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Progress in

MEDICAL GENETICS

VOLUME I

Edited by

ARTHUR G. STEINBERG

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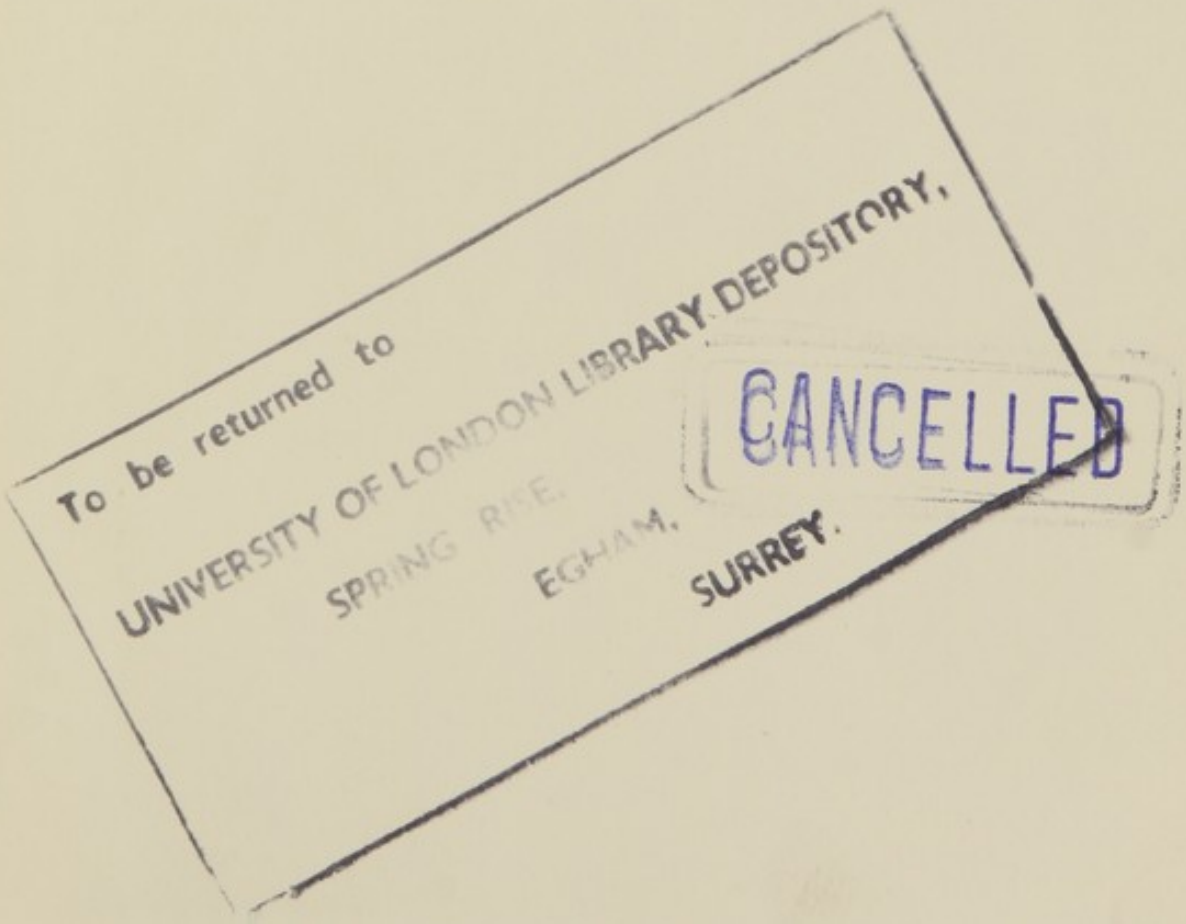

PROGRESS IN

Medical Genetics

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Medical Genetics

Volume I

Edited by

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Foreword

Recent years have seen a burgeoning of interest in medical (human) genetics. This growth has involved the activities of specialists in every discipline concerned with man as well as those trained in genetics. The result is that each problem has been considered from many aspects and has been reported in an awesome number of journals. The complaint that it is becoming impossible to "keep up with the literature" is most apt when applied to genetics. The clinician, and others whose interest in genetics is peripheral, though great and sincere, cannot devote the time necessary to "comb" the literature to find original sources. This series has been planned to help them. It is designed, also, to serve students as an introduction to the thought patterns and to the literature in the several topics reviewed and to serve the geneticist and others as an aid in keeping abreast of the areas which do not coincide with their specialties.

The late (late in relation to activity in other areas of genetics) development of interest in medical genetics does not mean there was no interest in this area earlier, for it is probable that the earliest interest in genetics was in human genetics. The Old Testament, for example, offers evidence that the ancient Hebrews had some understanding of the transmission of hemophilia. Certainly active interest in medical genetics was present in the last century (Galton and Darwin among others), and early in this century (1908) Garrod, under the felicitous title "Inborn Errors of Metabolism," advanced the hypothesis that hereditary disorders characterized by the accumulation of an abnormal metabolite resulted from the genetically determined absence of an enzyme necessary for the removal of the metabolite. This concept, supported by much recent work, is still accepted by geneticists interested in metabolic disorders.

Despite the interest on the part of a few giants in the field of biology and despite the brilliant insight of a giant in medicine (Garrod), general interest lagged until the fifth decade of this century. It is always difficult and always risky to advance reasons for a surge of interest in a scientific discipline; but it is highly probable that the initial impetus came from

the brilliant demonstration of the role of the Rh blood types in the causation of hemolytic disease of the newborn, from the immediately following successful demonstration of many other genetically determined blood types, and from the demonstration of their variable distribution among the populations of the world.

These developments coincided with a great increase in the number, variety, and efficacy of antibiotics in the physicians' armamentarium. The resulting reduction of the importance of infectious diseases as causes of dangerous illnesses gave physicians an opportunity to turn their attention to the less acute diseases, many of them genetically determined. The result has been an extraordinary growth in interest, activity, and knowledge in medical genetics during the years since 1945.

The exposure of the world population to increased amounts of radiation has stimulated interest in the measurement of mutation rates in man and of the effects of natural selection in man. Both these phenomena are related to genetic effects on morphogenesis and consequently to congenital anomalies. Recent studies on the interaction of the genotype and the environment in causing congenital anomalies in man and in other mammals have greatly advanced our understanding of this area (Chapters 1, 2, and 3).

We have already mentioned the role of the genetics of blood groups in stimulating interest in medical genetics. This interest inevitably turned to an analysis of the question of what role the blood groups play in health and disease, and to the question of what selective forces maintain them in the human species. Much new material has been reported in these areas in recent years (Chapters 4 and 5).

While the genetics of the blood types may have supplied the initial impetus to the modern growth of interest in medical genetics, the discovery of electrophoretic differences among genetically determined hemoglobin types greatly helped to expand and to continue this interest. The remarkable determination of the precise chemical change in the hemoglobin molecule resulting from a single gene substitution is the only demonstration of the exact effect of a mutation. The peculiar distribution of the hemoglobin types among the peoples of the world raises important questions related to evolution and mutation, as well as to epidemiology, and to clinical medicine (Chapter 6).

I have already mentioned the importance of increasing our knowledge of mutation and the mechanisms of selection. Until recently the effects of consanguineous marriages on morbidity of children have been neglected as a source of essential basic information for the study of these problems. New procedures for analyzing such data are now available,

and these have opened the way for enhanced utilization of this important source of information (Chapter 7).

Probably the most striking advance in medical genetics in the last five years was the demonstration, in 1956, that man has 46 chromosomes and not 48 as we had thought for so long. This discovery has been and is being followed by a series of discoveries relating chromosomal changes to various clinical entities. The progress in this field is so rapid that an article on it is apt to be out of date on the day of its publication (Chapter 8).

The chapters in this first volume of *Progress in Medical Genetics* discuss these topics with uniformly high competence and clarity.

It is our plan to maintain the high standards set by these pioneer authors in the future volumes of this series. The volumes will appear as advances seem to require them.

A. G. S.

DEDICATION

This volume is dedicated to Dr. David Adlersberg, who was to be co-editor of this volume. His untimely death deprived us of a cherished colleague and mentor. David Adlersberg was a pioneer in the treatment of diabetes, an authority on sprue and other metabolic diseases, and an internist who very early became interested in genetics. It was this interest that brought us together in 1952. Our association was a constant source of delight and of instruction. We worked together congenially, and I hope constructively, until his death early in 1960. His many friends and colleagues on all the continents will miss him greatly.

Arthur G. Steinberg

Chapter 1

Mutation in Man

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Introduction

The study of mutation has been a central part of research in genetics since this branch of biology had its modern beginning 60 years ago. The continuous refinement in cytogenetic analysis of aberrant phenotypes, the inventiveness and increasing precision of mutation rate measurement, the discovery of ways to modify mutation rates, and the beginnings of a chemical understanding of the mutation process make this one of the areas of greatest advance in biological insight.

At the same time knowledge of human mutation relative to that of the best experimental organisms is scanty and uncertain. In most ways man is a very unpromising object for genetic study. The rigor and resolution of salivary chromosome analysis on Drosophila, the sharp criterion of pollen transmissibility as a way of sifting out minute chromosomal aberrations in maize, and the fine structure analysis in viruses and bacteria cannot be remotely approached in man. Nowhere in human genetics is there the efficiency and certainty with which lethal mutations can be detected in Drosophila, the accuracy and internal checks provided by the chemostat for bacteria, or the gain in efficiency by selective methods in several microorganisms. Certain classes of mutants are inherently difficult to study in man, e.g. dominant lethals, early embryonic lethals, and mutants causing sterility or a small decrease in viability. Finally, almost nothing is known of human induced mutation rates.

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Despite these shortcomings of man as an experimental animal, a considerable amount of information about human mutation rates has been accumulated. For one thing hospital records, census data, and surveys made for other purposes frequently can be used for mutation rate information. Secondly, the investigator's ingenuity is challenged to the utmost and the result has been a succession of improved methods that are capable of extracting information from the most refractory sources. Although there is some information about spontaneous mutation, the study of induced mutation rates in man will probably have to wait for new methods.

A discussion of mutation is complicated by semantic difficulties. In the first place the word is used to describe both a process and the result of that process. Ordinarily this causes no confusion, but when a distinction is necessary I shall use *mutation process* for the first meaning, and *mutant allele, mutant chromosome,* or *mutant phenotype* for the second.

A more basic difficulty arises from the inability to distinguish small chromosome aberrations from "true" mutations. In common usage a mutation is a change that cannot be shown to depend on a detectable chromosome rearrangement or to some sort of recombination mechanism. Modern research has revealed a number of mutation-like processes such as conversion,[40] paramutation,[8] transposition,[39] and phase variation.[36] None of these, if they exist in man, could be distinguished by present methodology. Throughout this article, the word mutation will be used to designate all heritable changes not due to segregation or recombination. As knowledge increases, identifiable classes can be separated out. The first step of this kind has already been taken, with the discovery of several whole chromosome aneuploid types.[21, 31, 37, 62] Clearly, mutation will be a wastebasket category in human genetics for some time to come, as indeed in experimental organisms also.

Finally, in man the words dominant and recessive are sometimes confusing. For most "dominant" mutants the homozygous state is unknown, so there is no real measure of dominance. Likewise many "recessives" have been found to have detectable heterozygous effects. I shall use *dominant* when the effect under discussion is detectable in the heterozygote; when this effect requires a double dose of the allele, it will be called recessive. By this criterion, sickle cell hemoglobin is dominant whereas sickle cell anemia is recessive. For a discussion of this terminology problem see Allen.[1]

Mutation in Other Organisms

DeVries[20] first used the word mutation to describe inherited changes in the evening primrose, *Oenothera*. Later research has shown that these were for the most part segregation products from complex rearrangements rather than mutations, as they are now thought of. But the concept was correct—not the first time that the right conclusion has been reached from wrong evidence.

The early Drosophila workers showed that mutations do in fact occur and that by crossover analysis the change can be shown to be highly localized. Furthermore the mutation of a gene was shown to be independent of any change in its neighboring genes and analysis of somatic mutations showed that mutation of a gene is independent of (or at least not strongly dependent on) any change in its allele.

Refined methods of scoring mutants in maize by Stadler and in Drosophila by Muller led to the possibility of measurement of mutation rates and the finding of values of the order of 10^{-5} to 10^{-6} per gene per generation. Muller's CLB method and its host of improved and extended successors have also made possible the measurement of whole chromosome or genome mutation rates. This in turn added precision to the measurement so that it became possible to test effectively various environmental factors. First the temperature dependence of mutation was shown and then came Muller's and Stadler's famous discovery that the mutation rate can be greatly enhanced by high-energy radiation.

The discovery of giant salivary gland chromosomes in Drosophila larvae introduced a new order of precision in cytogenetic analysis. The absence of a visible cytological change now formed the basis of a distinction between gene mutations and chromosome aberrations which, along with criteria of passage through the male gametophyte in plants and the differential effects of ultra-violet and ionizing radiation, made possible an operational distinction between chromosome aberrations and gene mutations.

The possibility of chemical alteration of mutation rates was suggested by several experiments during the 1930's, but the first convincing demonstration of chemical mutation induction was Auerbach's discovery that nitrogen mustard was a potent mutagen.[5]

This was followed by a series of new chemical mutagens. Yet the early hope that chemical correlations among mutagenic compounds would lead to specific knowledge of the chemistry of the gene or of the nature

of mutation was vain. The only thing that effective mutagens had in common was a high alkylating activity.

The possibility of more specific chemical mutagenesis was realized with the invention of the "chemostat" by Novick and Szilard.[60] This device made it possible to test much milder substances and to detect minor changes in mutation rates. Furthermore the method was remarkably free of gratuitous assumptions and provided a number of internal checks to show that the process measured was in fact mutation and not some subtle form of selection.

Novick and Szilard showed for the first time that some substances were anti-mutagenic, thus making quite compelling the frequent assertion that the "spontaneous" mutation rate is determined by the balance between mutagens and antimutagens in the cell. Secondly they discovered that nucleic acid analogs were mutagenic or antimutagenic. The fact that some of the analogs were ribosides offers evidence that RNA may be involved in mutation.

The nearest approach to specific mutagenesis has been Freese's work on bacteriophage.[22] Bromouracil is strongly mutagenic, presumable by causing substitutions for thymine in the DNA molecule. The pattern of mutations obtained after this treatment is different from that following spontaneous mutation or from the use of other chemicals. So it is no longer true that mutagenic agents act simply to enhance all mutations in a nondiscriminating way. Some selectivity has been achieved, and with it a much deeper understanding of the nature of the mutation process itself.[23]

The hope that specific mutagens might be found—*i.e.* substances that will mutate a specific gene and no other—has not been realized. This seems unlikely of attainment, for the mutagen, in order to seek out and mutate a particular gene, would seem to have to have informational complexity comparable to that of the gene itself.[35]

Along with much deeper insight into the chemical basis of heredity has come some findings that are undoubtedly destined to be important, but whose role cannot at present be seen. Such factors as phase variation in Salmonella,[36] mutable genes in maize,[71] controlling elements,[39] paramutation,[8] antigenic and mating-type variation in Paramecium,[75] and the various phenomena associated with segregation-distortion in Drosophila[72] are too frequent to be ignored. Whether such changes are truly mutational or nucleic in the sense of altering the basic informational content of the gene, or more concerned with changes at the level of gene products (epinucleic) remains to be resolved.

Phenotypic Effects of Mutations

In Drosophila the great majority of mutant genes are recessive. This is conspicuously true of mutants causing a visible phenotypic change, whether spontaneous or induced by radiation or chemical treatment. It is also true of lethal and viability-reducing mutants. At high radiation doses dominant lethals and position effects become frequent, but these are generally due to gross chromosomal changes.

Among known human mutant phenotypes the reverse is true. The U. N. Report[100] lists more than twice as many dominant as recessive diseases of known or presumed monogenic inheritance, with the total disease incidence due to dominants some eight times as great as that due to recessives. But, as has been pointed out many times, this is undoubtedly due almost entirely to the difficulty of detecting recessive conditions, or of identifying them as being of genetic origin. One approach is through the systematic study of the children of consanguineous parents.

That the high ratio of dominants to recessives in man is the result of incomplete knowledge rather than a reflection of the true situation is confirmed by the study of sex-linked loci, where recessives are detected as easily as dominants. Here the recessives are in the same overwhelming excess as in Drosophila.

When dominant mutants in Drosophila are examined, the majority are more accentuated or severe in homozygotes; frequently the homozygote is lethal. The homozygous effects for most human dominant mutants are unknown, but it is likely that they, too, would be more profound than the heterozygous effects and in many cases lethal.

At the same time, more and more instances have been found where the heterozygote for a recessive mutant has a detectable effect. Some of the best examples are provided by traits whose biochemical basis is well understood—galactosemia, phenylketonuria, sickle cell anemia, and primaquine sensitivity. It is also likely that morphological changes and statistically detectable effects on viability will be found for the heterozygotes of an increasing number of recessive factors. This is clearly the case for experimental organisms where careful tests have been made.

