Jaundice of Pregnancy

This rare condition is seen during the last trimester of pregnancy when the woman develops a cholestatic jaundice with prominent itching. These disappear after delivery but reappear with subsequent pregnancies. The condition seems to have a variation in geographic incidence which might represent an environmental rather than a genetic factor. However, the countries where the incidence seems to be particularly high, such as Chile (Katz, Velasco and Reyes, 1966), Scandinavia (Thorling, 1955) and Switzerland (Haemmerli, 1966) seem to have little in common. There seems to be no familial incidence.

These patients have an inherent predisposition to cholestasis as evidenced by their response to oral contraceptives. Eight of reported 20 patients who had suffered jaundice while taking the contraceptive pill in therapeutic dosage have previously suffered from the cholestatic jaundice of pregnancy (Sherlock, 1966). It is tempting to regard the cholestatic jaundice of pregnancy as an exaggerated response in a susceptible woman to some steriod or steriods normally produced in pregnancy. Before introducing this method of population control, it would probably be wise to ascertain whether or not the cholestatic jaundice of pregnancy is common in the population exposed. A more careful genetic study should be made both of patients with cholestatic jaundice of pregnancy and those who develop jaundice while taking the contraceptive pill.

Hepatocellular Jaundice

In this type it is impossible even to attempt to localize the exact mechanism of the disturbed bilirubin metabolism. The factors concerned

are multiple and include increased hemolysis, disturbed uptake and intracellular transport of bilirubin, problems in conjugation and also in excretion from the liver cell.

Giant Cell Hepatitis

This condition may be a cause of stillbirth or the infant may die soon afterwards. It usually begins in the first 2 weeks of life. Hepatic histology shows a hepatitis with many multinucleated giant cells. This picture may not be specific for any one form of neonatal hepatitis.

The appearance of the disease in successive siblings suggests a genetic factor. Families of patients with neonatal hepatitis show a higher incidence of jaundice than families of sufferers from atresia of the bile ducts. An autosomal mode of inheritance is suggested (Hsia et al. 1958). A more detailed genetic study was performed by Danks and Bodian (1963). In 58 cases of extrahepatic biliary atresia there was an absence of a familial tendency. This was also so for 7 patients with intrahepatic biliary atresia. The finding of 4 consanguineous unions among the parents of the cases of "neonatal" giant cell hepatitis was the basis for suggesting a mutant autosomal gene in homozygous form as the cause of the disease. It was also suggested that some infants with a genotype for the disease manifest it so mildly or perhaps so severely that a diagnosis is not made. The apparent excess of male over female cases may be other evidence of failure of manifestation of the genotype. Alternatively, more than one disease may be concerned. It seems very likely that many of the patients with familial "atresia" of the bile ducts may have been suffering from one or other forms of giant cell hepatitis where the genetic basis is much surer.

The literature concerning genetic factors in neonatal hepatitis has been well reviewed by Cassady, Morrison and Cohen (1964).

Galactosemia

This condition causes jaundice, diarrhea and weight loss in the neonatal period and later is shown by mental defect, cataracts and cirrhosis. It is inherited as a single, autosomal, recessive gene. The defect is shown by absence of the enzyme, galactose-1-uridyl transferase which is essential for galactose metabolism (Isselbacher, 1959). The enzyme is absent in the red cell of homozygotes and reduced in heterozygotes (Schwarz et al., 1956).

Wilson's Disease (Hepatolenticular Degeneration)

This is a rare disease, occurring predominantly in young people, characterized by cirrhosis of the liver, bilateral softening and degeneration of the basal ganglia of the brain, and greenish brown pigmented rings in the periphery of the cornea (Kayser-Fleischer rings). It is a condition involving disturbed copper metabolism, large quantities of which are deposited in the tissues. Jaundice is found under two circumstances. Hemolytic crises may mark the onset and punctuate the course. They may be related to destruction of red cells by copper released from the tissues (McIntyre et al., 1967). Jaundice may also develop in the terminal stages of liver failure. Transmission is autosomal recessive. This inheritance is associated with a high consanguinity rate. In Bearn's series (1960) of 32 cases in 30 families there were 14 consanguineous marriages. The cases fell into 2 groups as regards to ethnic origins and clinical biochemical features so that the probability of 2 alleles at one Wilson's disease locus was raised. In one study by Soothill and co-workers (1961) 39 relatives of 2 siblings with the disease were studied and biochemical changes were more severe in the parents than in other relatives. Patients with Wilson's disease have an increased incidence of whorl fingerprint pattern (Hodges, 1962).

The detection of heterozygotes is unsatisfactory. Symptomless homozygotes must also be found for if untreated they will develop the disease. In some heterozygotes serum ceruloplasmin is moderately lowered (Sternlieb et al., 1961). The slope of decline of radioactivity of intravenously injected isotopic copper may indicate a slightly increased body copper store. Incorporation of oral radioactive copper into serum ceruloplasmin is reported to be depressed in heterozygotes but not as much as in homozygotes (Sternlieb et al., 1961). A diminished uptake of radioactive copper by the liver may be seen in the heterozygote (Osborn, Roberts and Walshe, 1963). The claims that heterozygotes can be detected by biochemical tests are, however, quite unconvincing.

Needle liver biopsy can be performed in siblings for early diagnosis when the fatty change and nuclear glycogen are noted even before cirrhosis. The copper content of the biopsy should be estimated (Sternlieb and Scheinberg, 1963), high levels suggest a homozygote. The patient may have no Kayser-Fleischer rings and no neurological involvement (Fisher and Sherlock, 1964).

The genetic defect may cause difficulty in ceruloplasmin formation, although this theory is not supported by the cases of Wilson's disease with a normal ceruloplasmin value.

Idiopathic Hemochromatosis

This condition is believed to be an inborn error of metabolism in which there is increased intestinal absorption of iron. Jaundice is unusual except in the terminal stage of hepatocellular failure or where there is a complicating primary liver cancer. Proven familial cases are rare and the majority seem to arise sporadically. However, in a group of 50 sufferers 3 brothers of patients were found with the disease, 50 per cent of male offspring over 15 years showed raised serum iron levels and other relatives had excess pigmentation, diabetes or cirrhosis (Debré et al., 1958). Essentially similar reports have come from others (Bothwell et al., 1959; Morgan 1961).

Liver biopsy is a surer method of diagnosis than serum iron estimation and increased hepatic deposition of iron has been shown in siblings even when serum iron values are normal. In one series 28 of 46 relatives of 16 patients gave a positive reaction for free iron in the liver and in a further 6 the presence of excess iron was shown by electron microscopy (Williams, Scheuer and Sherlock, 1962). Clinical findings in the siblings and children of sufferers were minimal. The only abnormality was often pigmentation of the skin. One relative, a brother, had definite cirrhosis, this patient also showed the most marked iron deposition.

The disease is usually regarded as being inherited in a dominant fashion but this must be qualified by the term "incomplete penetrance" or expression to explain the small number of overt cases in the families, and relatives who showed some but not all features of the disease. The increased frequency of diagnosis of the disease when liver biopsies are used raises the possibility that the condition may be recessively inherited (Williams et al., 1962). This is also consistent with the less severe disease found in children as compared with the propositi for one would represent the homozygous and the other the heterozygous state. The full disease has been seen in the offspring of a consanguineous marriage where both parents had raised serum iron levels. Here, presumably, both parents carried the abnormal gene and the offspring were presumptive homozygotes (Heilmeyer, 1967).

The nature of the gene defect causing increased iron absorption is not known. At the time of diagnosis in hemochromatotic patients iron absorption is normal but this may reflect saturated iron stores. Absorption increases after venesection therapy (Williams et al., 1966).

MacDonald (1964) believes that the iron deposition in the liver of patients with hemochromatosis reflects increased consumption of iron, usually as alcoholic beverages, but also from iron pots used in cooking or

as medicinal iron. The patients have some other cause for the cirrhosis. These circumstances may well cause increased hepatic iron. This situation differs however, from that found in patients with genetically determined, familial hemochromatosis, most of whom have never consumed excessive quantities of alcoholic beverages or taken iron in other forms. Powell (1966) conducted an interesting family study of patients with idiopathic hemochromatosis and patients with cirrhosis of the alcoholic. There were significantly greater iron stores, and even cirrhosis, in the relatives of patients with idiopathic hemochromatosis than in those of patients with cirrhosis of the alcoholic.

Other Forms of Cirrhosis

The relation between genetic susceptibility and the occurrence of other forms of cirrhosis is not clear-cut.

Certainly the development of cirrhosis does not appear in every alcoholic, the incidence being about 8 per cent of alcoholics receiving psychiatric treatment. Alcoholic cirrhosis can, however, be seen in families. Color blindness is said to be more common in cirrhotics than in a control population (Cruz-Coke, 1964), although this may be a reflection of a malnourished alcoholic population rather than the liver disease *per se* (Fialkow et al., 1966). In Australia and Austria cirrhosis is said to be more frequent in association with blood group A at the expense of blood group O (Billington, 1956; Wewalka 1960).

Family studies of cirrhotic subjects are exceedingly rare. Antibodies to various endogenous tissue substances have been repeatedly demonstrated in patients with nonalcoholic cirrhosis. Antinuclear factor and hypergammaglobulinemia are the most frequently observed (Bouchier, Rhodes and Sherlock, 1964). Elling and co-workers (1966) conducted a family study of 96 siblings and 38 propositi with hepatic cirrhosis. Twenty-two per cent of siblings were found to have antinuclear factor, and 17 per cent gave a positive latex reaction for rheumatoid factor. Hypergammaglobulinemia and thyroid antibodies were not more common among the relatives than in 2 control series. A positive antinuclear factor test was more common in siblings of propositi with a positive test result. The relation of these serological factors to chronic liver disease remains uncertain and this finding should in no way be related to a possible autoimmune cause for the cirrhosis.

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Chapter 5

The Genetics of Muscular Dystrophy

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Introduction

For many years there was considerable confusion concerning the clinical and genetic aspects of progressive muscular dystrophy. However, during the last few years several extensive studies (Stephens and Tyler, 1951; Becker, 1953, 1955; Stevenson, 1953, 1955, 1964; Lamy and de Grouchy, 1954; Walton and Nattrass, 1954; Walton, 1955, 1956a; Blyth and Pugh, 1959; Morton and Chung, 1959) have helped to clarify the situation. From considerations of the known function and fine structure of genes as described in such organisms as *Escherichia coli* and *Neurospora* genetic heterogeneity in a group of disorders such as the muscular dystrophies might be expected. This point has been well argued by Boyer and Fainer (1963). However, the classification of progressive muscular dystrophy into various types is not just of academic interest but is of importance in determining the prognosis in the individual case and for giving reliable genetic counseling. Studies designed to further our understanding of the pathogenesis of these diseases also depend on accurate classification

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because the results of such studies are more meaningful when they relate to clearly defined disease entities. Finally, clinically and genetically different disease entities might be expected to respond differently to a particular therapy. What is effective in one condition may prove to be ineffective or even deleterious in another. Thus, there are good reasons for attempting to classify the muscular dystrophies into various types on clinical and genetic grounds, though only those "types" which prove ultimately to have the same basic biochemical abnormality can be considered real entities.

The terms muscular dystrophy and myopathy are not synonymous; for whereas all muscular dystrophies are myopathies not all myopathies are muscular dystrophies. Myopathy relates to any disorder in which there are signs and/or symptoms attributable to changes occurring in voluntary muscle fibers, where these changes are not secondary to a lesion in the central or peripheral nervous system. The term muscular dystrophy relates to a particular group of myopathies which are genetically determined (Walton, 1963). Though the underlying biochemical abnormality (or abnormalities) in the muscular dystrophies is not known there is no certainty that these diseases are solely diseases of muscle tissue. For example, in myotonic dystrophy the wide variety of manifestations which may occur (such as testicular atrophy, frontal baldness, frontal hyperostosis, small sella turcica and mental backwardness) suggests that the muscle involvement is merely one of the many pleiotropic effects of the mutant gene. Similarly in Duchenne muscular dystrophy several investigators have shown that a significant proportion of affected boys are mentally retarded (Allen and Rodgin, 1960; Worden and Vignos, 1962; Dubowitz, 1965a). The mental retardation could be secondary to metabolic changes in the muscle tissue but this seems unlikely, because there is apparently no progressive deterioration in intellect as the disease progresses (Dubowitz, 1965a). A more likely explanation is that the same biochemical abnormality is responsible for both the muscle dysfunction and the mental retardation. Until more is known of the fundamental biochemical nature of the muscular dystrophies an entirely satisfactory definition of these disorders is not possible. They are a group of hereditary myopathies of unknown etiology.

Based on clinical and genetic differences the more clearly defined types of hereditary myopathies may be classified as follows:

1. Muscular dystrophies
    A. X-linked
        a. Severe type (Duchenne)
        b. Benign types.

B. Autosomal recessive
   a. Resembling Duchenne
   b. Limb girdle.
C. Autosomal dominant
   a. Facioscapulohumeral
   b. Distal
   c. Ocular
   d. Oculopharyngeal.

2. Congenital myopathies
3. Myotonias (autosomal dominant)
   A. Myotonic dystrophy
   B. Congenital myotonia
   C. Congenital paramyotonia.

X-Linked Forms of Muscular Dystrophy

Duchenne type muscular dystrophy (Duchenne, 1868), begins in infancy or early childhood and is characterized by progressive muscle weakness affecting first the lower limbs and pelvic girdle musculature but later affecting the pectoral girdle musculature. Pseudohypertrophy, particularly of the calf muscles, occurs in about 80 per cent of cases. The weakness gradually progresses and ultimately the child becomes confined to a wheel-chair by about the age of 10 and dies at about 20 (Walton and Nattrass, 1954). Evidence that the disease is X-linked has been derived from several sources. In the few cases where affected males have survived to have children, their sons have all been unaffected (Walton, 1955; Morton and Chung, 1959), though it is possible that these cases with prolonged survival represent a different type of X-linked muscular dystrophy referred to as "benign X-linked muscular dystrophy." That boys with Duchenne muscular dystrophy may have the same mother but different fathers (Milhorat and Wolff, 1943; Walton, 1955) also suggests X-linkage but does not exclude the possibility of autosomal inheritance with limitation to the male sex. The latter possibility seems unlikely from the statistical evidence of Morton and Chung (1959). If a gene is rare the frequency of sporadic cases due to mutation is

$$m/(m+1) \text{ for an autosomal dominant gene with sex limitation}$$

and

$$mu/(2u+v) \text{ for an X-linked recessive gene}$$

where m is the coefficient of selection against affected males and u and v are the mutation rates in eggs and sperms. In a highly deleterious condition such as Duchenne muscular dystrophy m approaches unity and

if the mutation rates in eggs and sperm are equal, the expected proportion of sporadic cases approaches ½ if due to a highly deleterious autosomal gene with sex limitation or approximately ⅓ if due to a highly deleterious X-linked recessive gene. Morton and Chung (1959) arrived at a figure of 0.355 ± 0.050 and concluded that this indicated X-linkage.