In Drosophila there is strong evidence that recessive lethals typically have a deleterious heterozygous effect. Stern and his associates[80] and Muller and Campbell[49] showed about a 5 per cent reduction in preadult viability of flies heterozygous for chromosomes that produced a lethal effect when homozygous. Stern's group studied a mixture of spon-

taneous and X-ray induced sex-linked mutants while Muller and Campbell's mutants were autosomal and ultra-violet induced.

These experiments do not show that all, or even a majority of lethal mutants have a deleterious heterozygous effect; they could, for example, consist of half with 10 per cent reduction in viability and half with none. A statistical analysis of Muller and Campbell's data suggested that this was not the case,[48] but a much more definitive result comes from the work of Hiraizumi.[30] Hiraizumi studied lethals extracted from natural populations of Drosophila. This is a sensitive procedure for finding lethal-bearing chromosomes that are neutral or beneficial in the heterozygotes, for these should persist in the population longer than those with a heterozygous disadvantage and thus make a disproportionate contribution to the average value of the heterozygous effect. The depressed viability in heterozygotes was about 2.5 per cent for pre-adult stages, and there were also effects on adult viability and fertility. Other workers have obtained similar results. Indirect evidence suggests that this partial dominance is also characteristic of mutants whose homozygous effect is small.[24]

The conclusion that these studies suggest is that practically all recessive alleles have some heterozygous effect large enough so that this is the major factor in the determination of the allele frequency. (This is a possible explanation of the results of Neel et al.[56] showing similar frequencies of a number of recessive conditions in Japan and in Western countries, despite large differences in the amount of inbreeding, at least in the recent past.) The effect of the heterozygote is deleterious in most cases, but a small number are advantageous as heterozygotes, leading to polymorphisms.

In summary, it is likely that most mutants are partially dominant. Nevertheless it is still convenient to classify a mutant as dominant or recessive accordingly as the mutant phenotype is ordinarily detected in the heterozygous or homozygous condition, as mentioned in the introduction. Galactosemia is classed as a recessive despite the fact that transferase activity in the heterozygote is approximately the average of the two homozygotes. Likewise chondrodystrophy is classed as a dominant, though the homozygote has never been recognized.

In Drosophila the range of mutational effects is from complete lethality to the smallest effect that the statistical resolving power of the experiment will detect. The qualitative effects are of all sorts, the chief limiting factor being the ability of the observer to notice minor departures from normality.

The best opportunity in the human species to observe the range of effects of recessive mutant alleles is in families where the parents were

consanguineous. Such studies, reviewed by Morton in this volume,[46] have revealed substantial increases in a number of abnormalities and diseases and in the death rate. Some of the abnormalities are recognized as due to single recessive factors. On the other hand part of the increase in morbidity and a substantial fraction of the excess mortality in consanguineous marriages is not due to known recessive factors. It is likely that some of the mutational damage is carried by the population in the form of mildly deleterious factors whose effect is to cause only a slight weakening. Such a factor may occasionally trip the balance between life and death so that a circumstance from which a hardier person would have survived is lethal for the child of a consanguineous union. That the effect of homozygous chromosomes is not due to environment-independent lethals is shown by the fact that the death rate in consanguineous marriages decreases with an improved standard of living just as does the rate for children of non-consanguineous marriages.

It is not possible at present to determine what fraction of the hidden load in man is due to monogenic lethals as contrasted to a larger number of mildly deleterious factors. There is evidence from Drosophila that full lethals may make up approximately half of the load in natural populations[24] and some data on bison (Slatis, unpublished) support this conclusion. For a discussion, see Morton's article in this volume.

Mutation Rates

As early as 1921 Danforth[18] noted that the equilibrium frequency of mutant genes was determined by the product of the mutation rate and the average number of generations the mutant persists before being eliminated from the population. Noting that the incidence of both polydactyly and syndactyly was roughly 1/1000, or one in 2000 genomes, and that each persisted on the average at least three generations, he concluded that the mutation rates must be less than 1/6000.

Modern mutation estimates began with Haldane[27] and Penrose[35] who reported mutation rates for hemophilia and epiloia, respectively. These marked the beginning of a number of studies, all based on general principles that owe their origin largely to Haldane. The methods are described in Haldane's 1949 paper[29] along with the results as of that time.

Direct Measurement of the Rate of Occurrence of Dominant Mutants

In principle the measurement of the rate of occurrence of highly penetrant dominant mutations is simple. Any instance of the mutant phenotype in a child of normal parents is a new mutant and from the

frequency of such occurrences the mutation rate can be computed. The mutation rate is conventionally measured as the rate per locus per generation. Thus the rate is ½ the frequency of mutant children from normal parents, the factor ½ coming from the fact that either of two genes could have mutated to produce the observed effect.

For example, Takahashi[85] reports 10 children with chondrodystrophy whose parents were normal among 80,435 births. If these are all due to mutations, then there are 10 mutant genes among 160,870 genomes and the estimated rate is 10/160,870, or 6.2×10^{-5}. This value is close to those of Mørch[42] for the same condition, but there are reasons to regard this as an overestimate.

Despite this seeming directness and freedom from sources of error there are several difficulties. For one thing it is necessary that the disease be correctly diagnosed and that no cases be missed. In most diseases this requires a detailed knowledge of the disease itself, and may call for elaborate diagnostic procedures. A further necessity is that the disease must be rare enough or have a sufficiently great effect on survival and reproduction that the possibility of mistaken paternity can be effectively ruled out.

Another problem is that several different mutant genes at different loci may produce the same phenotype. To the extent that this is true, the rate that is measured is the total for several loci rather than for a single locus. Further resolution may come from finer clinical criteria. For example, chondrodystrophy is said to exist in two forms, which may reflect two kinds of genetic causation. Similarly, different forms of muscular dystrophy have been distinguished, and there are now several diseases that superficially resemble hemophilia. Muscular dystrophy and hemophilia both have sex-linked forms, which enables a separation of entities on the basis of mode of inheritance. Yet, there are two sex-linked forms of thromboplastin deficient diseases, hemophilia and Christmas disease. When the disease can be caused by any of several indistinguishable autosomal dominant genes, it is inevitable that the total rate will be an overestimate of the per locus rate. This is one of the areas where linkage analysis will be helpful. Morton[43, 44] showed that there is a distinct heterogeneity in the amount of linkage between the gene for elliptocytosis and the Rh blood factors. The data are consistent with there being two loci causing elliptocytosis, one closely linked to the Rh factor and the other independent.

In some cases non-hereditary conditions (phenocopies) may mimic the mutation and thus lead to an overestimate of the mutation rate. This has been demonstrated most convincingly in retinoblastoma. Vogel[93] and

Tucker, Steinberg, and Cogan[88] have shown that sporadic cases who reproduce transmit the disease to considerably less than the expected half of their children.

Another source of bias in the direct method is low penetrance, particularly if the fitness is not extremely low. However, with some of the indirect methods to be discussed later complete penetrance is not necessary, provided that genetically competent persons who fail to manifest the trait do not have an altered viability or fertility.

For all these reasons, direct estimates of mutation rates are likely to be too high. Errors in paternity can be minimized by careful choice of traits to be measured, and is largely eliminated if the condition is very rare. The proportion of phenocopies can be estimated for those conditions that permit reproduction, but this isn't always true. And, as mentioned, by a finer resolution, by clinical or genetic methods, it is sometimes possible to increase the probability that a single locus rate is being measured.

These forms of bias are not likely to make the estimate too high by more than an order of magnitude, and probably by much less in the best cases. But there is another factor that is more difficult to assess. This is the fact that the characters chosen for mutation rate study cannot be too rare, or they would not have been found at all. A mutation whose rate of occurrence is in the order of the reciprocal of the population number will frequently be missed entirely.

It is quite possible, indeed highly likely, that there are genes whose rates of mutation are much lower than those whose rates have been studied. Various authors have called attention to the disease, ichthyosis hystrix gravior ("porcupine skin"), that has had so much attention in genetics textbooks. Whether this is Y-linked or autosomal,[78, 89] it has been recorded only once in medical history. Yet it is so conspicuous and has such a clear hereditary basis that it is not likely to have been overlooked. This suggests a very low mutation rate, 10^{-8} or 10^{-9} or less. There are other conspicuous dominant traits that have been observed only once. Another example is the "black blood disease" of Tamura and Takahashi[86, 87] which, from the author's description has very little effect on viability or reproduction and should be highly conspicuous, at least in all light skinned races.

Therefore, even granting that those rates which are measured are done well, it is likely as Haldane pointed out much earlier[29] that the measurements of human rates are mainly a sample of the more mutable loci.

Are there factors tending to bias in the opposite direction? One possibility is that, especially for severe diseases, the child dies before birth

or before diagnosis. But in general such factors are probably not as important as those tending to cause an overestimate of the average rate.

It might perhaps be thought that the blood groups or other common traits would be suitable for mutation study since the phenotype is measured with such precision. One could look for exceptions not explainable on paternity errors, *e.g. O* mothers with *AB* children, or *M* mothers with *N* children. Such exceptions have been reported. Cotterman (personal conversation) has mentioned several such exceptions in a large blood grouping center where mother and infant were studied. But most such instances have not been examined in detail. One case was studied carefully, and the mother was indeed *O* by the criterion of failure of her cells to be agglutinated by a number of anti-*A* and *B* agglutinins of animal and plant origin while the child was clearly *AB*. Yet the mother was unusual in that she carried no anti-*A* antibody, so she presumably carried an *A* gene which was somehow not expressed in such a way as to be detected in a standard test, yet prevented the development of anti-*A* antibodies. The Bombay blood phenotype appears to depend on a locus independent of the *ABH* alleles, hence can easily lead to seeming exceptions to normal inheritance.[9]

This means that the simple finding of exceptions to normal inheritance in blood groups does not constitute an acceptable measure of mutation rates. The same kind of difficulty is likely to complicate investigation of other loci where the alternative alleles are all fairly frequent in the population, for a rare modifier can simulate a mutation. Most information on mutation rates has come from the study of rare phenotypes, where it is less likely that a rare exception is due to a modifier.

Indirect and Semidirect Measures of Mutation Rates

The direct methods discussed thus far are applicable only to dominant traits. It is important that some way of measuring mutation rates for recessive mutants be found. This can be done by an indirect method due primarily to Haldane.[27, 29]

This method makes the fundamental assumption that there is an equilibrium between the rate of occurrence of new mutants and the rate of elimination of old mutant genes by selection. This necessitates an estimate of the fitness of the persons carrying the mutant genes.

If s is the proportion of reduction in fitness due to the mutant gene, x is the frequency of the mutant phenotype, and μ is the mutation rate per gene per generation, we can state the equality of new mutations and eliminations in this form:

$$sx = 2\mu \qquad (1)$$

The factor 2 comes from the fact that a mutation can arise in either the egg or the sperm, so there are two alleles that can mutate. Hence, μ can be estimated simply as half the product of s and x. This assumes that the gene is so rare that homozygotes can be ignored.

As an example of the application of this method, Reed and Neel[70] studied all persons known to have Huntington's chorea in the lower peninsula of the state of Michigan, roughly 9/10 of the state's population. The population incidence of the disease was estimated as 1.01×10^{-4}. The choreics were estimated to have a fitness 81 per cent of normal, that is $s = .19$. Therefore the estimated mutation rate is $(.19)(1.01 \times 10^{-4})/2 = 9.6 \times 10^{-6}$.

Note that if the fitness were to be .90, μ would equal 5.05×10^{-6}. This example illustrates the sensitivity of the estimate to small errors in the estimation of fitness if s is small. For this reason, the most accurate estimates by this procedure are for mutants whose effect on fitness is large; the nearer the fitness is to zero, the better from this standpoint.

There is another advantage to studying mutant genes that reduce the fitness greatly, namely, the short interval between the occurrence of the mutant and its elimination. If this interval is too long, the environmental conditions may have changed enough that the present measurement of fitness is not relevant to much of the period during which the gene frequency was determined.

Another procedure, like the indirect in depending on population incidence, but like the direct in its identification of individuals from normal parents, has been used by Patau and Nachtsheim[61] and Haldane.[29] Patau and Nachtsheim used this method to estimate the mutation rate for the Pelger anomaly. Nachtsheim[51, 52] has published new estimates based on a greater amount of data. Of 56 cases there were three who had come from normal parents, thus the fraction of new mutants is 3/56. The population incidence is about 1/3000. Hence the mutation rate is estimated as $(1/2)(3/56)(1/3000)$ or about 10^{-5}.

The high fitness of this trait makes it important to be sure that the penetrance is high. Nachtsheim[52] asserts that this is true, the only seeming exception being a probable mixup of blood streaks. With Chondrodystrophy, the penetrance becomes relatively unimportant because of the extremely low viability of the affected persons unless there is a strong viability effect of the non-penetrant gene.

The indirect method is applicable also to sex-linked loci. If x now stands for the frequency of the sex-linked trait *among males*, the equilibrium condition is given by

$$3\mu = sx \qquad (2)$$

which serves as a mutation rate estimating equation. The factor 3 comes from the fact that one third of the X chromosomes are in males. It is assumed that the selection in heterozygous females is negligible compared with that in hemizygous males and that the gene is rare.

Andreasson[2] found 81 hemophiliacs among 1,820,000 Danish males. Haldane[29] estimates the fitness as .29, so $s = .71$, giving $\mu = 3.2 \times 10^{-5}$.

An additional procedure has been introduced by Morton,[44] and may be called a "semidirect" method. It is like the direct in that it does not assume an equilibrium between mutation and elimination, but it is like the indirect in that the new mutant cases are estimated rather than identified. For example, Morton and Chung[47] estimated by equation (2) that Duchenne type muscular dystrophy had a mutation rate of $\mu = 8.9 \times 10^{-5}$. By segregation analysis, they estimated the proportion of sporadic cases as .355. Hence the mutation rate is estimated as $.355x$ or 9.9×10^{-5}, in excellent agreement with the other estimate.

In Table 1 are a number of mutation rates estimated by the foregoing methods. In general the dominants cluster around 10^{-5} with a few conspicuous exceptions. Whether these exceptions represent mutable genes or have some other explanation is not yet known. One argument for viewing the neurofibromatosis data as suspect is the absence of a parental age effect in sporadic cases. This is discussed below.

The recessive rates appear to be generally higher, if one can judge from two diseases.

Estimating Mutation Rates for Recessive Genes

The same kind of equilibrium equations as have just been used for dominant and sex-linked recessive factors can in principle be used to estimate the mutation rate to autosomal recessive alleles. But in this case the assumptions become much more restrictive, for it is necessary to assume something about the amount of selection in heterozygotes during the long interval between occurrence of a mutation and its homozygous expression.

The first attempt to estimate the mutation rate to an autosomal recessive factor was made by Neel and Valentine,[59] who studied the gene for thalassemia. If they assumed that there was no selection for or against heterozygotes and that fitness of homozygotes was practically zero, the mutation rate could be estimated at 4×10^{-4}. Such an estimate is, of course, extremely sensitive to small amounts of selection in the heterozygotes, which makes the mutation estimate of dubious utility,

especially since the mutation rate must be assumed to differ in different areas. As early as 1949 Haldane[29] urged that the possibility of a heterozygote of superior fitness should be considered, and pointed out that an advantage of the order of 2 per cent would be sufficient to maintain the gene frequency with no mutation at all. He even made the prophetic suggestion that the heterozygote might be resistent to malaria!

Now it is known that the high "mutation rate" is almost certainly the consequence of the high adaptive values of heterozygotes and the even higher rates needed to account for the incidence of the sickle cell gene have the same explanation.

Estimates of mutation rates for recessive factors have been made assuming no selection in heterozygotes and with estimates of the amount of inbreeding. These values have been published for albinism, ichthyosis congenita, total color blindness, amaurotic idiocy, amyotonia congenita, microcephaly, and phenylketonuria.[53, 56, 65] These all give values between 1 and 5×10^{-5}, which are in the range of values for the sex-linked recessives, though higher than for most of the dominants.

However, the same kind of calculation for cystic fibrosis of the pancreas leads to an estimate of about 10^{-3}. We may reject this as being implausibly high but in that case we are in the curious position of estimating only those mutation rates whose value agrees with what we think a mutation rate should be. For discussion see Penrose.[64]

One may choose, as some have,[54,57] to estimate no recessive mutation rates. There is one alternative though. That is to measure only those rates where there is some evidence, though it is weak in some instances, about the selection in heterozygotes. Though it is difficult to rule out the possibility that the frequency of, say, phenylketonuria is due to some selective balance mechanism rather than mutation, it is possible to bring much stronger evidence to bear on a trait like congenital deafness where a consanguinity analysis can be made.