Typical Duchenne muscular dystrophy has been described in 2 females with XO sex chromosome constitutions (Walton, 1957a; Ferrier, Bamatter and Klein, 1965) and this also suggests X-linkage. In summary, evidence in favor of X-linkage seems very convincing. However, close linkage has not been demonstrated between the gene for Duchenne muscular dystrophy and the Xg blood group locus (Clark et al., 1963; Blyth et al., 1965) or the color vision loci (Philip, Walton and Smith, 1956; Emery, 1966).

It is generally assumed that the gene for Duchenne muscular dystrophy is fully penetrant that is, all males hemizygous for the mutant gene manifest the disease. However, there have been reports which suggest that this is not always the case. Thompson, Ludvigsen and Monckton (1962) described 2 families in which the gene appeared to be nonpenetrant in certain males. In one family, which the authors described in detail, the maternal grandfather had a sister with 4 affected sons and though he himself was apparently unaffected, one of his daughters had a son with Duchenne muscular dystrophy (the proband). It is possible that the proband's mother was not a carrier and that the disease in the proband was the result of a new mutation. Unfortunately no details are given as to whether the carrier status of the proband's mother was determined by serum enzyme studies.

Richterich and co-authors (1963) have also suggested that the gene for Duchenne muscular dystrophy may not always be fully penetrant. In 5 of their families the carrier state, as recognized by a slightly raised level of serum creatine kinase, seemed to follow the pattern of inheritance of an X-linked recessive trait through several generations. However, only single cases of the disease were encountered in each family. Fifteen males in these kindreds were expected to be affected, but none was observed. The authors suggest that Duchenne muscular dystrophy may exist in 2 clinically distinct forms, one with typical manifestations of the disease and a high level of serum creatine kinase, and a latent form with no clinical signs and possibly a slightly raised level of serum creatine kinase. The argument rests heavily on the correct identification of carriers by using serum enzymes and in 2 pedigrees (Nos. 17 and 19), 2 fathers with the apparent "latent" form of the disease (a raised serum level of creatine kinase but no symptoms) had affected sons which would preclude

X-linkage. If individuals hemizygous for the mutant gene do not always develop the disease, one possibility is the operation of one or more environmental factors which prevent the disease being manifest in those who are genetically predisposed. If this were so, it might prove to be of the greatest practical importance. It is important that these observations be confirmed and extended.

Various estimates of the mutation rate for X-linked muscular dystrophy have been obtained: 95 X 10^{-6} (Stephens and Tyler, 1951), 43 X 10^{-6} (Walton, 1955), 60 X 10^{-6} (Stevenson, 1955), 47 X 10^{-6} (Blyth and Pugh, 1959) and 89 X 10^{-6} (Morton and Chung, 1959). These mutation rates are considerably higher than direct estimates for autosomal dominant traits. It may be that genes on the X-chromosome are more subject to mutation than autosomal genes but a more likely explanation is that there are several distinct loci with similar effects (Penrose, 1961). For instance, the above estimates are based on surveys where a distinction between the severe and benign forms of X-linked muscular dystrophy was not made.

The birth of sporadic cases of Duchenne muscular dystrophy, a proportion of whom are presumably the result of new mutations, does not appear to be related to maternal age (Lamy and de Grouchy, 1954; Morton and Chung, 1959). This suggests that the abnormality is the result not so much of gonial age but of a defect in gene replication which in itself may be under genetic control. There is certainly ample evidence that the spontaneous mutation rate in animals is influenced by genetic factors as demonstrated for example, by Demerec (1937) and Neel (1942). Also recent work has shown that specific points, referred to as "hot spots," along the DNA molecule of bacteriophage are more prone to mutation than other points (Freese, 1963). The relevance of these latter findings to man is, of course, conjectural. The finding of a pair of identical twin boys who were discordant for Duchenne muscular dystrophy (de Grouchy et al., 1961) suggests that the disorder may also arise as a consequence of a mutation occuring during early embryogenesis.

In recent years a more benign form of X-linked muscular dystrophy has been recognized by Becker (1955; 1957; 1962). Others have also described families with X-linked muscular dystrophy which began later and in which the progression was slower than in the classical Duchenne type of muscular dystrophy (Levison, 1951; Lamy and de Grouchy, 1954; Walton, 1955, 1956a; Blyth and Pugh, 1959; Emery, 1964a). In the Becker type of muscular dystrophy the disease usually manifests itself in the teens or early twenties, affected persons become chair ridden only after 25 to 30 years and the average life expectancy is only slightly

decreased. Apart from the late onset and benign course of the disease the clinical picture is similar to that of Duchenne muscular dystrophy: weakness begins in the pelvic girdle musculature, only later affects the upper limbs and there is pseudohypertrophy of the calf muscles. In view of the findings of Blyth and Pugh (1959) that severe and mild forms of the disease never occur in the same family, it seems reasonable to assume that the 2 diseases are distinct entities. The genes responsible for the 2 conditions may be allelic but because of the relative crudity of linkage studies in man this may be easier to disprove than to prove (McKusick, 1962). The results of recent linkage studies with the Xg(a) blood group (Blyth et al., 1965) suggest that the genes for the Duchenne type and the Becker type of muscular dystrophy may not be allelic. However, in this study only 2 families with Becker type muscular dystrophy were informative and more families need to be investigated in order to answer this question.

Recently 2 more large families have been described with a benign form of X-linked muscular dystrophy which the authors claim may be different from the Becker type of muscular dystrophy. In the type described by Mabry et al. (1965) it differed in its age of onset and the greater degree of disability which it produced. In the type described by Emery and Dreifuss (1966) the age of onset was earlier than in the Becker type of muscular dystrophy, there was no pseudohypertrophy and muscle contractures were an early and prominent manifestation. Some of the differentiating features between the 4 types of X-linked muscular dystrophy are given in Table 1.

Since the essential biochemical nature of the muscular dystrophies is unknown the subdivision of the benign X-linked form on clinical grounds is somewhat speculative but the clinical grouping of apparently dissimilar types would seem to be an important first step toward an understanding of the pathogenesis of these diseases.

TABLE 1—*Differentiating Features between the 4 Types of X-Linked Muscular Dystrophy*

Type	Usual Age of Onset of Weakness	Course	Pseudo-hypertrophy	Myocardial Involvement	Contractures
Duchenne	<5	severe	+	+	+ (late manifestation)
Becker	5-25	benign	+	?	—
Mabry	11-13	benign	+	+	—
Emery & Dreifuss	4-5	benign	—	+	+ (early manifestation)

Morton and Chung (1959) did not estimate separately the incidence and prevalence rates for the severe and benign types of X-linked muscular dystrophy but Kloepfer (1964) obtained these values by multiplying their combined values by the proportion of cases represented by each genotype. Assuming that 90 per cent of cases of X-linked muscular dystrophy were of the severe type, he estimated the incidence defined as the number per million births who will develop the disease of the severe type to be 250.9 x 10^{-6} from Morton and Chung's Wisconsin data and 159.6 X 10^{-6} based on their pooled data. The respective prevalence rates are 59.2 X 10^{-6} and 37.6 X 10^{-6}. With regard to the benign forms of X-linked muscular dystrophy the incidence rate would be 27.9 X 10^{-6} from Morton's and Chung's Wisconsin data and 17.7 X 10^{-6} based on their pooled data. The respective prevalence rates would be 6.6 X 10^{-6} and 4.2 X 10^{-6}.

Autosomal Recessive Forms of Muscular Dystrophy

Duchenne's original description of the type of muscular dystrophy which now bears his name (Duchenne 1868) was based on 13 patients, including 2 girls. A number of families have since been described in which both brothers and sisters have been affected with a disorder which clinically closely resembles the severe X-linked form of Duchenne muscular dystrophy (Lamy and de Grouchy, 1954; Kloepfer and Talley, 1957; Hammond and Jackson, 1958; Blyth and Pugh, 1959; Dubowitz, 1960; Jackson and Carey, 1961; Skyring and McKusick, 1961; Johnston, 1964). These studies indicate that Duchenne muscular dystrophy may sometimes be due to an autosomal recessive gene. Out of 343 families collected from the literature where adequate details were given, brothers and sisters were affected in 5 sibships, and in 7, a girl was the only affected person (Emery, 1964a). Even if some of the sporadic cases in males were due to an autosomal recessive gene the proportion of cases due to the X-linked form of the disease would still be considerably greater. In some instances the recessive form of Duchenne muscular dystrophy has been described in certain inbred groups (Kloepfer and Talley, 1957; Hammond and Jackson, 1958; Jackson and Carey, 1961). This would be expected if the disease were due to a rare recessive gene.

The autosomal recessive form of Duchenne muscular dystrophy appears to be clinically very similar to the X-linked form though it may be slightly later in onset and somewhat slower in progression than the commoner X-linked type (Jackson and Carey, 1961). The 2 types of Duchenne muscular dystrophy have been distinguished using the algebraic sum of the R and S waves in the first precordial lead of the

electrocardiogram (Skyring and McKusick, 1961). The recognition of the autosomal recessive form is important from the point of view of genetic counseling. An unaffected sister in a sibship in which a brother and/or a sister have the autosomal recessive form of Duchenne muscular dystrophy will not have affected children herself unless she marries a heterozygote, which is most unlikely with such a rare condition. On the other hand, an unaffected sister who has brothers with the X-linked form of the disease stands a 1 in 4 chance of having an affected son. Based on the pooled data of Morton and Chung (1959), the incidence and prevalence rates for this form of muscular dystrophy have been estimated to be 29.6×10^{-6} and 6.9×10^{-6}, respectively (Kloepfer, 1964). In this form of muscular dystrophy the fertility of affected individuals is virtually zero therefore the mutation rate from these figures may be estimated to be 29.6×10^{-6}.

Another autosomal recessive form of muscular dystrophy is the limb girdle type (Becker, 1953; Stevenson, 1953; Walton, 1955, 1956a; Morton and Chung, 1959, Morton, Chung and Peters, 1963). The onset of the disease is in young adult life and affects the proximal musculature of the limbs. Pseudohypertrophy is uncommon and usually the disease is relatively slowly progressive, death occuring in middle age. "Myopathy confined to the quadriceps muscles" may be a form of limb girdle muscular dystrophy (Walton, 1956b, 1963; Mumenthaler et al., 1958; Dubowitz 1965b), using segregation analysis Morton and Chung (1959) found that the proportion of sporadic cases of limb girdle muscular dystrophy (41 per cent) was in fact in excess of the proportion expected by chance if the disease were due to an autosomal recessive gene with full penetrance only in the homozygote. The sporadic cases were significantly less inbred than familial cases but were not clinically distinguishable (Chung and Morton, 1959). These sporadic cases are not due to dominant mutations since all of the 110 children of the sporadic index cases in Morton and Chung's study were normal. Morton and Chung concluded that a likely explanation for these sporadic cases is that some may represent expression of a usually recessive gene in a heterozygote. Morton and Chung calculated that if only 17 out of every 10,000 heterozygous carriers of recessive genes for dystrophy developed manifestations of the disease, either as a result of exogenous factors or the modifying effects of genes at other loci, this would account for the excess frequency of sporadic cases of limb girdle muscular dystrophy. Though some sporadic cases may be due to occasional expression in the heterozygote, some are probably phenocopies due to chronic polymyositis (Walton and Adams, 1958; Pearson and Rose, 1960). Occasionally an

affected heterozygote, by mating with an unaffected heterozygote may have affected children. This has been referred to as "pseudodominance" (Morton et al., 1963).

Based on the figures given by Morton and Chung (1959) and assuming that 59 per cent of cases of limb girdle muscular dystrophy are due to an autosomal recessive gene and 41 per cent are sporadic it can be calculated that for the autosomal recessive form of the disease the incidence is 38.5 X 10^{-6} from Morton and Chung's Wisconsin data and 46.9 X 10^{-6} from their pooled data. The respective prevalence rates are 11.7 X 10^{-6} and 14.2 X 10^{-6}. Comparable calculations for sporadic cases of limb girdle muscular dystrophy give an incidence of 26.8 X 10^{-6} for the Wisconsin data and 32.6 X 10^{-6} for the pooled data. The respective prevalence rates are 8.2 X 10^{-6} and 9.9 X 10^{-6}.

Fertility of affected persons is reduced to about 25 per cent of normal and Morton and Chung (1959) have estimated the mutation rate for the autosomal recessive gene for limb girdle muscular dystrophy to be 31 X 10^{-6} if the effects in heterozygotes are negligible, and 36 X 10^{-6} if all sporadic cases are due to penetrance in heterozygotes. Linkage with any of the recognized autosomal marker traits has so far not been demonstrated.

Autosomal Dominant Forms of Muscular Dystrophy

Facioscapulohumeral muscular dystrophy usually begins in adolescence and is characterized initially by weakness of the facial and pectoral girdle muscles and only after 20 or 30 years does the pelvic girdle musculature become affected. Pseudohypertrophy is very rare. This form of muscular dystrophy is inherited as an autosomal dominant with complete penetrance in both sexes (Bell, 1943; Boyes et al., 1950; Tyler and Stephens, 1950; Becker, 1953; Walton, 1955, 1956a; Morton and Chung, 1959; Morton et al., 1963). As with most autosomal dominant traits facioscapulohumeral muscular dystrophy shows wide variations in expressivity. Some individuals are so mildly affected that they may be unaware that they are suffering from the disease and may go unrecognized unless examined carefully. This is important if a true impression of the incidence of the disease in a particular family is to be obtained (Walton, 1963). Morton and Chung (1959) found the incidence rate for this form of muscular dystrophy to be about 3.8 X 10^{-6} in their own data from Wisconsin and 9.2 X 10^{-6} when based on pooled data from several surveys. The prevalence rates from these same data were 2.3 X 10^{-6} and 5.6 X 10^{-6} respectively. The fertility in this type of muscular dystrophy is nearly normal and the mutation rate has been estimated to be no more

than 50 X 10^{-6} (Morton and Chung, 1959). Linkage with any of the recognized autosomal marker traits has so far not been demonstrated.