I shall therefore consider only characters for which there is some evidence that the frequency is in fact maintained by mutation. This restricts the estimate, insofar as present methods are used, to genome rates rather than single locus rates. However, this may not be a disadvantage; for many purposes the genome rate is what is wanted.

Mutation Rates from Consanguinity Analysis

Data from consanguineous marriages provide an estimate of the amount of hidden recessive genetic impairment, or the genetic load.[15, 48] There is the difficulty that the social concomitants of inbreeding, such as differ-

ence in economic status, may be confounded with genetic effects; but with careful selection of control data this can be minimized.

Morton, Crow, and Muller[48] expressed the relationship between parental consanguinity and viability in the form

$$S = e^{-(A+BF)} \tag{3}$$

where S is the proportion of survivors, F is the inbreeding coefficient of the child, and A and B are constants determined by the weighted regression of ln S on F.

This formulation requires only the assumption that the different genetic and non-genetic causes of death act independently. This seems justified as a first approximation by the absence of large deviations from linearity in animal and plant inbreeding experiments and by the fact that only low levels of inbreeding are studied in man where such interaction effects would be minimized. At the low death rates encountered in human populations the simple linear approximation, $Deaths = A + BF$, gives practically the same results.

When $F = 0$ (random mating) the expressed load is measured by A. When there is complete homozygosity ($F = 1$) the total load is $A + B$. Morton, Crow, and Muller[48] showed that the total genetic load in the population, including that which is hidden, is given by Σqs where q is the allele frequency and s is the probability of death when that allele is made homozygous. The derivation of these relationships is given by Morton et al.[48] See also a discussion by Steinberg.[76]

The load, as defined by Σqs and measured in *lethal equivalents*, lies between B and $A + B$. A lethal equivalent[48] is a group of mutant genes of such a number that, if dispersed in different individuals, they would cause on the average one death.

If the load is due to recessive factors maintained by recurrent mutation and the population is at equilibrium, the mutation rate is given by the total load multiplied by the harmonic mean of z, where z is the sum of the inbreeding coefficient of the population, the frequency of pre-existing mutant alleles, and the amount of dominance of the recessive alleles.

If it is assumed, as seems plausible from Drosophila data and from what human evidence is available, that the usual means of elimination of mutants is through heterozygous effects, the mutation rate is given by the total load multiplied by the harmonic mean of the dominance. This value is unknown in man, but Drosophila data[30] suggests a value of about 2.5 per cent.

Data from various sources were used to estimate the total genetic load as about 1.5-2.5 lethal equivalents per gamete for all loci contributing to

deaths from late fetal to early adult ages.[48] More recent data are in approximate agreement, except for those from Kure and Nagasaki, Japan, which may be a consequence of special circumstances. For a discussion see the article by Morton in this volume.

If this load is interpreted as mutational in origin the estimated mutation rate is .03-.05 per genome.[48] Newer data[82] do not change the estimate appreciably. It is likely that the better environment of recent times has lowered the death rate so that some former drastic alleles are less harmful now. Therefore current data underestimate the load, which in principle should be measured during the past while the present gene frequencies were being established. This is another argument for collecting data on effects of consanguinity in primitive societies while they still have a low living standard.

Earlier it was pointed out that mutation rates for recessive factors are unreliable because of the absence of information about selection in heterozygotes. In particular an allele that is favored in the heterozygote will give a spuriously high estimate of the mutation rate if its frequency is used as the basis for such an estimate. The same question arises in the theory of lethal equivalents.

The argument that the load is mutational rather than segregational has been given by Crow.[15] The ratio B/A is less than the average number of mutually heterotic alleles maintained in the population at each locus. Since this value was high for the data used, Morton et al.[48] concluded that the load was mutational. This seemed preferable to postulating dozens of alleles, heterotic in all heterozygous combinations, maintained in balanced polymorphism at each relevant locus.

The extension of the theory of lethal equivalents to traits not measured as mortality has been made by Morton's discovery of an approach to the problem when consanguinity is ascertained retrospectively.[11, 45, 46] See also Kimura.[32]

If the effects of fully penetrant, recessive genes can be isolated then the total load, expressed as frequency of the trait, is Σq. By segregation analysis[44] this component has been isolated for muscular dystrophy,[47] deafness[11] and low grade mental deficiency.[45]

The estimated mutation rates per genome of all the alleles contributing to the effect being measured are 6×10^{-5} for recessive muscular dystrophy, 45×10^{-5} for deafness, and 155×10^{-5} for mental defect as shown in Table 1.

These values are calculated on the assumption that elimination is largely through consanguinity and the average value of a is taken as .005. If the elimination is by heterozygous effects with 2.5 per cent of

the homozygous effect on fitness, the estimates are two-three times as high.

The values in Table 1 are total genome values. A crude idea of the

TABLE 1—*Estimated Mutation Rates in Man*

Trait	Mutation Rate per gamete	Remarks	References
		Dominant Factors	
Epiloia	0.8×10^{-5}	One of the earliest measurements	(25)
Aniridia	0.5×10^{-5}	Corrected value from Penrose	(41, 65)
Microphthalmus	0.5×10^{-5}		(73)
Wardenberg's syndrome	0.4×10^{-5}	See also (11)	(94)
Facioscapular muscular dystrophy	0.5×10^{-5} $<0.5 \times 10^{-6}$	Direct Indirect	(7) (47)
Pelger Anomaly	$.9 \times 10^{-5}$	Indirect	(61, 51, 90)
Amyotrophic lateral sclerosis	3×10^{-5}	But see (52) for critique	(26, 90)
Myotonia dystrophica	1.6×10^{-5}		(33)
Myotonia congenita	0.4×10^{-5}		(33)
Huntington's chorea	0.5×10^{-5} 0.2×10^{-5} 1.0×10^{-5}	Upper limit More probable estimate Indirect, large error	(70) (70) (70)
Chondrodystrophy	4×10^{-5} 6×10^{-5} 6×10^{-5}	Probably more than one locus, and overestimates for other reasons also (58)	(29, 42) (65) (85)
Retinoblastoma	1.5×10^{-5} 2.3×10^{-5} 0.6×10^{-5} 0.4×10^{-5}	Probably include phenocopies Bilateral cases only Corrected for phenocopies	(65) (55) (38) (93)
Neurofibromatosis	$13\text{-}25 \times 10^{-5}$ $8\text{-}10 \times 10^{-5}$	Direct Indirect	(16) (16)
Deafness	4.7×10^{-5}	Semidirect. Estimate of all loci causing dominant deafness	(11)

TABLE 1—Continued

Trait	Mutation Rate per gamete	Remarks	References
	Sex-linked Recessive Factors		
Hemophilia	2.0×10^{-5}	A very early measurement	(27)
	3.2×10^{-5}		(2, 28, 29)
	2.7×10^{-5}		(91)
Muscular dystrophy	9.5×10^{-5}		(77)
Sex-linked	5.5×10^{-5}		(81)
	4.3×10^{-5}		(95)
	8.9×10^{-5}	Indirect	(47)
	9.9×10^{-5}	Semidirect	(47)
	Autosomal Recessive Factors		
Limb-girdle Muscular dystrophy	6×10^{-5}	Total for all recessive loci	(45, 47)
Deaf mutism	4.5×10^{-4}	Total for all recessive loci	(11, 45)
Low-grade mental defect	1.5×10^{-3}	Total for all recessive loci	(45)
Factors causing death from birth to early adulthood	4×10^{-2}	Total for all factors, based on data from rural France	(48)

rate per locus can be gotten by assuming that all loci have the same frequency. With this assumption the number of loci can be calculated.[45] This will, of course, be a minimum estimate if the alleles are not equally frequent.

The estimated minimum number of loci is 2 for muscular dystrophy, 35 for deaf mutism, and 71 for mental defect. The corresponding mutation rates per locus,[45] which are therefore maximum, are 3.4, 1.3, and 2.2×10^{-5}.

These values are quite consistent with the other mutation rates for recessive alleles given in Table 1. They are based on a number of assumptions and should not be taken as being more precise than they are, but it is gratifying to have agreement between these values and those obtained by entirely different procedures. If heterozygous elimination is assumed, the values are increased by two-three fold.

Once again it is necessary to be sure whether the load is mutational and not segregational. The question cannot be answered definitely, of course, but there are two arguments. One is the high B/A ratio. The other is the large amount of selection required to maintain this many loci in a polymorphic condition. The theory of how the total amount of selection can be measured from knowledge of a single allele has been worked out by Morton and Crow and is given in Morton's article in this volume and will not be repeated here.

The values given for autosomal recessives in Table 1 represent a first, in many ways crude, attempt to measure *genomic* as opposed to single locus mutation rates. I hope that the further development of the theory of lethal and detrimental equivalents plus more data from man and experimental organisms will make possible genomic mutation rate estimates of greater and greater precision.

Factors Affecting Mutation Rates

With the lack of precision of human mutation rate data it is not surprising that little is known about the factors influencing it. There is little hope for much greater knowledge in the future from existing methods; single locus rates are too low to permit detection of modifications and genomic rates are based on recessive factors. The best possibilities appear to be through somatic cell studies and of course through deeper fundamental knowledge arising from experimental organisms, which may open up possibilities not now foreseeable.

The effect of parental age on mutation is definite. To my knowledge it was first pointed out by that person to whom so much of human genetic methodology is owed, Weinberg.[96] Long ago he suggested parental age as a criterion for mutational etiology.

Penrose[63, 64] has summarized the data on parental age of sporadic cases of chondrodystrophy, acrocephalosyndactyly, epiloia, neurofibromatosis, retinoblastoma, and mongolism. The first two showed a striking increase in parental age, particularly of the father. This not only argues for a mutational origin, but the greater paternal age effect suggests that the number of cell divisions prior to meiosis rather than the lapsed time is the important factor in mutation number. Mongolism, of course, showed the expected maternal age effect.

On the other hand, the other three conditions showed no significant age rise. For retinoblastoma there is already evidence that a large fraction of sporadic cases are phenocopies. Perhaps the same is true for neurofibromatosis which has a strikingly high mutation rate.

Data so far are fragmentary but parental age analysis offers promise as a useful analytical tool. Unfortunately there is little data on experimental mammals to provide interpretive guidance.

A distinction between cell division-dependent and a time-dependent mutation mechanism could in principle be made by studying sex-linked mutants. If the rates are the same in the two sexes 1/3 of the cases should be new mutants[17] for a gene that lowers the fitness to zero or $s/3$ for one that causes a selective disadvantage of s.

Following a suggestion by Haldane that the mutation rate for hemophilia is higher in males than in females, a detailed analysis of this and of muscular dystrophy has been made.[10, 47] When ascertainment bias is corrected and more realistic assumptions are made, there is no convincing evidence of a difference in the sexes.

The possibility of a "premutation" was raised by Auerbach[6] to account for the simultaneous occurrence of several sporadic cases in a pedigree. By analogy with some of the results of chemical mutagenesis in Drosophila she suggested that this might be an example of delayed mutation. But see Vogel.[92]

Of the external environmental effects chemicals and radiation are of course the most discussed. But, at the moment, there is no evidence from man on chemical effects and very little on radiation. Since the latter topic has been reviewed several times[50, 57, 98, 100] it will not be discussed here.

Somatic Mutations

The study of human somatic cell mutation is primitive, but it promises to grow into a major source of genetic knowledge. The distinction between mutational and recombinational events is usually not possible, but we can look forward to refinements that can make distinctions of a subtlety that may rival those possible with microorganisms.

In Vivo Studies

Somatic mosaicism has been observed in man many times, but rarely under circumstances that provide any quantitative information or insight into the mechanisms. A striking bilateral mosaic for a skin abnormality was reported by Zlotnikoff.[97] Heterochromia of the iris is not uncommon. That the explanation is not simple somatic mutation with a low but constant risk per cell is demonstrated by several reports of multiple instances in a single pedigree.

The red blood cell antigens have had the most study thus far. Cotter-

man[12] has reported two families with multiple instances of 0-A_2 mosaicism; in one instance two sisters were affected, in the other a mother and son. In all four cases the A_2 cells were in the minority, comprising 8-12 per cent of all the cells. The mosaic producing mechanism, whatever its nature, must be hereditary to account for the double occurrence in each pedigree. Another fact of interest is that one of the two sisters also had a mosaic iris.

A variety of mechanisms have been demonstrated or postulated to account for somatic changes in experimental plants and animals[83] and there is currently great interest in such mechanisms as possible models for differentiation[75] and tumorgenesis.[34] It is difficult to distinguish among crossing over, conversion, transposition, chromosome rearrangements, and point mutations even with the most favorable material. Yet the exploitation of finer methods for detecting small numbers of exceptional cells, selective procedures, and cytogenetic analysis can be expected to make such distinctions possible. It might be thought that, because of the lack of evidence for somatic synapsis, recombinational mechanisms might not be found; yet they have been discovered in other places that are, *a priori*, just as unlikely. This topic has been reviewed recently by Stern.[79]

Atwood[4] has developed a technique for finding a small number of nonagglutinable cells in a heterogeneous population. The procedure uses a lectin of high anti-A activity which, fortunately, is available in large amounts. One difficulty is that when the cells are sufficiently dilute even competent cells fail to agglutinate. Atwood circumvented this with a method similar in principle to isotope dilution. The cells are labeled with radioactive chromate, then suspended with anti-A lectin and the agglutinated cells removed. At this point fresh unlabeled cells are added and the whole process repeated until the activity of the unagglutinated supernatant becomes constant. From the amount of dilution and the ratio of final to initial activity the fraction of nonagglutinable cells can be calculated.

From A_1O, A_2O, A, B, and A_2B donors exceptional cells, not agglutinable with anti-A, were found in proportions of the order of 10^{-3}. A person thought to be homozygous A_1A_1 gave a smaller proportion of inagglutinable cells, but not as much smaller as independent mutation at both of two loci would predict. A later paper[3] offers further difficulty to interpreting the results by a kinetically simple mutation model. A polycythemic patient of group A_20 treated with P-32 yielded a tenfold increase in O and a doubling of A_2 cells, but the frequencies soon returned to normal.

For all these reasons it is far from clear what the nature of the changes

is, but the power of the technique is such that more definitive results may be expected.

In Vitro Studies

Techniques are now available for growing mammalian cells in clones and many of the experiments of microbial genetics can now be done with such cultures.

Variant clones are easily produced. Puck and his associates have found clones that differ in nutritional requirements, morphology, and resistance to virus destruction.[67, 68] Selective methods are also available for isolation of variants. Drug resistant strains are easily selected and several have been reported.[84] DeMars[19] has developed a technique for selecting auxotrophic mutants. This makes use of the fact that in the presence of aminopterin and with restricted thymine and adenine the cells stop dividing but continue protein synthesis and after a few days become permanently unable to grow, even if aminopterin is removed. On the other hand, an auxotrophic mutant with an additional requirement (glutamine, in this case) does not synthesize protein and later when the aminopterin is removed and the missing amino acid is supplied grows normally. This permits a 10^5-fold increase in cell survival of auxotrophs and offers an efficient technique for discovery of new mutants.

Mammalian cells in tissue culture have shown a high susceptibility to killing by X-rays. The mean lethal dose, computed from the linear proportion of the log survival-dose curve as the dose giving a survival fraction of 1/e, is about 50 roentgens for diploid cell cultures. Much of the lethal effect is probably due to chromosome breakage, some of which is directly observable cytologically.

General Remarks

This review has not included anything about the mutagenic effects of radiation on human germ cells and the possible effects of radiation on future generations. There has been so much written on this subject (see, for example, references 50, 57, 98, 100) that there is nothing I could add.

The assessment of the effect of mutation on the population is complicated not only by lack of information about mutation rates and the action of selection, but also by the still more elusive questions of how to assess the effect of genetic changes on human well being in future environments.

The most general and elegant unifying idea is the Haldane-Muller principle that equates the decrease in fitness (defined in a strict Darwinian sense) with the total mutation rate. This is discussed fully by Muller[49] and others.[13, 14, 49, 57, 100] But others have argued that this formulation leaves out so much of what is important in human society as to be of limited use. See especially Neel and Schull.[57] Wright[99] has suggested an approach to the problem.

Improved estimates of mutation rates of the kind given in this review may be expected because of better facilities for collecting, retrieving, and processing data. This has been stimulated in large part by the increased public concern about radiation risks. In Scandinavia and Germany there are large data centers and some risk figures have been published (e.g. Verschuer;[90] but see Nachtsheim et al.[52] for a scathing criticism).

Increased knowledge of biochemical genetics and the development of in vivo and in vitro somatic cell methods have already brought human genetics into the laboratory, where the methods that have been so fruitful in experimental organisms, especially microorganisms, can be applied. The first significant steps in human cytogenetics have already been taken with the recognition of trisomics, monosomics, and polyploids as distinct from mutation.[21, 31, 37, 62] The experimental study of induced mutation is now possible in somatic cells with such possibilities as tests of mutation theories of differentiation, aging, and carcinogenesis.

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Chapter 2

Natural Selection in Man

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I write this chapter under a severe handicap. The nearest library with an adequate coverage on human genetics is in Bombay. I shall therefore miss many important data. Personally I prefer to live in a country where the assortment of segregating human genes differs from that of Europe and North America to a country with good libraries.

The first point to make is very simple. Darwin postulated natural selection to explain evolution. In my opinion he was fully justified in doing so. But the kind of natural selection which he postulated is exceptional, and although it has been observed and measured in animals it has not yet been observed or measured in men.

Darwin thought of natural selection as a process which slowly changes the mean of a population, as it can be rapidly changed by human "methodical selection" (his phrase; he did not write of artificial selection). The first serious measurements of natural selection were made by Bumpus (1899) and Weldon (1901). They both found strong selection against extremes. That is to say, if the characters which they measured were inherited (which they did not prove), the effect was mainly to conserve the norm of the populations in question. It is now obvious enough why this was so. In a fairly rapidly evolving line like the ancestors of horses, measurable characters change by about 4 per cent per million years. Though much quicker changes sometimes occur, they can only be found in rare cases.

Let us now define selection. The definition is easiest when generations are separate, as in annual animals and plants. Suppose we study an area into which there is no immigration. We count the animals or plants in it, and classify them both for sharply defined characters (like blood

group membership, color blindness, or hemophilia in men) and for continually variable characters (like stature, longevity and intelligence quotient in man). We call this population S. A generation later there is a new population S^1, which may or may not differ from S in the frequency of the various characters concerned. This change, again, may or may not be due to selection. The rising generation in India has a larger fraction of literates than its parents. But now we define a fictitious population P, consisting of the parents of S^1, each individual occurring once for each members of S^1. That is to say, a member of S who had no children is not a member of P. One who had six children is represented six times in P, and so on. It is important that S and S^1 must be enumerated at the same age, preferably as early as possible.

If P differs from S significantly in the frequency of any character, we say that selection has occurred. It may occur because some members of S died before they could become parents. This is still very important in India. Or it may be because some members of S had more children than others who lived as long or longer. This is the main selective agency in hygienically advanced countries. Where, as in man, generations overlap, we can use Fisher's (1930) concept of the reproductive value of an individual rather than counting his or her descendants.

In the case of sharply defined characters selection is a simple affair. We ask whether the fraction of hemophilics in P is the same as that in S. It is of course smaller, but how much smaller we shall consider later. For metrical characters such as height we must ask whether the distributions in S and P differ. If so their mean values may differ. Also the variances (or other measures of spread round the mean) may differ.

So far we have only considered *phenotypic* selection; that is, to say selection based on characters which are fairly readily determined or measured, but whose genetical determination may not be known. We have next to ask whether this selection is *effective*. We say that selection is effective if we believe that the next generation S^1 would have been different had P been a random sample of S. Selection within a pure line is known to be ineffective. I shall later consider how far selection among humans is effective.

In some cases we can observe and measure *genotypic* selection; that is to say, we can discover that the frequencies of various genotypes differ in S and P. This can be done in rather few cases. For example the frequencies of the M, MN, and N phenotypes, each of which corresponds to our genotype, are about the same among parents as among adults or children, but I know of no systematic study which might disclose small differences. In the case of rare dominants homozygotes are

extremely rare. So almost all humans fall into two classes, normals and "mutants," e.g. brachydactyls, epiloiacs, or persons with blue sclerotics. In such cases we can determine whether the gene frequency differs in S and P. In many cases we cannot do so, owing to dominance. Thus there is evidence of a shortage of A children of O mothers by *AO* fathers. This must diminish the frequency of the *A* gene. But in other types of union AO may be at an advantage over *AA*. Owing to the dominance of *A* such an effect is very difficult to detect.

In very few cases are there adequate figures on the difference between S and P. We must remember that theortically S should be studied at birth, or earlier if that were possible. There is at present no way of finding out whether there is phenotypic selection for or against high stature in adults. In some populations at least the shorter adults have larger families (Boas, 1911). But it is possible that this selective tendency is counterbalanced by the premature death of persons who would have been very short if they had lived to be adult, or that it is entirely ineffective, large families and short stature both being due to poverty.

I shall later consider some of the data on natural selection in man in some detail. Meanwhile it is worth considering the consequences of selection. In the first place phenotypic selection in a given direction does not necessarily cause a change of gene frequency in the corresponding direction, even in the absence of mutation. Consider an autosomal recessive lethal gene *L*, say the gene for one of the amaurotic idiocies. Suppose its frequency is exactly 1 per cent in a population of a million. It is convenient to think of the two million genes in the relevant locus of these million people immediately after fertilization. Twenty thousand of these genes were *l*. If there were no inbreeding there would have been about 100 *ll* homozygotes, carrying 200 of these genes. Let us suppose that owing to inbreeding there were 130 homozygotes. Thus 260 of the 20,000 genes had no opportunity of leaving descendants. The other 19,740 genes were in that number of heterozygotes in a population of 999,870. If these heterozygotes were exactly as fit as normals the gene frequency would fall from 1.0 per cent to 0.987 per cent. If, however, the heterozygotes were 1.5 per cent fitter than homozygous normals, the number of *l* genes in the next generation would rise from 20,000 to 20,036 per million. That is to say, the slight advantage of the heterozygotes would more than compensate for the lethality of the homozygotes. It will be seen that the precise advantage needed in the heterozygotes for balancing depends on the extent of inbreeding. Probably a good many genes in human populations are close to stable equilibrium frequencies, that is to say, the frequency changes little from

one generation to another. This may be because of opposing selections, as in the above example, or because selection is balanced by mutation or migration. It is commonly stated that opposing selections only balance when the heterozygotes are fitter than homozygotes. This is not so necessarily true when a gene substitution increases the fitness of one sex and decreases that of the other (Haldane, unpublished). It is however usually true. Very commonly selection is balanced by mutation. This is the case with all rare dominant and sex-linked recessive characters, provided the latter are truly recessive; that is to say, women heterozygous for them do not differ from the "wild type" appreciably in fitness.

Thus natural selection sometimes results in a stable equilibrium and again sometimes there is reason to think that human populations are near such an equilibrium, and may be supposed to be in equilibrium without much error.

In other cases we believe that natural selection is changing genotype frequencies at an appreciable rate, in fact that it is playing the part which Darwin ascribed to it. If so we ask why the population is not in equilibrium. This may be for at least any of the following reasons.

1. Relative fitnesses of genotypes have recently altered. Here "recently" means in the last thousand or so generations, in fact since the upper paleolithic. We may be sure that this is true in very many cases, because of the very great changes in human environment which have occurred in the last 25,000 years. These include climatic changes, changes due to human activity, such as the inventions of agriculture, cities, and birth control, and finally changes due to microbial evolution, which has led to the origin of new diseases.

2. Mutation rates have altered. In the last 30 years they have probably risen owing to increased use of x-rays in economically advanced countries. There may have been much larger changes due to increased or decreased amounts of mutagenic substances in human diets. We know nothing on this matter.

3. Migration from a region where relative fitnesses are different prevents the attainment of equilibrium in a given region. For example sparsely pigmented humans (so-called white men) in India have a much higher death rate from skin cancer than the well-pigmented Indians.

4. One or more mutations have occurred recently which give rise to genotypes favored by natural selection, whose frequency is therefore increasing. They occur so rarely that they had never had the opportunity to spread earlier. Most gene mutations known to us have fre-

quencies of 10^{-6} or more. If some occur with frequencies of the order of 10^{-12} they may be really novel. But no such are known. Chromosomal changes such as inversions may occur with such very low frequencies. That is to say, the frequency with which a chromosome is broken in two particular places, followed by rotation of the central portion and a reunion, and incorporation into a zygote, may be so rare as to be unique in the history of a species. Nothing is yet known of such changes in man.

5. Mating systems have altered. This is the cause of upset of equilibrium of which we know most. For over 1000 years up until 1830 or so, most of the people of western Europe lived in villages, and migration between them was slow. The Catholic, orthodox, and Swedish Lutheran churches tried to discourage inbreeding; but it must have occurred, as few people even now know their 16 great-great grandparents, and thus may unwittingly marry their third cousins.

Consider a community in which the mean value of Wright's coefficient of inbreeding is .01. This is about as high a value as has yet been recorded in a human community, but in primitive rural communities the inbreeding is probably much higher than this. It would be reached in one generation if 16 per cent of all marriages were between first cousins. Suppose an effectively lethal recessive gene, such as that for juvenile amaurotic idiocy, occurs by mutation with a frequency of 10^{-5}. In equilibrium as many genes must be destroyed by death as appear by mutation. So the frequency of recessives must be 10^{-5}. This implies a gene frequency of .000917. If the population begins to mate at random, the frequency of recessive homozygotes falls to 8.4×10^{-7}, or about one twelfth of its former value. Hence only about one twelfth of the new genes arising by mutation are destroyed, and the gene frequently rises very slowly to an asymptotic value of .00316, giving a frequency of recessive homozygotes of 10^{-5}, and a new equilibrium. However this process is very slow. The time needed to reach even half way to the new equilibrium may be several thousand years.

In all European countries, in North America, and many parts of South America the frequency of lethal and sub-lethal recessives is now far below equilibrium, though probably rising very slowly. In India a large drop may be expected as the teaching of Mahatma Gandhi on the desirability of inter-caste marriages is adopted.

Speculation on the effects of natural selection on human populations has been almost entirely focused on the effect of changes in relative fitness. The following are often thought to be true. "As the result of improvements in medicine, many persons who would previously have died young survive to breed. Hence undesirable genes spread through

the population. Secondly, as the result of birth control and late marriage among the well-to-do, and of diminished infantile and maternal mortality among the poor, the poor increase more rapidly than the rich. But genes for intelligence, perseverance, and so on, tend to make their possessors rich. So such genes are being eliminated."

Let us examine these statements. Undoubtedly more persons with undesirable autosomal dominant genes (e.g. that for rectal polyposis and consequent rectal cancer) and sex-linked genes (e.g. that for hemophilia) survive than did so 60 years ago. The frequency of such genes will be increased by mutation until a new equilibrium is reached unless persons carrying them are persuaded not to breed or prevented from breeding. This increase is clearly undesirable. There is also good reason to think that resistance to infection is largely genetically determined. Susceptibility depends to a small extent on non-specific genes, such as that for agammaglobulinemia, but probably (to judge both from human data and those on mice and poultry) mainly on specific genes. Mice selected for immunity to "mouse typhoid" do not gain immunity against other bacterial or virus infections.

We have good evidence (Allison, 1954; for a general discussion see Harris, 1959) that persons heterozygous for sickle-cell anemia are resistant to *Plasmodium falciparum* malaria, though probably not so to other types. The gene in question is however nearly lethal when homozygous, and seems to be disappearing in the descendants of Africans imported into North America, where *P.falciparum* is rare or absent. Within 20 years it should be rare in Africa, and the gene will disappear there also. Such disappearance is desirable.

Now consider tuberculosis. It killed vast numbers of Europeans during the nineteenth century. But it was becoming less dangerous long before modern chemotherapy rendered it almost trivial. This was probably due it part at least to selection of genes increasing resistance to this disease. If these genes, or any of them, diminish human fitness in the absence of serious mortality from tuberculosis they will gradually disappear. In consequence our descendants will become susceptible to tuberculosis (in the absence of streptomycin, isoniazid, etc.) but fitter in some other respects. There is no way of evading this argument. If the genes for tuberculosis resistance had made for increased fitness in the absence of tuberculosis, they would already have spread through the population. If they do not make for lowered fitness they will not disappear at a measurable rate. Thus the prevention and cure of tuberculosis is not a dysgenic measure, and is probably a eugenic one. This important argument is drawn from L. S. Penrose.

According to Fisher's theory of the evolution of dominance, dominant genes gradually become recessive owing to the selection of modifiers which bring the heterozygotes back towards normality. If these modifiers are not absolutely neutral, they must slightly lower the fitness of their carriers, so that their spread is harmful to the species. To take a concrete example, heterozygotes for one type of cystinuria excrete moderate and variable amounts of cystine (Harris, 1959). If modifiers are being selected to lower this amount or to alter the urine in some other way which will diminish their tendency to form calculi, this slight change in physiology will probably be slightly harmful, or it would have occurred already. If by preventive or therapeutic measures these heterozygous cystinurics are made as healthy as normals, the selection in favor of modifiers will cease and may be reversed.

We may put the matter as follows. If any mutant lowers the fitness of heterozygotes or homozygotes, modifiers will be selected which increase the fitness of the mutants but lower that of others. The net effect of such selection is a gain in mean fitness. But if the mutants can be brought to normal fitness by medical care, the gain in fitness will be still greater. We may have to reconcile ourselves to the prospect that our descendants will need more artificial aids, such as spectacles, injections of antigens, and so on, than our ancestors. But the most determined eugenists do not suggest that we should give up clothes because they protect weaklings from frost-bite and sunburn. Our environment is likely to be increasingly artificial, and we should welcome selection which will make us fitter in that environment, rather than some other.

Let us now examine the argument as to the selective elimination of the intelligent. Some of this is merely centripetal selection balanced by elimination of mental defectives (Penrose, 1948). But there is probably a genuine selection against intelligence in some societies. Eugenists often state that this is because the intelligent are prudent, and therefore marry late, and have small families. It seems likely, however, that Kinsey, Pomeroy, and Martin (1948) came nearer to the truth. Educated males in the U.S.A. are much less active sexually than uneducated males. Educational level appears to be more important in this respect than income, race, or religion. In other societies this has not been the case. In particular in some societies where marriages are arranged, the parents of a girl will try to choose her a learned bridegroom. This was the case among Jews through the last 1500 years, and is still the case in some groups in India.

Our ignorance as to the quantitative detail of natural selection in man is enormous. We hardly ever have data comparing the population P with

S. We have not even got adequate data as to the intensity of selection against such characters as hemophilia. Many pedigrees of this condition have been collected but it is obvious that they are likely to include hemophilies two generations or more ago who begot numerous daughters. They therefore give too high an estimate (about half the normal) of the fertility of hemophilics. To avoid this bias it is not enough to exclude the ancestors of the propositus (the abnormal person from whose observation the pedigree starts). The more fertile an abnormal the more likely he is to appear as an ancestor. Since fertility is genetically determined, one should exclude ancestors' brothers also. The most satisfactory method would no doubt be to follow up a hundred or so hemophilic boys from birth. But this is very difficult, and has not yet been done. It seems likely, however, that the mean fitness of hemophilics is about .25.

Usually we have data as to survival over a certain fraction of the life cycle, or liability to certain causes of death. For example Karn and Penrose (1951) showed that there is an optimal birth weight. Babies whose weight at birth falls short of this optimum or exceeds it by 500 Gm. or more have a smaller chance of surviving the hazards of birth and the first month of life than those whose weight is near the optimum. It is possible, but improbable, that this centripetal selection is reversed in other parts of the life cycle. It must be remembered that there is a low correlation between weight at birth and at maturity. Perhaps those whose mean birth weight exceeded the optimum by one Kg. are so much more fertile as adults than the average as to more than make up for their high early death rate. This is unlikely, but we may be fairly sure that results as unexpected as this will be obtained.

Attempts have been made, beginning perhaps with Haldane (1954) to estimate what American workers such as Muller (1950), Morton, Crow, and Muller (1956) and Crow (1958) call the genetic load. This is the over-all effect of gene substitutions in depressing the mean fitness of animal (including human) populations below that of their fittest genotype. Kimura (1958) divides this load into three components. The segregational load is the loss of fitness due to the fact that homozygotes at some loci are less fit than heterozygotes. The mutational load is the loss of fitness due to recurrent mutations. The evolutional or substitutional load is due to the fact that genes at many loci persist, which are gradually being replaced by others as the result of natural selection, for example genes making for susceptibility to tuberculosis in populations where this disease is a major killer. Only the last of these loads is a cause of effective natural selection. Various attempts have

been made to estimate these loads, and current estimates of each of them are of the order of 10 per cent. My own guess is that the evolutional load is much heavier than this. But I also think that it is largely futile, the main selective agencies in the last few thousand years, since populations become dense, having been pathogenic bacteria, protozoa and viruses. But since micro-organisms can evolve much quicker than men, new diseases, or new forms of old ones, are continually appearing, and it would be foolish to expect the evolution of a universally resistant human stock.

However from a humanistic point of view this classification is shallow and may be misleading. The fittest from a Darwinian point of view are not the most socially desirable. Some religions teach that complete celibacy, and therefore zero fitness, is the highest human goal. Before rejecting this view (as I do) biologists should at least consider the social Hymenoptera and Isoptera. My own view is that a human society as far beyond our own as ours is beyond lower paleolithic society would probably keep its numbers stable mainly by clonal reproduction, a minority only being sexually reproduced, and regarded as experimental organisms whose qualities are only partly predictable, and of whom a very small fraction would be eligible for clonal reproduction or further sexual breeding. In such a society natural selection would hardly exist, undesired genotypes being prevented from breeding by human choice. Meanwhile a good deal of the genetic load may be desirable. It is not, I think, desirable that every one should be as fertile as the most fertile existing genotypes. It is certainly undesirable in a society where family planning is not universally accepted.