The distal type of muscular dystrophy usually begins in middle age and is characterized by muscle weakness and wasting which mainly affects the distal musculature of both upper and lower limbs. Welander (1951) estimated the frequency of this condition to be about 1 in 55 among 1,859 inhabitants of a Swedish "isolate," though it appears to be rare in other parts of the world. She recognized various forms of the disease: "typical" cases in which only the distal muscles were affected and where the disease was slowly progressive, and "grossly atypical" cases where the proximal muscles were also affected and where the disease pursued a more rapid course. She considered the disease to be inherited as an autosomal dominant trait, the typical cases being heterozygous for the mutant gene, whereas the grossly atypical cases were presumably homozygotes. Some support for this view comes from her observation that a marriage between 2 affected persons produced 16 living children, 7 of whom were affected and 2 had the grossly atypical form of the disease with widespread muscle involvement and might be regarded as homozygotes (Welander, 1957). However, this interpretation of the mode of inheritance does not adequately explain Walton's findings (1963). He studied 6 cases of the distal type of muscular dystrophy and though there was no evidence of the disease in any of their relatives, the proximal musculature was affected in all cases and 3 of the patients were unable to walk within 10 years from the time of onset. Perhaps the disease in the north of England is genetically different from that in Sweden.

The ocular type muscular dystrophy is also rare. In this type of muscular dystrophy there is ptosis with weakness of the orbicularis oculi and eventually complete bilateral ophthalmoplegia. Histology of the extrinsic eye muscles and the orbicularis oculi reveal characteristic dystrophic changes (Moses and Heller, 1965). There may also occasionally be some involvement of the neck, trunk and limb muscles (Walton, 1963). The disease usually begins in early adult life (Kiloh and Nevin, 1951) but may even be manifest in infancy (Fuchs, 1890). In their extensive review of the literature Kiloh and Nevin (1951) commented that a family history of ptosis or ophthalmoplegia is found in about half of the recorded cases, and that in these cases it appears to be inherited as an autosomal dominant trait affecting both sexes with about equal frequency. Whereas published pedigrees of such families clearly indicate autosomal dominance the large number of isolated cases described in various reports and reviewed by Kiloh and Nevin (1951) and by Lees

and Liversedge (1962) suggest either that penetrance of the gene is often incomplete, a high mutation rate, or that the condition may be due to the manifestation of more than one gene and that whereas one such gene is clearly an autosomal dominant others may be recessive.

Another type of muscular dystrophy in which the dystrophic process is limited to only a small group of muscles is the so-called "oculopharyngeal" type. In this type of muscular dystrophy ptosis is also a prominent feature but is associated with progressive dysphagia due to dystrophy of the pharyngeal muscles. It usually begins in middle age and is inherited as an autosomal dominant trait. A large proportion of the reported cases have been of French-Canadian extraction (Taylor, 1915; Victor, Hayes and Adams, 1962; Hayes et al., 1963; Peterman, Lillington and Jamplis, 1964). However, in isolated cases of ocular myopathy of late onset reported from many parts of the world (Walton, 1963) pharyngeal involvement has been reported frequently and Bray, Kaarsoo and Ross (1965) have shown that the single most useful distinguishing feature between the ocular and oculopharyngeal cases is the age of onset. Here again, whereas dominant inheritance of the oculopharyngeal cases is often found, the frequent occurrence of isolated cases raises the possibility that sometimes the condition may be due to an incompletely penetrant gene, to spontaneous mutation or to a recessive gene with similar clinical effects. It is interesting to ask why these rare restricted types of muscular dystrophy should often begin late in life and why the dystrophic process affects only a restricted group of muscles. As yet there appears to be no satisfactory answer to these questions.

Carrier Recognition in Muscular Dystrophy

In recent years there has been much interest in devising tests for detecting carriers of Duchenne muscular dystrophy as an aid to genetic counseling. There have been several reports of female carriers of X-linked Duchenne muscular dystrophy with manifestations of the disease (Emery and Lee, 1964). The only manifestation may be enlarged calves (Kryschowa and Abowjan, 1934; Dubowitz, 1963; Walton, 1964b) but sometimes there may be definite muscle weakness (Sidler, 1944; Levison, 1951; Chung et al., 1960; Garcia del Rio and Alaba, 1960; Emery, 1963; Moser et al., 1964; Murphy et al., 1964) and at least one carrier has been described who was so severely affected that this led to her death (Fraser, 1963). However, clinical manifestations in carriers are uncommon and their absence is of no help in genetic counseling. The use of serum creatine kinase for detecting carriers was first employed by Okinaka et al. in 1959 but with little success. Since then, there have been refinements in

the technic of measuring the amount of creatine kinase in serum and roughly 65 per cent of carriers have been found to have significantly raised levels of this enzyme (Table 2). It appears that a much smaller proportion of carriers of benign X-linked muscular dystrophy have raised levels of serum creatine kinase (Wilson, Evans and Carter, 1965; Emery, 1965a).

Several investigators have explored the possibility of using other methods to detect carriers. With regard to electromyography, there have been claims that in a proportion of carriers there are abnormalities of the interference pattern on maximum voluntary contraction (Barwick, 1963), an increase in the mean number of cycles per second in polyphasic potentials (van den Bosch, 1963), and a reduction in the absolute refractory period of muscle (Caruso and Buchthal, 1965). However, routine electromyographic technics do not seem to be of any use in detecting perfectly healthy female carriers of Duchenne muscular dystrophy (Caruso and Buchthal, 1965; Emery, Teasdall and Coomes, 1966).

Démos et al. (1962) have reported a significant reduction in the "peripheral circulation time" in a proportion of carriers but the method is complex and hardly a practical proposition in most laboratories. Estimations of total limb blood flow by the method of venous occlusion

TABLE 2—*Serum Creatine Kinase in Carriers of Duchenne Muscular Dystrophy*

Method (± modifications)	Number Total	Number Abnormal	Percent Abnormal	References
Kuby et al. 1954	10	5	50	Sugita & Tyler 1963
	22	15	68	Milhorat & Goldstone 1965
	3	2	67	Stephens & Lewin 1965
Ennor & Rosenberg 1954	53	38	72	Dreyfus et al., 1965
	15	12	80	Hughes 1963
	15	10	67	Pearce et al. 1964a
	17	15	88	Wilson et al. 1965
	37	24	65	McAlpine & Thompson 1966
Tanzer & Gilvarg 1959	8	4	50	Pearson et al. 1963
	26	22	85	Richterich et al. 1963
	7	6	86	Rotthauwe & Kowalewski 1965
	14	11*	78	Wiesmann et al. 1965
	30	20	67	Emery 1965d
Total	257	184	72	

*After ischemic exercise

plethysmography have revealed no significant difference between a number of carriers and healthy women of comparable age chosen as controls (Emery and Schelling, 1965).