Nevertheless it would be immensely desirable to know something more about natural selection as it is acting on human beings at the present moment. We do not know many of the questions which we should like to ask. But we do know enough to make them worth asking. Let us consider a possible program. In the first place it is essential to follow up several large samples from birth, or earlier, to the age of 50 years, when further reproduction is negligible, and if possible till death. The samples should if possible be chosen in countries where migration is not intense, for example rural Sweden and rural India.

In the first place we need quantitative evidence about the mutational load. This involves following up at least 100 hemophilics from birth, and similarly with other abnormalities from the time of their first detection. It is very important, because the load is equal to the mutation rate and independent of the fitness. Other evidence can be obtained from the study of the offspring of cousin marriages.

The segregational and evolutional load, can, in principle, be studied on fairly large samples of ordinary populations. The most immediate results would probably be to demonstrate that natural selection for many characters—for example, hair color and blood group membership—was too slow to be detected in samples of only a thousand; and that there was appreciable centripetal selection for other characters, notably weight at birth and later—the centripetal selection probably but not certainly, contributing to the segregational load. But it is reasonably certain that if the work was properly carried out, it would lead to unexpected results.

However until we know more about the relation between genotype and phenotype in man, such a project will yield relatively little information. It can be argued that it should be postponed until enough knowledge of human genetics has accumulated to make it worth while. I suspect that this argument may be equally valid a century hence, but that if nothing is done now our descendants will regret their ignorance of the facts about natural selection in man before infectious diseases were abolished and family planning socially enforced.

Until such a project is carried through, our knowledge of human natural selection will be extremely incomplete. Above all, because some genotype or phenotype is associated with a high death rate at some particular period, it will be assumed to lower fitness. This may be so. But it is not necessarily so. It may lower death rates or raise fertility at other periods of life.

We can be sure that if this project is attempted the environment will be in a far from steady state. Old diseases may be abolished and new ones arise. Radioactivity may increase. If there is a third world war it will probably become the principal selective agency, though it is unlikely that the survivors will be able to study its selective effects in a quantitative way. If other planets are colonized the founding fathers and mothers may be heavily selected for resistance to radiation.

But it is likely that many centuries will elapse before the human environment is again as stable as it was in most regions up to a century ago. And by then we may be embarked on a program of artificial selection. I believe that such a project should be started as quickly as possible.

This conclusion is unsatisfactory. I now believe that we know much less about natural selection in man than I thought was known 30 years ago. It is easy to make out a case for the rapid deterioration of civilized races owing to the lesser fertility of the able, and the protection of the genetically handicapped. If these arguments are correct the inhabitants of Africa should have a much greater mean intellectual capacity than

those of European stock. I know of no evidence for this. It is almost equally easy to support the optimistic theory that mankind is evolving under natural selection towards better adjustment to his environment. This was Darwin's belief. Thirdly, it can be argued that owing to the various processes which Lerner (1954) calls genetic homeostasis, and which resist humanly directed artificial selection, the genetical composition of human populations is changing so slowly that the change may be neglected for some centuries without danger. Finally, it is possible that each of these theories may be true for a group of characters, but none of them true in general.

We do not know. We shall know if enough people want to know.

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Chapter 3

Genetics and Congenital Malformations

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This is an exciting time to live in, for those interested in the gene and its role in development. Progress in fundamental genetics and experimental embryology is rapidly increasing our understanding of the nature and function of genes, and of the developmental processes that they direct. Most of these advances are too recent to have been applied to the problems of congenital malformations in humans. Yet there seems little doubt that ultimately they will be applied, and so it seems appropriate to review some of them, briefly, here.

I. Genetics in Embryology

A. The Gene in Normal Development

1. Transmission of Genetic Information—the Template Hypothesis

The nucleus of the fertilized egg contains a set of maternal and a set of paternal chromosomes, which carry in them the information that

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will direct the development and functions of the individual. Whatever it is that carries the information must be able to reproduce it and send it to the cell cytoplasm. There is now good evidence that this is done by the chromosomal deoxyribonucleic acid (DNA).

According to the ingenious model of Watson and Crick (1953), the DNA unit is a pair of helical strands of nucleotides, each nucleotide consisting of deoxyribose, phosphate, and a purine (adenine, guanine) or pyrimidine base (cytosine, thymine). The strands are held together by hydrogen bonds between the base pairs, and the structural relationships are such that adenine on one chain pairs with thymine on the other, and guanine with cytosine. Thus there is a very specific complementary relationship between the two chains. If they unwind and separate, each can synthesize a chain complementary to itself, and the result is two pairs of chains identical with the original.

Briefly, the template hypothesis holds that the sequence of the DNA base pairs constitutes the code that carries genetic information from the organism's parents to the nucleus of the fertilized egg. The code is reproduced, by mitosis, in all the nuclei of the somatic cells of the organism. The information it carries must then be conveyed to the cytoplasm. This is done by the ribonucleic acid (RNA), some of which is synthesized in the nucleus, where the information carried by the DNA is imprinted on it. The RNA then migrates to the cytoplasm, multiplies, and in turn imprints the appropriate parts of the information it carries on the proteins being synthesized there, just as a template conveys a pattern from type to printing plate. The supporting evidence comes from a wide variety of sources, and though some steps in the process have not yet been fully demonstrated, the concept forms a highly productive working hypothesis.

The best evidence that DNA carries the genetic information comes from bacterial genetics. In the pneumococcus, for instance (and in several other species as well), when cells of a particular serological type are exposed to DNA from another type, a certain number will change their genetic constitution to the type from which the DNA came —an example of directed mutation, and a conclusive demonstration that DNA can carry information that will determine the nature of the recipient cell (Avery et al., 1944; Braun, 1953). The genetic fine structure of DNA has been analyzed in extraordinary detail in bacteriophage (Benzer, 1957), to the point where it seems that a functional alteration in the genetic material (mutation) may involve as little as one or two nucleotide pairs. Further evidence for the genetic role of DNA is well reviewed by Brachet (1957).

How the information is transmitted from the nuclear DNA to the cytoplasm is much less clear, but the bulk of the evidence suggests that it is ribonucleic acid (RNA) which serves as the intermediary agent.

In those viruses, such as the tobacco mosaic virus, that contain only RNA, it has been possible to separate the RNA from one strain and combine it with the protein of a genetically different strain. In the descendants of the "hybrid" virus, the RNA and the protein coat were the type from which the original RNA came, showing conclusively that RNA is capable of carrying the genetic message which determines the specificity of the viral protein (reviewed in Brachet, 1957).

Radioactive isotope and other studies show that RNA is synthesized in the nucleus (Brachet, 1957; Zalokar, 1960) and migrates to the cytoplasm, where it appears in small particles called microsomes. By a remarkable series of electron micrographs of the giant chromosomes of Drosophila salivary glands, Gay (1960) has shown that, at the time when secretion granules appear in the cytoplasm, the nuclear membrane develops blebs containing material which is continuous with certain specialized regions of the chromosome. The blebs are then released into the cytoplasm and become involved in the formation of secretion granules. It seems likely that this is a direct visualization of the passage of genetic information carried by the RNA from chromosome to synthesizing cytoplasm. There is also a wealth of evidence that RNA appears in high concentrations in the cytoplasm when proteins (including enzymes) are being rapidly synthesized, and is probably essential to protein synthesis (Brachet, 1957; Spiegelman, 1957).

How the information is passed from DNA to RNA to protein is still not known, but the template hypothesis (Spiegelman, 1957) suggests that the base-pair sequences of the DNA determine a surface pattern which acts as a sort of mold (template) conferring a corresponding pattern on the microsomal RNA, and that the RNA in turn acts as a mold to guide the construction of the protein molecules. Hoagland (1959) describes in the cytoplasm a special type of water-soluble RNA of small molecular size called "transfer RNA," that appears to be the link connecting the code in the microsomal RNA template with the sequence of amino-acids in the proteins being synthesized. Each amino-acid molecule attaches itself to a molecule of transfer RNA at a specific spot determined by the RNA pattern. The transfer RNA molecule then attaches itself, with its amino-acid, to the microsomal RNA at a spot where it finds its complementary nucleotide sequence. Thus the amino-acids are lined up to form protein in a sequence which is determined by the pattern of the RNA template, which in turn depends on the sequence of

nucleotide pairs in the chromosomal DNA. The reader is referred to Hoagland's article for a graphic depiction of this process.

It can be seen from this brief review that great progress is being made in clarifying the extraordinarily specific relationship between a gene and the amino-acid sequence of a particular protein molecule, as so beautifully demonstrated in the genetically determined abnormalities of hemoglobin structure, reviewed elsewhere in this volume (Rucknagle and Neel, Chapter 6).

Possible relevance to human malformations. The discovery of bacterial transformation—a directed, heritable change in a characteristic of one type of bacterium by exposure to DNA from another type—raised the question of whether this could also be done in higher organisms. This would, of course, have very practical applications. For instance, it might be possible to cure hemophiliacs, if one could induce transformation of the gene for hemophilia to its normal allele in a sufficient number of somatic cells to supply adequate amounts of anti-hemophilic globulin. The same would apply to a variety of other "inborn errors of metabolism;" but it is difficult to see how this method could be used to prevent hereditary malformations, since one would have to know in advance which embryos to treat, and would probably have to transform a relatively large number of cells, early in development. So far, even in bacteria, very few of the cells exposed to the transforming principle actually undergo genetic change. Alternatively, one might hope to transform the mutant genes in the parental gonads, but again, one would have to affect a majority of the cells exposed—and have some safeguard against transforming other normal genes to mutant ones, since the existence of recessive genes makes it impossible to say that if DNA is normal at one locus it is normal at all loci. In any case, the human race does not yet appear sufficiently advanced, morally, to be entrusted with a method of rapidly altering its genetic constitution!

A group of French investigators claims to have induced genetic transformations in ducks by injecting DNA from one race into another (Benoit et al., 1957), but at least one group has failed to confirm this (Svoboda and Haśkova, 1959). An attempt to induce somatic transformations by DNA (in rats) was also unsuccessful (Bearn and Kirby, 1959).

2. *Differentiation*

If all the somatic cells of the body have the same complement of chromosomes (with the exception of certain tissues that are polyploid), how is it that there are many different kinds of cells in the adult organ-

ism—i.e. how does differentiation occur? It probably begins as a result of the fact that the cytoplasm of the fertilized egg is not homogeneous, but varies from region to region. Cleavage then divides the egg into a number of cells, each of which, because of this, will be cytoplasmically different from its neighbors, and each of which contains a totipotent nucleus. Each nucleus now has a different substrate to act upon. The same nuclear message, sent to different cytoplasmic receivers, can be expected to produce different responses. Also, different cytoplasms may evoke different messages from the DNA—i.e. some parts of the DNA may become active in some cytoplasms and others in others. Furthermore, the nucleoprotein must replicate itself from its protoplasmic substrate, and if this varies in different cells the nucleoprotein itself will become different, so that the DNA in one type of cell may not be able to send the same messages as DNA in another type—nuclear differentiation has occurred.

Evidence to support this concept of differentiation by a progressive series of interactions between nuclei and cytoplasm comes from a variety of sources. The existence of cytoplasmic differentiation in the egg (sometimes to a remarkable degree of specialization) has been well demonstrated in a wide variety of organisms (Waddington, 1956; Brachet, 1957). Many facts demonstrate that certain genes appear to act only in certain types of cells; for example, the gene causing pink eye in the mouse acts by preventing melanoblast differentiation in the Harderian gland, but does not have a detectable effect in other areas of the body (Markert, 1958)—thus the effect of the gene must depend on the metabolic pattern of the cell concerned. Further evidence of differential genetic activity comes from studies of the giant chromosomes of Diptera. These chromosomes have characteristic patterns of banding, which undergo specific modifications, such as the formation of puffs and rings, in certain regions of the chromosome in particular tissues at particular stages of development, suggesting that the regional activity of the chromosomes varies with the physiological activity of the cells they are in (Beerman, 1956).

Evidence that the nuclei themselves become differentiated during development comes mainly from the elegant experiments of King and Briggs (1956), who developed a method for removing the nuclus from an unfertilized frog egg and replacing it with a nucleus taken from a cell in the late blastula or early gastrula. Many of these eggs completed development normally, showing that the donor nucleus was still totipotent. When the donor nucleus came from the endoderm of a late gastrula the host eggs fell into three classes—they either completed development nor-

mally, or showed various degrees of abnormal development (particularly of *ectodermal* structures), or failed to develop beyond the gastrula stage because of defects in the chorda mesoderm. Thus, within a group of endodermal cells that all look alike, some have nuclei that are still totipotent, while in others the developmental potential has been restricted to various degrees. Furthermore, serial retransplantation of nuclei from the three classes of embryos showed that they retained the same developmental potentials as they showed in the original transplantation—i.e. some of them had become—within the limits of the experiment—permanently differentiated.

Finally, the presence of self-producing particles in the cytoplasm, and their possible role in differentiation, should be mentioned (Catcheside, 1958). Such particles have been demonstrated in a number of cases, and it has been suggested that cytoplasmic differentiation could occur as a result of competition between various types of such particles, with one type gaining the ascendancy, depending on the cellular environment. Sonneborn (1951), for instance, has shown that *Paramecium* can exist in one of several antigenic types. These behave as if determined by a system of cytoplasmic particles. The genes determine which types of particle may be present, but which one actually determines the antigenic quality of the cell depends on the conditions of culture (Beale, 1958). Thus genetically identical descendants of one Paramecium may have different antigenic reactions, depending on their environments. It has been suggested that the descendant cells from a fertilized egg may become different from one another in a similar way.

From this all-too-brief review one can see that rapid progress is being made in analyzing the exceedingly complex biochemical interactions between genes and cytoplasm which form the basis for differentiation. Since many malformations involve errors in differentiation, progress in this field has an important bearing on teratology. But studies of the individual cell, however elegant, cannot throw very much light on the developmental mechanisms of multicellular organisms. What is the biochemical basis for the mysterious forces that allow cells to recognize one another, to undergo migrations to highly specific locations, and to form themselves into structured masses which perform the complicated involutions and other marvelous movements of morphogenesis?

3. *Embryonic Induction and Morphogenesis*

The inductive process is obviously basic to the problem of abnormal development, and progress in this field will be followed with great

interest by those concerned with the causes of malformations. Many intriguing problems of development are now being actively investigated, such as the relation of cell membrane properties (particularly adhesiveness) to mass cell movements in morphogenesis (DeHaan, 1958), enzyme induction in embryonic cells and bacterial populations, and the immunological mechanisms involved in the regulation of growth and differentiation (Ebert, 1958). Only a few of them can be mentioned here. Others are well reviewed elsewhere (McElroy and Glass, 1958).

Induction involves the initiation, by one region of an embryo, of morphogenetic processes in another. Thus transplantation experiments have shown that the region of the blastopore (the "primary organizer") will cause the surrounding tissue to form neural tube, and the optic cup, protruding from the primitive brain, induces formation of lens in the overlying ectoderm. A great deal of effort has been spent trying to identify, biochemically, the inducing principles, but the picture is still far from clear. Evidence is accumulating that they may be nucleoproteins. For instance, Niu (1959) has shown that cultures of gastrula ectoderm cells grown in extracts of adult organs such as kidney, liver, or thymus, develop characteristics of the organ from which the extract came, and that the active component of the extract is probably RNA.

Another intriguing approach to the problem of RNA's role in induction has been reported by Ebert (1959). The (RNA containing) Rous sarcoma virus when placed on the chick chorioallantois produces characteristic ectodermal and mesodermal sarcomatous lesions. A mixture of virus and microsomes from chick heart muscle was digested with hyaluronidase, and the extracted microsome fraction was inoculated on chick chorioallantoic membranes. The resulting growths contained, besides the typical sarcoma cells, large numbers of muscle-like cells and fibers, some with the characteristic cross-striations of cardiac muscle. No tumors resulted from inoculation of heart microsomes alone, and no muscle cells were found in tumors produced by the virus alone. Clearly, the virus facilitates the transfer of "heart-muscle-forming" information from the RNA of the heart microsomes to the tumor cells. As Dr. Ebert says, ". . . the questions are many, the answers few," but further developments in this field should be illuminating.

B. Mutant Genes and Abnormal Development

It seems well established that all stages of development are guided by genes. In a few cases it can be shown that the egg itself carries the imprint of the maternal genes, which can influence its pattern of cleavage,

gastrulation, or later embryogenesis (Waddington, 1956). In the mouse there is a series of mutant genes at the *T* locus, one of which (t^{12}) in the homozygote kills the embryo in the morula stage about four days after fertilization. Another allele (t^{o}) suppresses mesoderm formation and leads to death at the time of gastrulation (five-six days), and still others kill at later stages of development (Dunn, 1956; Bennett et al., 1959).

On the other hand, one can equally well say that all stages of development are guided by the environment. A genetically normal embryo may develop abnormally if exposed to a noxious environmental agent (teratogen). If the resulting anomaly resembles that produced by a mutant gene it is called a "phenocopy" of that gene. Furthermore, it is well known that the effect of a mutant gene may be altered by its environment (which of course includes the other genes of the organism). For instance, the Yellow (*AY*) gene in the mouse, which when heterozygous causes a yellow coat color, kills the homozygous embryo at the time of implantation, but homozygous embryos that were transplanted to non-Yellow mothers lived longer than those that grew in their own (Yellow) mothers (Robertson, 1942).

Mutant genes that bring about abnormalities of development provide valuable opportunities for finding out how malformations develop. As we have seen, some genes, at least, act by controlling the synthesis of protoplasmic constituents in highly specific ways. If this applies in general, it should be possible to identify, in biochemical terms, specific abnormalities underlying gene-determined malformations, which would be a tremendous step forward in our understanding of the mechanisms of abnormal development. So far, I am not aware of any examples where this has been done in mammals, but biochemical techniques are advancing so rapidly that success does not seem far off. In order to identify the biochemical defect in a particular case it will first be necessary to identify, histologically, when and where in the embryo the first signs of abnormal development occur. This in itself contributes to the understanding of abnormal development, since it requires tracing back, through embryological history, the chain of abnormal events that eventually manifests itself as a malformation. The gene, in effect, can be used as an experimental tool, that alters development at a specific point; by studying the abnormal consequences of this one can infer what the normal inductive and other developmental relationships may be. This approach has been ably presented elsewhere (Gluecksohn-Waelsch, 1954; Gruneberg, 1957). Only a few of the many interesting examples can be touched on here.

A series of studies that demonstrates well the use of mutant genes to

analyze developmental mechanisms has been described by Zwilling (1956). The limb buds of chicks (and some other vertebrates, including mammals) begin as mesodermal thickenings covered by ectoderm. The ectoderm develops a thickened ridge, the apical ectodermal ridge, along the free edge of the bud. When this ridge is removed, the distal end of the limb does not form, showing that the ridge is essential for normal development of the distal wing. In the "wingless" mutant, the apical ectodermal ridge degenerates, and no wing is formed. Grafts of genetically normal apical ectodermal ridge to "wingless" mesoderm also regress, suggesting that the mutant mesoderm lacks something necessary for the maintenance of the ectodermal ridge. When the apical ectodermal ridge from a genetically normal chick was grafted to the limb bud mesoderm of a chick carrying a gene for polydactyly, the resulting wing was polydactylous, whereas a polydactylous apical ectodermal ridge grafted to normal mesoderm resulted in a normal wing, showing that the stimulus for extra digit formation came from the mesoderm. In the polydactylous mutant the apical ectodermal ridge was larger than normal, and the excessive growth occurred preaxially, over the portion of the limb that formed the extra digit. Thus normal limb development depends on a complementary relationship in which the apical ectodermal ridge promotes continued outgrowth of the underlying mesoderm, but also receives from the mesoderm a stimulus necessary for its maintenance. Each depends upon the other. Abnormal development results from a deficiency in the maintenance factor in one case (wingless) and an excess in the other (polydactyly).

In the mouse, the series of mutations at the "T" locus is interesting from many points of view. The dominant gene *T* in the heterozygous condition causes shortening of the tail. The gene appears to affect primarily the notochord (Gluecksohn-Waelsch, 1954), perhaps by altering its surface properties (Gruneberg, 1958b). In the homozygote the gene has much more severe effects on the notochord, neural tube, and somites, and the homozygous embryos die on the eleventh day of gestation. There are now a large number of alleles known at the T locus, all affecting the axial structures (if present) at various stages (Dunn, 1956). In t^{12}/t^{12} embryos development ceases in the morula stage, and there is evidence of a deficiency in RNA and failure of the cells to organize themselves into a blastocyst. Embryos homozygous for t^{o} die at about nine days of gestation, and show duplications of the neural axis. A group of *t* alleles recently described by Bennett et al. (1959) produce their effects in the latter half of gestation, and cause pyknosis and degeneration in the ventral part of the neural tube, and, later, abnormal development of the

head skeleton, including cleft palate. Studies of combinations of these alleles suggest that *T* is a compound locus, participating in the induction mechanisms of the developing embryonic axis, that different alleles represent mutations at different sites within the main gene (Dunn, 1956), and thus that different regions of the gene are involved with different aspects of the inductive mechanism.

An experimental analysis of these inductive mechanisms has been begun by Bennett et al. (1958), with somewhat surprising results. Neural tube from *T/T* embryos, cultured *in vitro* with somites from a normal embryo, will induce cartilage formation in them. On the other hand, *T/T* somites will not form cartilage when cultured with normal tube, but *T/T* limb buds will produce cartilage in culture, showing that, although there is no fundamental biochemical block to cartilage formation in *T/T* embryos, the somites cannot respond to the neural tube's inductive stimulus. Bennett suggests that the failure of cartilage formation in *T/T* somites may be due to a lack of a "pre-inductive" stimulus, perhaps from the notochord, which the somites require before they can respond to the neural tube. On the other hand, it may be that the gene affects directly the capacity of the somites to respond, though this does not seem likely in view of the histological evidence of abnormalities appearing first in the notochord. Further results are awaited with interest. It seems clear that it is not a simple matter to identify the developmental stages at which alterations at the *T* locus interfere with the embryo's inductive mechanisms; how much more difficult it will be to specify the biochemical changes involved.

Another example of the use of a gene-determined developmental error in the analysis of embryological mechanisms is the "Danforth's short-tail" (*Sd*) mutant in the mouse, investigated by Gluecksohn-Waelsch (1954) and others. The gene is semi-dominant, producing a shortening of the tail, reduction of the vertebral bodies, and reduction or absence of the kidneys in the heterozygote. Here again the importance of the "genetic environment" is demonstrated by the fact that the kidney anomalies appear in certain strains and not others, though both are carrying the same mutant gene. In the homozygote there is absence of the tail, rectum and anus, partial or complete absence of bladder, urethra and kidneys, and spina bifida. Studies of mutant embryos demonstrated that the absence or reduction of the kidneys resulted from a failure of the ureter to induce differentiation in the kidney anlage. Gruneberg (1958a) has shown that all the various manifestations of the mutant gene can be understood as stemming from an abnormality in the primitive streak—perhaps, he suggests, a change in its surface properties. This would give rise to an

abnormal notochord which degenerates, leading to the anomalies of the vertebral bodies and loss of the tail. The notochordal anomaly is also held responsible for the reduction of cloaca and tail gut, with consequent absence of bladder and urethra. Delay in establishment of contact between the reduced cloaca and Wolffian ducts may interfere with growth of the ureter buds and lead to their failure to induce the metanephros. Here again effects of a mutant gene on a specific developmental process have provided an opportunity to gain a better understanding of inductive mechanisms in the mammalian embryo.

An even more striking example of the extraordinary effects a gene can have on development has been described by Hummel (1958). A semi-dominant gene, "Disorganization," in the mouse, in the heterozygote may produce no abnormalities (i.e. it shows reduced penetrance) or one or more of a wide variety of malformations including exencephaly; small ectopic or missing eyes; absence or reduction of mandible and maxillae; clefts of the alveolus; cleft palate; microstomus or macrostomus; branchiogenic cysts; accessory tongues; rachischisis; rib malformations; extra or missing limbs; extra long bones; gastroschisis; bladder exstrophy; duplication of large intestine and rectum; and hemartomas. Apparently the gene interferes in some way with a variety of inductive mechanisms, but the embryology is yet to be worked out. Penetrance varies with the genetic background, and the intra-uterine environment, but the type of malformation produced does not. One wonders if some of the bizarre multiple malformations seen in humans might represent dominant mutations to a gene of this sort.

Possible relevance to human malformations. It has been proposed that inherited malformations may turn out to be "molecular diseases" comparable to sickle cell anemia in which a mutant gene produces an abnormality in the structure of cytoplasmic large molecules, and that it should be possible, by the appropriate biochemical methods, to identify a specific molecular abnormality for each type of inherited malformation. This is very probably so, since no other mode of gene action is recognized, but it may not be possible to detect the molecular abnormality post-natally. It seems likely that the action of a gene in altering an inductive or morphogenetic process may be a fleeting one, such as a change in the surface properties of certain cells, or an alteration in a metabolic gradient in a particular area only at a certain time in development, and not detectable at birth, no matter how powerful the biochemical technique. It would seem that identification, in biochemical terms, of the action of teratogenic genes, will, in many cases, have to be done at or before the time in embryogenesis when the first morphological abnormal-

ity appears. Thus there is a great need for intensive biochemical and biophysical studies on normal and abnormal embryos.

C. Genetic Differences in Response to Environmental Teratogens

Many workers, studying the embryological effects of a variety of teratogens, have reported strain differences in the frequencies of malformations produced by a given treatment (Kalter and Warkany, 1959), yet little use has been made of the opportunities so provided for analysis of the mechanisms of abnormal development.

For instance, in tracing back through embryogenesis the sequence of changes resulting in a congenital malformation, it is useful to know that a high proportion (preferably all) of the embryos studied early in development would have had the malformation at birth. If one is studying cleft palate, for instance, produced by a method that causes only a few of the embryos in each litter to be affected, it is difficult to tell whether changes observed before palate closure are occurring in those embryos that would have had cleft palates at birth. But if the treatment is known to produce cleft palate in all the embryos in a litter, then it is clear that changes observed in the palate before the time of closure actually precede its failure to close. Thus it is advantageous to use strains of animals that produce high and low frequencies of the malformation being studied. For instance, by comparison of the A/Jax inbred mouse strain, in which the appropriate maternal treatment with cortisone produces cleft palates in virtually all of the resulting offspring, with the more resistant C57BL/6Jax strain, Walker and Fraser (1957) were able to show that the malformation in this case was caused by a delay in the movement of the palatine shelves from their vertical position on either side of the tongue to the horizontal position, overlying the tongue.

Strain differences in response to a teratogen are also significant in emphasizing that even when the embryo is exposed to an environmental teratogen, its genetic constitution is important in determining the likelihood that it will be malformed. Maternal treatment with a given dose of cortisone produced cleft palates in virtually 100 per cent of the embryos of the A/Jax mouse strain, but in only about 20 per cent of the embryos in the C57BL strain (Fraser et al., 1954). Studies of crosses between the two strains showed that the frequency of cleft palate produced by cortisone depends not only on the genotype of the embryo but that of the mother (Kalter, 1954), thus demonstrating the importance of the (genetically determined) uterine environment in determining the developmental pattern of the embryo.

Strains that give high and low frequencies of a malformation following maternal treatment with a teratogen also provide opportunities for gaining information about the mechanism by which the teratogen acts, and the factors that influence its action. Studies on the time of palate closure in the untreated A/Jax and C57BL strains, crosses between them, and back-crosses to the A/Jax strain, show a correlation between the normal time of palate closure and the frequency of cortisone-induced cleft palate—the later the time of palate closure in untreated animals, the higher the frequency of cleft palate appears to be in treated animals (Trasler and Fraser, 1958). Furthermore, the appearance of acid-mucopolysaccharide in the embryonic palatine shelves before closure appears to differ in the two strains and to be influenced differently by maternal cortisone treatment, although this has not yet been confirmed. In any case the example shows that inter-strain comparisons may be helpful in gaining insight into the complicated interactions of mother, embryo and teratogen.

D. The Embryonic Genotype and the Intra-Uterine Environment

As already mentioned, the development of the embryo depends not only on its own genes, but on its environment, which is influenced by its mother's genes and by her environment (which of course includes the developing embryo). A great deal of attention is now being given to the effects on the embryo of alterations in the mother's environment, such as nutritional deficiencies, hypoxia, radiation and drugs (Kalter and Warkany, 1959), but relatively little attention has been given to the effects of the mother's genotype on the pattern of embryonic development, and this is a problem that might merit more intensive investigation. We have already mentioned, for example, the relationship between the frequency of cortisone-induced cleft palate and time of normal palate closure in the mouse. In the genetically similar F1 hybrids between the A/Jax (late closing) and C57BL (early closing) strains, the palate closes later when the mother is from the A/Jax strain and the father from the C57BL strain than in the reciprocal cross, showing that the mother's genotype influences the pattern of embryonic development, with important consequences when the embryo is exposed to a teratogen. Other examples of this phenomenon have been described by Landauer (1954) in the chick.

An elegant approach to the problem of interaction between embryonic and maternal constitution involves the transfer of fertilized ova of one

genotype to mothers of another. In this way, eggs of the same genetic constitution and with the same cytoplasm can be grown in different uterine environments, and the effects of the uterine environment can thus be separated, experimentally, from those of the genotype and egg cytoplasm. McLaren and Michie (1958) have shown by this technique that variations in the number of presacral vertebrae in mice can be determined partly by the effect of the mother's genes on the uterine environment. Using a similar technique but different strains, Green and Green (1959) demonstrated that genetically determined differences in the cytoplasm of the ovum may also influence vertebral differentiation. Obviously the situation is complicated, but the approach should be a useful one for analyzing some of the problems of abnormal development.

Perhaps the most puzzling aspect of the interaction between embryo and its uterine environment is the fact that when genetically similar embryos, in the same uterus, are exposed to the same teratogen, some may become malformed and others not. So far no clear evidence is available as to the nature of the environmental variables involved. The same question applies to the "spontaneous" occurrence of malformations in certain inbred lines, in the absence of any known environmental teratogen. For instance, about 10 per cent of the offspring of the inbred A/Jax mouse strain are born with a cleft lip, but only a few animals in any one litter are affected. Why? One clue is supplied by the fact that animals with a cleft lip are more likely to occur in the uterine position next to the ovary than in other positions (Trasler, 1960), but what it is about this uterine site that makes the difference remains unclear, in spite of intensive efforts to identify the predisposing factor. One is led to suppose that the intra-uterine variable must be a rather subtle one. It is clear that the A/Jax strain genotype determines that the animal is likely to have a cleft lip, since the defect does not appear in other strains in the same environment. It follows that the A/Jax genotype may be making the embryo's lip closure mechanism sensitive to alteration by relatively small environmental disturbances. If so, it might be more rewarding to search for the biochemical characteristics of the embryo that make its lip closure mechanism unstable, than to try to identify the minor, and probably multiple, intra-uterine variables that upset the unstable process.

An indirect approach to this problem would be to study the effects of specific chemicals on the frequency of "spontaneous" malformations within a strain. Landauer (1957) has shown, for instance, that treatment with insulin produces higher frequencies of rumplessness in strains of chicken that have an appreciable (5 per cent) spontaneous frequency

of the defect than in strains with much lower frequencies. This would seem to suggest that the genes causing instability of the rump-forming embryonic system act by altering something that is easily upset by a pharmacological effect of insulin. The report of Woollam and Millen (1960) that maternal treatment with thyroxine lowers the frequency of "spontaneous" cleft lip in the A mouse strain is an encouraging indication that this approach may be a productive one in mammals as well.

Possible relevance to human malformations. It seems unlikely that the experimental production of malformations by teratogens will tell us much about teratogens in humans, since most teratogens are effective only in non-physiological doses, to which humans are most unlikely to be exposed. On the other hand, the "sporadic" malformations that occur with characteristic frequencies in some inbred lines of experimental animals may be much more comparable to the common human malformations. Both are characterized by a genetic constitution that predisposes the embryo to the effects of little understood environmental factors. If the biochemical characteristics of the embryo associated with this predisposition could be identified in experimental animals, and if means could be found to correct the biochemical abnormality, the results might well suggest prophylactic measures in humans.

II. Genetics of Human Malformations

Apart from one major break-through (the discovery that some malformations are due to chromosomal excesses or deficiencies), progress in the genetics of human malformations has been slow. Perhaps the most encouraging indication of progress is the gradual disappearance of the tendency to take sides in the argument about whether malformations are hereditary or environmental.

The current attitude about the causes of malformations may be summarized in the following two statements:

1. Some malformations are determined by gene mutations or chromosomal anomalies, others mainly by environmental teratogens, and others by a complicated interaction between multiple genetic factors and a variety of ill-defined environmental factors. The latter group is by far the commonest.

2. Any specific clinical category of malformation is likely to be etiologically heterogeneous, containing examples of all three classes described in statement 1.