A number of investigators have demonstrated abnormalities in the histology of muscle biopsy specimens in a proportion of carriers of X-linked Duchenne muscular dystrophy (Dubowitz, 1963; Emery, 1963; 1965b; Pearson, Fowler and Wright, 1963; Macciotta, Cao and Scano, 1965; Stephens and Lewin, 1965; Pearce, Pearce and Walton, 1966). Others have detected changes in the isoenzyme pattern of muscle lactate dehydrogenase in a proportion of carriers. (Emery, 1964b, 1965a; Wilkinson, 1965). Possibly a muscle biopsy for histological and isoenzyme studies may be a useful adjunct to the estimation of serum creatine kinase for detecting carriers of X-linked Duchenne muscular dystrophy. However, if this is to be established these investigations will have to be carried out on many more carriers.

The various clinical, histological and biochemical manifestations in carriers of X-linked Duchenne muscular dystrophy can be explained in terms of the Lyon hypothesis (Lyon, 1961, 1962) concerning gene action in the X chromosome in females. Presumably in those carriers with clinical manifestations, high serum levels of creatine kinase, and abnormal muscle histology the proportion of their cells in which the active X chromosome is the one bearing the muscular dystrophy gene is greater than in carriers who have no clinical manifestations, normal serum levels of creatine kinase, and normal muscle histology.

Congenital Myopathies

Amyotonia congenita (myatonia congenita, Oppenheim's disease) refers to the syndrome of generalized muscular hypotonia, feebleness of voluntary movements and depressed or absent tendon reflexes which is present at birth or is manifest during early infancy. Batten (1910) was the first to recognize that the syndrome could be myopathic in origin or result from a lesion in the anterior horn cells of the spinal cord (infantile spinal muscular atrophy or Werdnig-Hoffmann's disease). More recent studies have made it clear that the syndrome may in fact result from many causes (Brandt, 1950; Walton, 1956c, 1957b). In a follow-up study of 109 cases diagnosed as "amyotonia congenita," 67 (61 per cent) were found to have Werdnig-Hoffmann's disease and 22 (20 per cent) had a myopathy of one form or another. The remaining 20 (19 per cent) cases included patients with cerebral palsy (6), mental defect (8), scurvy (2), arachnodactyly (1), multiple congenital defects (1), congenital dislocation of the hip (1), and spinal ganglioneuroma (1) (Walton, 1956c). The

congenital myopathies themselves are a heterogenous group though they all have in common muscular weakness dating from birth or early infancy which is apparently not due to a disease outside the muscular system. An understanding of the pathogenesis of this group of diseases is urgently needed both for genetic counseling and for determining the prognosis in the individual case. Serum enzyme studies and refinements in histological and histochemical technics may soon make this possible.

An entirely satisfactory classification of the congenital myopathies is not yet possible but with our present knowledge the following classification seems reasonable:

1. Congenital muscular dystrophies proper.
   a. Rapidly progressive.
   b. Slowly progressive or relatively nonprogressive.
2. Exceptional early onset in forms of muscular dystrophy which usually begin in childhood.
3. Congenital "myopathies."
   a. Benign congenital hypotonia.
   b. Congenital universal muscular hypoplasia.
   c. Central core disease.
   d. Nemaline myopathy.
   e. Mitochondrial abnormalities.
4. Arthrogryposis multiplex congenita (myopathic form)

In the congenital muscular dystrophies proper the histological findings are identical with those found in the muscular dystrophies with onset in childhood or later life (variation in fiber size, focal necrosis of fibers, phagocytosis of necrotic tissue by histiocytes, connective tissue proliferation and fatty infiltration). Electromyographic findings may be misleading in this group of disorders (O'Brien, 1962). Though serum levels of aldolase and creatine kinase are very high in preclinical cases of X-linked Duchenne muscular dystrophy (Pearson, 1957; Pearson et al., 1961; Walton, 1964b; Vassella, Richterich and Rossi, 1965) they are usually normal or only slightly raised in congenital muscular dystrophy (Pearson and Fowler, 1963; Vassella et al., 1965; Wharton, 1965). Cases of congenital muscular dystrophy with rapid progression leading to death in infancy or early childhood have been described by several authors (Howard, 1908; Councilman and Dunn, 1911; de Lange, 1937; Gilmour, 1946; Banker, Victor and Adams, 1957; O'Brien, 1962; Short, 1963; Wharton, 1965). In all these cases there was histopathological confirmation of the diagnosis of muscular dystrophy. In 5 of these reports there was more than one affected sibling in the same family (Howard, 1908; de

Lange, 1937; Banker et al., 1957; Short, 1963; Wharton, 1965) which suggests that this form of congenital muscular dystrophy is inherited as an autosomal recessive trait. Lewis and Besant (1962) have also described 2 siblings with a rapidly progressive form of congenital muscular dystrophy but this may be a different disease because the dystrophic process was almost entirely limited to the diaphragm, the skeletal muscles having an apparently normal histological appearance.

Cases of congenital muscular dystrophy have been described where the course of the disease ran a more benign course, being either very slowly progressive or relatively nonprogressive (Turner, 1940; 1949; Turner and Lees, 1962; Richter and Humphreys, 1955; Greenfield, Cornman and Shy, 1958; Fukuyama, Kawozura and Haruna, 1960; Pearson and Fowler, 1963). There was histological confirmation of the diagnosis of muscular dystrophy in all these reports though the histological findings in Turner's patient who came to autopsy (Turner, 1949) have been interpreted as possibly not being evidence of dystrophy but of changes secondary to infective endocarditis from which the patient suffered (Walton, 1956c). Perhaps Turner's cases would be better considered as having a congenital "myopathy" rather than a muscular dystrophy (see below). With one exception (Richter and Humpreys, 1955) in all these reports there were other affected siblings in the same family which suggests that this form of congenital muscular dystrophy is also inherited as an autosomal recessive trait.

It is conceivable that recognized forms of muscular dystrophy which usually begin in childhood or early adult life might occasionally begin in infancy or even be manifest at birth. Clinical manifestations of muscle disease during the neonatal period or early infancy have been described, for example, in cases of Duchenne muscular dystrophy (Walton and Nattrass, 1954), paramyotonia (Hudson, 1963), myotonic dystrophy (Vanier, 1960; Dodge et al., 1965), ocular muscular dystrophy (Fuchs, 1890), in a distal type of muscular dystrophy (Magee and de Jong, 1965), and in limb girdle muscular dystrophy (Silvestri, 1909). Vanier (1960) reported 6 cases of myonic dystrophy in children all of whom had been described at birth as being "floppy." This suggests that the occasional "floppy" infant in whom a muscle biopsy is indicative of muscular dystrophy may have one of the recognized forms of muscular dystrophy but with unusually early onset. Conceivably the investigation of a suspected case of congenital muscular dystrophy should therefore include a search for evidence of myotonia, to exclude myotonic dystrophy, and a serum creatine kinase estimation to exclude X-linked Duchenne muscular dystrophy.