A. Malformations with a Clearly Identified Environmental Cause

So far, in spite of intensive search, relatively few specific environmental agents have been shown to cause malformations in human beings. These include radiation during pregnancy (Murphy, 1929; Plummer, 1952), maternal infection with rubella (Hill et al., 1958), or toxoplasma (Feldman, 1958), acute maternal folic acid deficiency during pregnancy (Thiersch, 1952), and maternal treatment with synthetic progestins (Wilkins et al., 1958). (The evidence for Asian influenza is at present inconclusive—Coffey and Jessop, 1959; Walker and McKee, 1959). The environmental teratogens fall outside the scope of this discussion, except for the fact that exposure to the teratogen at a time when the embryo is known to be sensitive, does not always cause a malformation. Nothing is known of why some embryos are affected and others are not, but results of experimental teratology (see above) suggest the possibility that the difference may lie in the genetic constitution of embryo and/or mother. This will be hard to test in humans, since it would require studying a series of cases in which the mother was exposed to the same teratogen in several pregnancies. It might be possible to obtain indirect evidence, e.g. by seeing if the frequency of malformations was increased in the near relatives of children with the prenatal-rubella syndrome (which would suggest a genetic predisposition to maldevelopment, exacerbated by the rubella infection), but so far as I know, no such studies have been done.

B. Malformations with a Clearly Identified Genetic Cause

1. Single Gene Mutations

There are a large number of congenital malformations that show Mendelian patterns of inheritance—i.e. each appears to be caused by a change at a particular genetic locus. Although each one may be very rare, together they compose an appreciable part (though probably under 10 per cent) of the total malformation load (United Nations Scientific Committee, 1958). A comprehensive listing of them is not available, but a number of authors have attempted to tabulate them with varying degrees of incompleteness (Fraser, 1954; Fraser, 1958b; Gates, 1946; Stevenson, 1959; Cuendet, 1957; Francois, 1959—for eye diseases; Witkop, 1958—for dental conditions).

a. *Autosomal dominant inheritance.* The conditions easiest to identify as being hereditary are those showing dominant inheritance, i.e. (as the term is commonly used in human genetics) conditions caused by a single gene in the heterozygous state. Strictly speaking, a dominant mutation should be one in which the heterozygote resembles the mutant homozygote. When the heterozygote is intermediate between the mutant and normal homozygote for the character concerned, one speaks of "intermediate dominance." But mutant genes causing diseases or defects are usually so rare that the homozygote may never have been observed, and so one cannot make the distinction between "complete" and "intermediate" dominance. Furthermore, the decision as to whether a trait is dominant or recessive may depend on how closely one looks for its expression in the heterozygote. The gene for sickle cell hemoglobin, for instance, can be considered dominant, in so far as its presence can be detected by special tests on the blood of the heterozygote, or it can be considered recessive on the grounds that it produces clinical disease only in the homozygote. For the present discussion, a dominant gene for a malformation will mean one that causes the malformation in the heterozygote.

In malformations showing dominant inheritance, each affected individual has an affected parent (except in the case of mutation), each offspring of an affected individual has a 50:50 chance of being affected, and the sexes are equally affected. Malformations showing this pattern of inheritance, at least in some families, include achondroplasia (Mørch, 1941), dentinogenesis imperfecta (Hursey et al., 1956), the "nail-patella syndrome" (dystrophic nails, absent patellae, deformed head of radius, iliac spurs—Wildervanck, 1950), hydrotic ectodermal dysplasia (Clouston, 1929), lobster claw defect of hands and/or feet (Stiles and Pickard, 1943), and Madelung's deformity (Paus, 1951).

b. *Sex-linked inheritance.* Sex-linked genes are those carried on the X chromosome. (There do not seem to be any well-established examples of diseases caused by genes on the Y chromosome—Stern, 1957.) A recessive gene on the X chromosome will not express itself in heterozygous females, but will express itself in males since there is no normal allele on the Y chromosome. This is the basis for the characteristic pattern of inheritance—transmission through unaffected females, appearing in half the sons of these females. Malformations showing sex-linked recessive inheritance include the anhydrotic type of ectodermal dysplasia (Halperin and Curtis, 1942), one form of gargoylism (Herndon, 1954), one form of hydrocephalus (Bickers and Adams, 1949), and the testicular feminization syndrome, or male pseudohermaphroditism (Burgermeister, 1953).

Sex-linked dominant inheritance resembles autosomal dominant inheritance except that all the daughters and none of the sons of affected males will be affected. Although a few diseases showing this type of inheritance are known in humans (e.g. hypophosphatemic rickets—Graham et al., 1959), I am not aware of any malformations that do so.

c. *Autosomal recessive inheritance.* A recessive gene, not on the X-chromosome, is defined as one that expresses itself phenotypically only when homozygous. Since malformations caused by recessive genes are rare, affected children almost always come from unaffected parents, who are both heterozygous. Each sibling of an affected child therefore has one chance in four of being affected, but only rarely are there affected relatives other than siblings, unless there is inbreeding in the family. With a 25 per cent probability of recurrence in siblings, and the small size of most modern families, it follows that most cases of a recessively inherited disease will be "sporadic"—i.e. the family history will be negative. Since marriage between relatives increases the probability that both parents will carry the same genes, the frequency of consanguinity is higher in the parents of children with recessive diseases than in the general population.

Examples of recessively inherited malformations include female pseudohermaphroditism due to adrenal hyperplasia (Childs et al., 1956); chondrodystrophia calcificans congenita (Fraser and Scriver, 1954); chondroectodermal dystrophy (dwarfism, polydactyly, defective nails and teeth, and other malformations—Metrakos and Fraser, 1954); one type of gargoylism (Jervis, 1950); and some cases of microcephaly (Komai et al., 1955).

d. *The problem of the "sporadic" case of a rare malformation.* Even when a malformation is known to show Mendelian inheritance as a rule, the geneticist or counselor may, from time to time, be confronted with a "sporadic" case of the malformation—i.e. a case in which there are no affected relatives in the family. Whether one is interested in the genetics of the condition from a research point of view, or because the case has been referred for genetic advice to the family concerned, the interpretation of the sporadic case presents a knotty problem. A variety of possible interpretations exist, including the following:

a. Recessive genes: As mentioned previously, the combination of small family size and a one in four probability of a child being affected means that most cases of recessively inherited diseases in humans will be sporadic. This presents a problem to the genetic counselor confronted with the sporadic case. It is well known that clinically similar diseases may show different modes of inheritance. For instance, achondroplasia is usually the result of an autosomal dominant gene, but occasionally it

shows autosomal recessive inheritance. Thus the counselor, in evaluating the sporadic case, must consider the possibility that it is caused by a recessive gene, in which case the risk of recurrence for siblings would be one in four, rather than the very low figure if a dominant mutation is involved.

b. Phenocopies: It is well established in experimental genetics that a genetically normal organism may be made to acquire the characteristics of a mutant organism by exposing it to an appropriate environmental change at a particular stage of development. Such organisms are called "phenocopies" (Goldschmidt, 1938). For instance, the cataract caused by prenatal infection with rubella might be regarded as a phenocopy of a clinically similar cataract caused by a mutant gene. A sporadic case of a malformation which is known to be inherited in some families may thus represent a phenocopy. The distinction is important, since, if a genetic change is involved, the probability of recurrence of the malformation in the family may be quite high, but if the malformation is a phenocopy the chance of recurrence will depend only on the chance of recurrence of the causal environmental factor, which may (as in the case of the rubella cataract) be extremely remote. Unfortunately, unless a prenatal factor such as rubella can be identified (and such recognized factors in humans are few), it may be quite impossible to distinguish this from other reasons for a negative family history. Statistical methods have been developed (Morton, 1958) for estimating the proportion of phenocopies in a series of cases of a particular disease, but we are still usually unable to tell which particular cases are phenocopies and which not.

c. Mutation: The significance of mutation in man is capably dealt with elsewhere in this volume (Crow, 1960), and will be mentioned here only in relation to the problem of the sporadic case. A mutation is a sudden and persisting change in the nature of the gene. A sporadic malformation may, of course, result from a dominant mutation occurring in the sperm or egg which gives rise to the child, or in the relatively immediate antecedents of these cells. If the sporadic case does in fact represent a dominant mutation, the malformation will have a 50:50 chance of occurring in each child of the malformed person. The risk for the siblings, on the other hand, is usually low. For instance, in children born with split hand and/or foot who had unaffected parents and negative family histories, only six out of 97 siblings were affected (Birch-Jensen, 1949), and some of these may have been due to inclusion of some cases of reduced penetrance (see next section).

Further evidence that at least some sporadic cases represent dominant mutations comes from studies of parental ages. For instance, achondro-

plasia usually occurs sporadically, although as with the lobster-claw malformation, the genetic pattern in the descendants of achondroplastic dwarfs is usually autosomal dominant. On theoretical grounds, the frequency of mutations should increase with increasing parental age, and more so in fathers than in mothers, since there are many cell divisions (and thus many opportunities for copying-errors in gene reproduction) in the testis during spermatogenesis, but few in the ovary during oogenesis. Penrose (1955) has shown that for achondroplasia the father's mean age at birth of the affected child is increased, thus supporting the idea that achondroplasia may arise by dominant mutation.

In malformations so severe as to prevent reproduction of affected individuals, parental age studies may be the only way to test the possibility that they are caused by dominant mutations. Patients with acrocephalosyndactyly, for example, almost never have children, but an association with increased paternal age has been demonstrated, suggesting that this malformation may be caused by dominant mutation (Blank, 1958). In view of the importance of knowing the frequency of mutations in man, and the effects of radiation on it, other studies of this type are needed.

d. Reduced penetrance: A dominant mutant gene is said to show reduced penetrance if some individuals heterozygous for the gene do not show its effects. Similarly, a recessive gene shows reduced penetrance if if fails to manifest itself in some homozygotes. In human pedigrees this is seen as a deficiency in the theoretically expected proportion of affected cases, and, in the case of dominant genes, as "skipping" of generations. Sometimes the cause of reduced penetrance is clear—e.g. a person, known to be carrying the dominant gene for Huntington's chorea by the fact that the disease appears in one of his parents and his children, may have died from some other cause before developing the disease, and will appear in the pedigree as unaffected. In other cases the cause of reduced penetrance is unclear. Experimentally it can be shown that the penetrance of a gene may vary according to both the genetic constitution and the environment. Thus mutant genes that show reduced penetrance in humans present a great challenge, since the identification of factors that reduce the penetrance of a gene causing a disease could lead to therapeutic measures.

In any case, reduced penetrance is one of the bug-bears of genetic counselors, since it represents one possible explanation of the "sporadic case." Sporadic cases of retinoblastoma, for example, may be fresh mutations (particularly if bilateral), in which case the risk of affected children is quite high, or phenocopies (particularly if unilateral), with a

relatively low risk for the children (Vogel, 1957). However, Macklin (1960) has described families where a case was thought to be sporadic, but intensive investigation of the family unearthed affected persons among the very distant relatives, suggesting that the gene could be transmitted for several generations without causing disease. A similar situation has been described for the lobster-claw malformation (Birch-Jensen, 1949). In such a case the risk for the children of an affected individual would be lower than 50 per cent, since not all persons inheriting the gene would have the defect. Unless penetrance is very low, this risk would be higher than it would for a phenocopy, but unless an affected relative is found, the counselor is unable to decide which category his particular sporadic case belongs to.

Thus the sporadic case of a rare malformation can be attributed to a variety of causes, with different prognoses as to the prospect of recurrence in other family members (Table 1). Unfortunately, in our present state of knowledge it is often impossible to distinguish between the various possibilities. For some conditions, such as retinoblastoma (Vogel, 1957) and the lobster-claw defect (Birch-Jensen, 1949) cases in which only one side of the body is affected are less likely to be hereditary than bilateral cases. Recessive inheritance may be suspected if there is parental consanguinity. Parental age studies may suggest what proportion of sporadic cases of a particular malformation may be due to dominant mutation (Penrose, 1955), and sibling distribution data may permit an estimate of the proportion of phenocopies in a series of cases (Morton, 1958). For the individual sporadic case the probability of recurrence lies between 0 and 50 per cent, and it may be possible to estimate, very roughly, where, within these limits the true value is likely to be, but no means of making a precise prediction are available.

TABLE 1—*Risks for Sibs and Children of a Person with a "Sporadic" Malformation Being Similarly Affected, for Various Genetic Situations*

Type of sporadic case	Risk for sibs	Risk for children
Phenocopy	Low	Low
Dominant mutation	Low	High (1/2)
Somewhat reduced penetrance	Intermediate	Intermediate
Recessive gene	High (1/4)	Low

2. *Chromosomal Anomalies*

A great advance in our knowledge of the causes of malformations has been the demonstration that some malformations are caused by chromo-

somal imbalance, i.e. the presence of an extra chromosome or the absence of one that should normally be present (aneuploidy). This breakthrough was made possible by the development of improved techniques for the examination of human chromosomes (Hsu and Pomerat, 1953), which led to the discovery that the normal number of chromosomes in human somatic cells is 46, instead of 48 as previously supposed (Tjio and Levan, 1956; Ford and Hamerton, 1956). The first report of a malformation due to aneuploidy was that of a patient with Klinefelter's syndrome, who had a somatic chromosome number of 47, containing two X chromosomes and a Y (Jacobs and Strong, 1959). Shortly after this, Lejeune et al. (1959) and Jacobs et al. (1959a) simultaneously and independently reported that mongolian idiots have 47 chromosomes, one of the small acrocentric chromosomes being represented three times instead of twice, while Ford et al. (1959a) reported a case of Klinefelter's syndrome in a mongolian idiot, who had 48 somatic chromosomes, XXY plus the extra acrocentric. Then it was reported that some cases of gonadal dysgenesis (Turner's syndrome) had only one X and no Y chromosome (Ford et al., 1959b). More recently, Jacobs et al. (1959b) have reported a female with dysplastic ovaries and infantile genitalia who had an extra X chromosome. Another type of teratogenic chromosomal aberration has been described by Lejeune and Turpin, who described a case of skeletal anomalies with mental deficiency in which part of one chromosome had been broken off and attached to another—a "translocation" (Anonymous, 1959). As more workers become interested in the field, there will doubtless be a flurry of newly discovered malformations due to chromosomal imbalances. The cytological aspects of the situation are presented elsewhere (Ford, 1960a and b) and the present discussion will touch briefly only on the developmental aspects.

Aneuploidy has been extensively investigated in experimental genetics, particularly in plants. In Datura, for instance, trisomics are known for each of the 12 chromosome pairs, and it is possible to tell, from the appearance of the plant, which of the 12 pairs has the extra representative (Blakeslee, 1934). The effects of the extra chromosome are widespread, affecting many parts of the plant, but little has been done to identify the biochemical changes involved. The discovery that mongolism is due to trisomy suggests that the developmental upset is caused by an excess of something normally produced by that particular chromosome pair, rather than a deficiency, as had previously been tacitly assumed, but the resulting biochemical abnormalities are probably multiple, and not analogous to those underlying the "molecular diseases" or "inborn errors."

In humans, the discovery of aneuploidy has had several other interesting implications. The fact that the presence, in Klinefelter's syndrome, of a Y chromosome in what would otherwise be an ordinary XX female produces a more-or-less male phenotype shows that the Y chromosome has an active part to play in development rather than being genetically inert, as previously supposed. Tanner et al. (1959), by comparing growth rates and skeletal maturation in Klinefelter's and Turner's syndromes, have concluded that genes on the Y chromosome are responsible for male-female differences in rate of development, and suggest that the sex difference in mesomorphy depends on a double dose of X chromosome inhibiting muscular differentiation and development in females. Furthermore, the fact that an embryo without gonads develops into a phenotypic female (Turner's syndrome) supports the findings in rabbits, where castrated male embryos developed female genital characteristics (Jost, 1953). This suggests that in humans, as in other mammals, the embryonic testis produces a morphogenetic hormone that directs the embryo along the male developmental pathway.

It is interesting to speculate about how many more kinds of abnormality will be found due to chromosomal imbalances, and what they will be like. By analogy with other organisms, it seems likely that surpluses or deficiencies of large chromosomes will be lethal (apart from the X, of course) and so there will not be many types of malformation in this class. Since chromosomal aberrations involve large groups of genes, they can be expected to give rise to multiple malformations, and no single, specific metabolic error would be expected. Conversely, those who search for chromosomal aberrations in cases of disease, such as phenylketonuria, known to be caused by a single gene change, are probably wasting their time. The malformations so far shown to be due to chromosomal imbalance do not show a strong familial tendency (except where there has been a translocation; see Mongolism, p. 72) and this is to be expected if the anomaly arises by non-disjunction or other error during gametogenesis.

At least two of the malformations due to chromosomal imbalance tend to occur more often in older mothers. These are mongolism and, to a lesser extent, Klinefelter's syndrome (Lenz, 1959). Does this mean that some physiological change associated with increasing age increases the frequency of non-disjunction in the egg? If such a change could be identified it might point the way towards preventive measures.

Furthermore, one might expect to find chromosomal anomalies in other conditions with a slight familial tendency, that occur with increasing

frequency in the offspring of older mothers, e.g. abortions (Warburton and Fraser, 1959).