The term "congenital myopathy," as distinct from congenital muscular dystrophy, is used here for a miscellaneous group of conditions which present as muscle weakness in infancy but there is little if any progression and the muscle histology differs from that observed in the usual form of muscular dystrophy. The muscle histology in these cases has been described as normal (benign congenital hypotonia), or characterized by a general smallness of the muscle fibers (congenital universal muscular hypoplasia) or there are characteristic abnormalities within the muscle fibers which can be recognized by special staining technics (central core disease, nemaline myopathy and, what are here referred to as "mitochondrial abnormalities"). Benign congenital hypotonia (Walton, 1956c, 1957b; Greenfield et al., 1958) refers to a condition in which there is generalized muscle weakness and hypotonia but the muscle histology appears normal with conventional staining technics. Eight of the 17 cases of benign congenital hypotonia studied by Walton (1957) recovered completely during childhood while the remaining 9 cases improved to some extent but showed evidence of persisting muscular disability throughout life. All of the cases described by Walton (1956c, 1957b) and Greenfield et al. (1958) were sporadic with no family history of any similar disorder in close relatives. A distinction has been made between this condition and that of congenital universal muscular hypoplasia (Krabbe's disease) in which the muscle histology is characterized by a general reduction in the size of the muscle fibers (Gibson, 1921; Krabbe, 1947, 1958; Schreier and Huperz, 1956; Ford, 1960). The validity of this distinction has been questioned by Walton (1957b) who considered that there is no essential difference between this condition and benign congenital hypotonia. Ford (1960) described a mother and daughter both having congenital universal muscular hypoplasia and Gibson (1921) described a large family where the disease appeared to be inherited as an autosomal dominant trait but with incomplete penetrance. The cases described by Krabbe (1947, 1958) and Schreier and Huperz (1956) were all sporadic.

In 1956 Shy and Magee described a congenital nonprogressive myopathy in five individuals in the same family which appeared to be inherited as an autosomal dominant trait. Muscle histology in these cases revealed some variation in fiber size but none of the usual changes seen in dystrophic muscle. However, using Gomori's trichrome stain it was found that the core regions of the fibers stained differently from the surrounding myofibrils: the central cores stained bluish whereas myofibrils normally stain red. In a patient with the same condition described by Engel et al. (1961) electron microscopy revealed that the central cores are devoid of

mitochondria and histochemical studies have shown that the cores are lacking in both oxidative and phosphorylase enzyme activity (Dubowitz and Pearse, 1960). It has also been shown that in contrast to the variation in enzyme activity between individual fibers of normal muscle, there is uniform enzyme activity in the muscle fibers in central core disease and also in at least some patients with benign congenital hypotonia (Dubowitz and Pearse, 1961; Dubowitz, 1965b).

Shy et al. (1963) have recently described another congenital nonprogressive type of myopathy but in this case the muscle fibers contained aggregates of rod-like bodies which could be readily demonstrated with Gomori trichrome stain. This has been referred to as menaline myopathy. The mother of the patient described by Shy and colleagues (1963) also had some muscle weakness but rod-like structures in the muscle fibers were not found in her muscle biopsy. However, Spiro and Kennedy (1965) have described histological evidence of nemaline myopathy in 2 generations of the same family. The nosological status of central core disease and nemaline myopathy is complicated by the recent finding of histological changes of both diseases in a woman with a congenital nonprogressive myopathy and who had a daughter similarly affected but in whom muscle histology showed changes only of central core disease (Afifi, Smith and Zellweger, 1965). Afifi and colleagues suggest that nemaline myopathy and central core disease are merely different manifestations of the same disease process. It is also possible that a proportion of the cases previously thought to have benign congenital hypotonia or congenital universal muscular hypoplasia may have had central core disease or nemaline myopathy for all the previous reports were based on conventional staining technics. This idea is substantiated by the report of Hopkins, Lindsey and Ford (1966) who have shown that by using special histological technics the affected daughter of a patient previously thought to have congenital universal muscular hypoplasia (Ford, 1960) is apparently suffering from nemaline myopathy.

In a child described by Shy and Gonatas (1964) with a proximal myopathy the muscle histology appeared normal when stained with hematoxylin and eosin. However, examination of Gomori trichrome stained material and electron microscopic studies showed giant abnormal mitochondria. The basic metabolic rate was normal. This condition appears to be different from that described by Luft et al., (1962) in which structural abnormalities of the mitochondria were also found; muscle weakness was not a prominent feature but there was severe hypermetabolism though thyroid function was apparently normal. The results of these studies on central core disease, nemaline myopathy and

mitochondrial abnormalities indicate that the full assessment of a floppy infant requires not only routine histological examination but also special staining technics including histochemical studies.

Before concluding this discussion on congenital muscular dystrophies mention should be made of arthrogryposis multiplex congenita. This term was introduced by Stern in 1923 to describe a condition in which there is congenital limitation of movement of various joints. Arthrogryposis is not a single entity but rather a syndrome which may result from a number of pathological processes. Three forms have been recognized (Adams, Denny-Brown and Pearson, 1962): what might be called an arthropathic form due to a developmental defect in the joints themselves, a neuropathic form resulting from a defect in the innervation of the skeletal muscles (Relkin, 1965), and a myopathic form where the deforming muscle contractures are secondary to prenatal muscle disease (Howard, 1908; Gilmour, 1946; Middleton, 1934; Banker et al., 1957; Greenfield et al., 1958; Pearson and Fowler, 1963). In all these reports of arthrogryposis associated with congenital myopathy the histological appearances were those of muscular dystrophy and the clinical course was rapidly progressive in at least 3 of them (Howard, 1908; Gilmour, 1946; Banker et al., 1957). The risk of recurrence of arthrogryposis in future siblings depends on the nature of the underlying disease: in the neuropathic form the chances are small because familial cases have so far not been recorded, whereas in the myopathic form they may be as high as one in four.

The Myotonias

The myotonias include myotonic dystrophy, congenital myotonia and congenital paramyotonia. All are inherited as autosomal dominant traits. Myotonic dystrophy (Steinert's disease) affects males more often than females and usually begins in late adolescence or early adult life with progressive weakness of the more peripheral muscles of the limbs, the sternomastoids, and the facial muscles. Myotonia may affect all the skeletal muscles but usually it is localized to the tongue and hands. The manifestations of dystrophy include frontal baldness, cataracts and gonadal atrophy. Skeletal abnormalities may also be found and include increased thickness of the calvarium, hyperostosis frontalis interna and a small sella turcica (Jéquier, 1950; Walton and Warrick, 1954; Isherwood and Mawdsley, 1963). Smooth muscle is involved as well as skeletal muscle in myotonic dystrophy (Harvey et al., 1965). The wide range of manifestations in this disease have been extensively reviewed by Bell (1947), Klein (1958, 1961), Caughey and Myrianthopoulos (1963) and

Welsh et al. (1964). It seems most likely that the wide variety of manifestations in myotonic dystrophy are all due to the pleiotropic effects of a single mutant gene, a situation comparable to that of sickle cell anemia. All extensive studies of myotonic dystrophy indicate that it is inherited as an autosomal dominant trait (Bell, 1947; Maas and Paterson, 1950; de Jong, 1957; Lynas, 1957; Klein, 1958, 1961; Caughey and Myrianthopoulos, 1963).

The clinical presentations are extremely variable: there may be marked incapacity in childhood (Vanier, 1960; Dodge et al., 1965) or at the other extreme the only manifestation of the disease may be cataracts producing symptoms in old age. Myotonic dystrophy is frequently quoted as an example of "anticipation" (the onset of hereditary disease at an earlier age in the offspring than in the parent), and the related phenomenon of "progression" (senile cataract in the first generation, presenile cataract in the second generation and juvenile cataract in the present generation). It is difficult to visualize any biological explanation for anticipation and progression. They are probably due to sampling error (Penrose, 1947; Caughey and Myrianthopoulos, 1963). Thus anticipation might seem apparent because only those individuals who are less severely affected have offspring; patients in whom the disease begins earlier and is more severe are more likely to be ascertained, and because the observer and the observed are in the same generation many siblings who at present appear to be unaffected may develop the disease later in life. With regard to progression this too is probably due to sampling error because over 90 per cent of patients who have inherited the gene for myotonic dystrophy have cataracts (Klein, 1958; 1961). However, in a large proportion of these cases the cataracts are visible only on slit-lamp examination and do not cause symptoms until middle age and their removal is usually not necessary until the sixth or seventh decade.

There have been numerous reports of various abnormalities in affected individuals as well as in apparently unaffected members of families with myotonic dystrophy (reviewed by Bell, 1947; Klein, 1958; Caughey and Myrianthopoulos, 1963; Pruzanski, 1965). Such abnormalities have included harelip and cleft palate, congenital heart disease, von Recklinghausen's disease, spastic paraplegia, amyotrophic lateral sclerosis, mental retardation, deafmutism, epilepsy, certain limb deformities including syndactyly and talipes, and eye defects such as microphthalmos, colobomata and optic atrophy. It is difficult to believe that all these abnormalities represent manifestations of the same pleiotropic gene. It is possible that at least some of the reported associations are fortuitous. In one study where the incidence of various congenital abnormalities in

families with myotonic dystrophy was compared with the incidence in 1,447 controls no difference was found (Henke and Seeger, 1928). The *simultaneous* occurrence of myotonic dystrophy and a specific, apparently *unrelated* abnormality *in more than one member of a particular family* appears to have been described in only 2 families: one with congenital deformities of the hands and feet (Fleischer, 1918) and the other where myotonic dystrophy was associated with bilateral cleinocamptodactyly in cousins (Klein, 1958). In a recent report of myotonic dystrophy and polycystic disease of the kidney in 3 siblings (Emery, Oleesky and Williams, 1967), investigation of all members of the family revealed that the 2 conditions, which at first seemed to be associated, were in fact inherited independently and that the association of the 2 diseases in the same individuals was probably fortuitous.

There have been several studies of the chromosomes in patients with myotonic dystrophy. It has been claimed that in a proportion of cells in some patients with myotonic dystrophy there is an additional small acrocentric chromosome and it has been suggested that these supernumerary chromosomes may play a part in determining the variation in clinical manifestations in this disease (Fitzgerald and Caughey, 1962). However, Jackson (1965) concluded that the number of aneuploid cells which he observed in his patients with myotonic dystrophy was not different from that which would be expected by chance. On the other hand, Mutton and Gross (1965) found that out of 12 patients with myotonic dystrophy 2 had a significantly higher frequency of chromatid and isochromatid breaks. The chromosome constitution in this disease is therefore still not clear.

In myotonic dystrophy the serum level of creatine kinase is only slightly raised. Out of 37 cases in which the serum level of creatine kinase was estimated, a slightly raised level was found in only 21 (57 per cent) (Colombo et al., 1962; Kuhn, Stehlin and Stein, 1962; Heyck and Laudahn, 1963; Okinaka et al., 1964; Pearce et al., 1964b; Vassella et al., 1965). Six patients who had either no symptoms or who were only minimally affected, had a normal level of serum creatine kinase even after exercise (Emery, unpublished observations). Thus, in contrast to Duchenne muscular dystrophy the serum level of creatine kinase is of little value in recognizing early cases of myotonic dystrophy. Genetic counseling to apparently unaffected siblings depends on whether there are any "micro" signs of the disease such as cataracts on slit lamp examination or myotonic discharges on electromyography. Even if the results of these tests are negative, not enough is known about the natural history of the disease to be sure that the person in question carries the

gene and will develop manifestations of the disease later in life. A prospective study of a number of first degree relatives of various ages is needed to answer this question.

The prevalence of myotonic dystrophy has been reported as 24 x 10^{-6} in Northern Ireland (Lynas, 1957), 49 x 10^{-6} in Switzerland (Klein, 1958, 1961) and 94 x 10^{-6} in New Zealand (Caughey and Barclay, 1954). The condition may be more frequent in certain population groups than in others but it is also possible that these differences in prevalence rates may reflect the completeness with which cases were ascertained in the various studies. Estimations of the mutation rate also differ depending on the estimated prevalence (8 x 10^{-6}, Lynas, 1957; 16 x 10^{-6} Klein, 1958, 1961).

In congenital myotonia (Thomsen, 1876) the myotonia affects the entire voluntary musculature and begins in infancy. It appears to be a fairly benign condition, there are no dystrophic manifestations and the disorder is compatible with normal survival. The condition usually appears to be inherited as an autosomal dominant trait, but Becker (1961) has suggested that there may also be a recessive form of the disease. In a series of 142 cases of congenital myotonia, 80 were sporadic, in 32 there were others affected in different branches of the family, and in 30 cases other siblings were affected but no one else. Out of the 30 cases in which siblings only were affected, 3 were the offspring of consanguineous marriages (Becker, 1961).

Congenital paramyotonia also begins in infancy and affects the entire voluntary musculature but in this condition myotonia only appears on exposure to cold. Congenital paramyotonia also appears to be inherited as an autosomal dominant trait (de Jong, 1957; Hudson, 1963).

The relationship between myotonic dystrophy, congenital myotonia and congenital paramyotonia is not clear. Some believe that they represent different manifestations of the same disease (Grinker, 1943; Klingler, 1948; Maas and Paterson, 1950; French and Kilpatrick, 1957; Hudson, 1963) whereas others believe that they are separate disease entities (Bell, 1947; Penrose, 1947; de Jong, 1957). These differences of opinion are largely due to the marked variability in clinical manifestations in these diseases. For example, myotonic dystrophy may begin in childhood whereas cases of congenital myotonia may only develop symptoms as late as the second decade. Myotonia can be widespread in its distribution in myotonic dystrophy and in the early stages of the disease dystrophic manifestations may be completely lacking. Alternatively a large family has been described in which all the affected members had myotonic dystrophy sine myotonia (Schotland and Row-

land, 1964). Furthermore, Drager et al. (1958) have shown that the attacks of muscular weakness which occur in paramyotonia are accompanied by a rise in serum potassium, while Gamstorp (1963) has noted that myotonia occurs in some patients with hyperkalemic periodic paralysis. Van't Hoff (1962) described under the title of "myotonic periodic paralysis" cases in which myotonia of the eyelids was demonstrated and in which attacks of weakness were accompanied by hyperkalemia. Shy (1958) suggested that paramyotonia and hyperkalemic periodic paralysis are probably one and the same condition but this suggestion seems premature and McArdle (1962) found that myotonia could not always be demonstrated in cases of periodic paralysis of the hyperkalemic variety. The relationship between these various disorders is ill-understood and will probably remain so until the underlying biochemical abnormalities are identified. As was emphasized in the introduction, from genetic considerations heterogeneity within a group of disorders such as the muscular dystrophies is to be expected. It will therefore probably be to advantage if, when considering etiology, the various types of myotonias are each regarded as separate entities.

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