On the other hand, at least one condition known to be caused by chromosomal imbalance does not appear to show a maternal age effect. The mean age of mothers who give birth to children with Turner's syndrome appears to be normal (Lenz, 1959). However, family studies using color blindness as a genetic marker show that (in some cases at least) the aberration occurs during spermatogenesis, in which case one would not expect a maternal age effect (Anonymous, 1959; Ford, 1960a).

C. Malformations Caused by a Genetic Predisposition Interacting with Environmental Factors

By far the largest category of malformations is that in which the defect shows a familial tendency, but there is no simple Mendelian pattern of inheritance. Twin studies usually show a higher concordance rate for the malformation in monozygotic than in dizygotic twins, suggesting that the familial tendency is based, in part at least, on genetic factors. However, the fact that many monozygotic pairs are discordant for the anomaly shows that intra-uterine environmental factors may determine whether or not an embryo genetically predisposed to abnormality actually becomes abnormal. I am not aware of any evidence, in humans, as to what these intra-uterine variables might be. Certainly they are not easy to identify, and are probably not gross differences. By analogy with "sporadically" occurring malformations in experimental animals it has been proposed that the predisposing genes may act by making the organogenetic process involved unstable, so that it can be upset by minor environmental fluctuations (Fraser, 1959).

Information about the genetic factors predisposing to malformations can be obtained in a variety of ways, which will be reviewed briefly here.

1. *The contingency method.* Propositi with the malformation to be studied are ascertained by some method which takes no account of whether there are affected relatives, and the frequency of the malformation is measured in various groups of relatives. This approach is useful in a number of ways. Firstly, of course, it demonstrates whether or not the malformation is familial. If there is a familial tendency, attempts can be made to fit the pattern of distribution within families to various genetical models. For the common malformations such attempts are usually unsuccessful. The pattern is likely to be so complicated that fitting it to a theoretical model of inheritance requires postulating so many

factors, both genetic and environmental, that the model has no practical utility. Furthermore, the model is likely to be different in different families. Nevertheless, the figures obtained are useful for genetic counseling, where they can be applied empirically (Fraser, 1958). For instance, the siblings of a child with harelip (whose parents are unaffected) have about a 5 per cent chance of having a harelip, but their chances of having some other sort of congenital malformation do not seem to be increased.

Secondly, studies of familial distribution may allow one to distinguish etiologically different types within the same clinical category. For instance, it could be shown that cleft lips and/or palates associated with labial pits show a different pattern of inheritance from those without labial pits (Van der Woude, 1954). By the same approach Fogh-Andersen (1942) was able to show that cleft palate without cleft lip is genetically different from cleft lip, with or without cleft palate.

Thirdly, family analysis may demonstrate that clinically different malformations are etiologically related. Thus anencephaly occurs with an elevated frequency in the siblings of children with spina bifida aperta, and vice versa, suggesting that the two malformations have some etiological factor in common (Penrose, 1957).

Finally, the contingency method may be useful in detecting *formes frustes* or minor signs of the genetic predisposition to the malformation in question. This would be helpful for counseling, but so far the approach has not been useful in the common malformations.

2. *The twin method.* The twin method is really a special type of contingency method. Twin pairs in which at least one has the malformation to be studied are selected in some manner that takes no account of whether the co-twin is also affected, and concordance rates are measured. On the assumption that monozygotic twins are genetically identical while dizygotic twins are not, and that the intra-uterine environment is equally similar for both types of twin, it is concluded that a higher concordance rate in monozygotic than in dizygotic pairs is evidence for a genetic factor in the etiology of the malformation. The biases that may affect this method have been discussed elsewhere (Metrakos, 1953; Price, 1950). For one thing, it is necessary to decide whether the twins are mono- or dizygotic. Dizygosity is established if the twins differ in any characteristic known to be controlled exclusively by heredity, such as a blood group. Monozygosity is a diagnosis by exclusion—if extensive tests fail to establish dizygosity, the twins are considered monozygotic. Certainly the number of placentas is *not* a valid criterion. The only almost conclusive proof of monozygosity is successful growth

of tissue grafted from one twin to the other, and this test, of course, is not practicable on a wide scale. Secondly, the twins must be selected by some method that guards against the tendency for unusual pairs to be included in the series more often than they should be according to their frequency in the population. The best safeguard here is to observe all the twin pairs in a given population in which at least one has the malformation in question. This often presents serious practical difficulties, but the results can be quite misleading if this is not done, as shown by Simonds (1957) in a study of tuberculosis in twins. Finally, the assumption that the environment is just as similar for dizygotic as for monozygotic pairs may not be valid. Those monozygotic twins that develop in a single chorion may have a more similar environment than the dichorionic pairs, and it would be most informative to see whether the concordance rates would be similar for monozygotic monochorionic and monozygotic dichorionic pairs. No such information is available, and until it is, the evidence from twin studies for genetic factors in the common malformations must be accepted with reservations.

Concordance rates have been measured for a variety of malformations. Most malformations show a somewhat higher concordance rate in monozygotic than in dizygotic pairs, suggesting (with the reservations noted above) that there is a genetic predisposition. However, the concordance rates in monozygotic pairs are usually nowhere near 100 per cent, suggesting that the causation is not entirely genetic. For instance, the concordance rate for monozygotic pairs in which at least one has a cleft lip (with or without cleft palate) is about 40 per cent, as compared to 5 per cent in dizygotic pairs (Metrakos et al., 1958). Corresponding figures for clubfoot are 23 per cent and 2 per cent, and for dislocated hip 41 per cent and 3 per cent (Idelberger, cited by von Verschuer, 1957). Mongolism is an exception, but here the cause is an extra chromosome, and the high concordance in monozygotic pairs is not surprising (Allen and Baroff, 1955).

It is interesting, and somewhat puzzling, that the frequency of malformations does not seem to be increased much, if at all, in twins. Since intra-uterine conditions are known to be less favorable for twins than non-twins, and since an unfavorable prenatal environment is postulated as one of the factors contributing to malformation, one would expect twins to be malformed more often than non-twins. Nevertheless, there does not seem to be any evidence of this for most malformations, which suggests that the intra-uterine factors predisposing to malformation are not those associated with the presence of an extra embryo in the uterus. It might be thought that the unfavorable effects of twinning occur only

at the later stages of gestation, after the organogenetic period, but against this is the fact that the frequency of spontaneous abortion in twin pregnancies appears elevated (Osborne and DeGeorge, 1959).

3. *The consanguinity method.* Since parents who are related to each other are more likely than unrelated parents to have similar genes, the parents of children with recessively inherited diseases are more often related than are parents in general. Thus an elevated parental consanguinity rate is evidence for recessive inheritance of the disease in question. However, the increase in parental consanguinity rate decreases as the frequency of the disease increases, so that the method is not useful for detecting common recessive genes. An increase in the consanguinity rate in parents of children with a common malformation would suggest that some cases of the malformation are caused by rare recessive genes, or by homozygosity for a number of gene pairs each of which is ineffective by itself. The frequency of consanguinity does not appear to be increased in the parents of children with cleft lip (Fogh-Andersen, 1942), and probably not in spina bifida aperta (Penrose, 1957), but there does appear to be an increase in the parents of children with congenital heart disease who have associated malformations (Lamy et al., 1957).

An alternative approach to the problem starts with the consanguineous parents and measures the frequency of malformations in the children. Any increase over the frequency in the general population is a measure of the contribution that recessive genes make to the malformation load of the population. The only series of consanguineous matings sufficiently extensive and well-controlled to provide large enough figures on malformations for statistical analysis is that of Schull (1958) and others, on a Japanese population. He found a slight increase in the frequency of malformations in the consanguineous group, particularly multiple malformations. It is not clear whether these represent syndromes caused by hitherto unrecognized recessive genes, or the result of increased homozygosity for groups of genes. It may be, as Neel (1958) suggests, that at least some portion of the load of malformations we bear is the price we pay for being genetically healthy in other respects. Further discussion of the question will be found elsewhere in this volume (Chapters 2 and 7).

4. *Racial comparisons.* Differences in frequency of a congenital defect between races suggest a genetic basis for the defect, although an environmental factor differing in the races is an equally valid interpretation of such findings, taken by themselves. However, racial studies may provide useful supportive evidence when treated in conjunction with

family and twin studies. Unfortunately there is very little reliable information on frequencies of malformations in various racial groups, and particularly the frequencies with which certain malformations occur together. Neel (1958) has discussed the significance of such findings in a large scale study of Japanese infants. He points out that the total frequency of congenital malformation is similar for all populations adequately studied, but that the frequency of specific defects varies widely from population to population. Furthermore, the major types of defect tend to occur together more often than expected by chance, and the pattern of association reflects, disproportionately, the frequencies of the specific defects in the populations concerned. For instance, cleft lip ± cleft palate occurs more frequently in Japanese than in Caucasian populations, the increase being less than twofold. In cases of central nervous system malformation, cleft lip ± cleft palate appears as an associated malformation much more frequently in Japanese than in Caucasian populations, the increase being about tenfold. This suggests that if the gene complex predisposing to a malformation acts by lowering a threshold of susceptibility, the gene complex for one type of malformation may lower the organism's threshold to another. This sort of interaction may have an important bearing on the mechanisms influencing the population frequencies of deleterious genes. It is only by collecting extensive data of this sort that we can learn to understand the ways in which the frequencies of genes for malformations are controlled, and how the effects of man-made environmental changes such as improved treatments and increased radiation will alter them.

V. Etiological Factors in the Common Malformations

The better we understand the causes of congenital malformations, the better we are prepared to develop preventive measures, and to advise parents of malformed children about the outlook for their future children. At present we are better equipped with figures for parental counseling than with preventive measures. Indeed, counseling is one of the few preventive measures available, and if parents of malformed children would decide not to have any more children this would probably lower the frequency of malformations more than any other preventive approach, especially in the case of those showing Mendelian, and particularly dominant, inheritance.

Perhaps the extent of our progress in understanding the genetics of malformations can best be demonstrated by summarizing what is known of the causes of some of the more common malformations.

a. *Anencephaly and Spina Bifida Aperta*

Penrose (1957) has recently reviewed the genetics of anencephaly. The malformation occurs about three times as often in girls as in boys. As previously mentioned, anencephaly appears to be etiologically related to spina bifida aperta, since they occur together not only in the same individual but in the same families more often than would be expected by chance. If a child is born with anencephaly, or spina bifida aperta, the chances are roughly 1 per cent that a subsequent child of the same parents will have anencephaly, and 2 per cent that it will have spina bifida aperta (Penrose, 1957; Record and McKeown, 1950; Böök and Rayner, 1950; Miller, 1959). Little data are available on the probability that children born after two affected children will be affected, but what little evidence there is suggests that the risk is roughly 10 per cent (Fraser, 1958a). These figures are useful as guides for genetic counseling, but it must be remembered that they are averages, and that the risk for a particular family may vary widely from the average. In some families there are too many affected individuals to account for reasonably on the basis of coincidence, and it seems likely that there is a rare type with a relatively strong genetic basis. Grebe (1953) has proposed that there are two forms of anencephaly—hereditary and non-hereditary—which are clinically indistinguishable; but the question is probably not quite so simple as this. Where does one draw the line between a malformation due to genetic susceptibility with intra-uterine factors pushing the embryo over a threshold of abnormality, and those due to an exogenous factor acting on an embryo whose response is influenced by its genetic constitution? Certainly not by the criterion of a negative family history. It would be a great boon to counselors if some means could be found for identifying families with a high recurrence risk after (or better still, before) the birth of their first affected child rather than the third or fourth. So far, however, our attempts to do this have been fruitless, except in Mongolism, see Mongolism, p. 72.

There have been remarkably few reports of anencephaly in twins whose zygosity has been adequately determined. Both discordant and concordant monozygotic pairs have been reported (Penrose, 1957), but the data are insufficient for establishing concordance rates, except to suggest that the concordance in monozygotic twins is low (Record and McKeown, 1951). The observation of Coffey and Jessop that anencephalics more often have group O mothers than one would expect by chance (cited by Penrose, 1957), needs further investigation.

Penrose has considered eight hypotheses to explain the genetics of

anencephaly, none of which is completely satisfactory. A dominant gene, or recessive gene pair with very low penetrance, or a susceptibility determined by multiple genes would all fit the data reasonably well. So would a chromosomal aberration such as a translocation. A normal chromosomal complement has been reported for some anencephalics (Anonymous, 1959), but it is quite possible that a chromosomal imbalance might be responsible in some families. Any of these explanations requires a subsidiary hypothesis to account for why the condition remains so prevalent in the population, and data on the fitness (in the evolutionary sense) of parents of anencephalics are needed.

A number of environmental variables have been shown to be associated with the occurrence of anencephaly. Penrose (1957) has drawn attention to the great geographic variation, ranging from 0.012 per cent of hospital births in Lyons to 0.671 per cent in Belfast. This could result from genetic differences between populations, but seems more likely to reflect environmental variability. However, there is no evidence as to what the environmental variable might be. There was a marked seasonal variation in frequency of anencephaly in Scotland (Edwards, 1958), with more cases in October to February than in March to September, but there was no evidence for an association with epidemics. There was also an increase in frequency in the lower social classes, and a tendency for anencephaly to occur more often in first-born than in later-born children (ibid.). It would be interesting to know if similar associations occur in other populations. The evidence that maternal infection with Asian influenza may be followed by an increased frequency of malformations, and particularly anencephaly, in the offspring (Coffey and Jessop, 1959) is puzzling. For one thing, in those cases where a febrile episode was reported it often occurred after the time when the neural tube normally closes (Warkany, 1960). It is possible that the increase in frequency of infection with Asian influenza in mothers of malformed children may reflect an association of these two things with some common factor (such as social class), rather than a causal association between Asian influenza and malformation. At present it must be admitted that no specific causal factor for anencephaly, either environmental or genetic, is known in human beings.

b. *Cleft Lip, With or Without Cleft Palate*

Since it has been demonstrated (Fogh-Andersen, 1942) that cleft lip (which may or may not have an associated cleft palate) is, in most cases, etiologically different from cleft palate without cleft lip, the two

will be discussed separately. In this section "cleft lip" will refer to clefts of the lip with or without a cleft palate.

The frequency of cleft lip varies from population to population (though not nearly as much as for anencephaly), with estimates varying around 1 per 1,000 births. It is lower in American Negroes and higher in Japanese than in Caucasians (Neel, 1958). Males are affected more often than females, the sex ratio being highest in the most severe cases (Fogh-Andersen, 1942). The incidence increases somewhat with increasing maternal age (MacMahon and McKeown, 1953). Babies with cleft lip have a higher-than-average frequency of other malformations as well. It may be that this group are etiologically different from the group without associated malformations, with a different (probably lower) risk of recurrence in siblings.

There are a number of rare, inherited, diseases that may include cleft lip and/or palate as one feature. For instance, *fistula labii inferioris congenita* shows dominant inheritance, with somewhat reduced penetrance, and about half the cases have an associated cleft lip and/or cleft palate (Van der Woude, 1954). These account for only about 1 per cent of all cases of cleft lip and/or palate. Cleft lip may also occur as one manifestation of the dominant gene for lobster-claw defect of the hands and feet (Fraser, unpublished).

Apart from these rare variants, it is generally recognized that congenital clefts of the lip occur in the near relatives of affected persons more often than they do in the general population, but that this cannot be accounted for by any system of genes with full penetrance. The parental consanguinity rate is probably not increased. Twin studies (Metrakos et al., 1958) suggest that the familial pattern has a genetic basis, since the concordance rate in monozygotic twins (40 per cent) is much higher than it is in dizygotic twins (5 per cent). On the other hand, the fact that in 60 per cent of monozygotic pairs where one member is affected, the other is not affected shows that environmental factors must be important, too. I have already discussed the possibility (The Embryonic Genotype, p. 50) that the genetic constitution for cleft lip makes the embryo's lip-closing mechanism unstable, so that it can be upset by otherwise unimportant environmental variables. The tendency for affected relatives to occur more often on the maternal than on the paternal side of the family (Fraser, 1955) suggests that some of the intrauterine environmental variables may be influenced by the mother's genotype.

A number of attempts have been made to account for the familial

distribution on the basis of a single gene (Fogh-Andersen, 1942; Carter, 1953), but so many variables are involved that such efforts do not appear particularly profitable in our present state of knowledge. From a practical viewpoint, however, it is important to know the probability of recurrence of the defect in various groups of relatives of affected persons, to use in counseling the families concerned. If the parents are normal, and they have had a child with cleft lip (excluding those associated with specific rare conditions such as labial pits), the probability of being similarly affected is about 5 per cent for each subsequent child. It must be emphasized that this is an empirically determined average figure, and that the prospect may vary either above or below this for any particular family. The average risk does not seem to increase when the parents are consanguineous (Fraser, 1956), or when there is an affected relative, other than parent or sib (Fogh-Andersen, 1942; Fraser, 1957). If a second affected child is born, the risk for subsequent children appears to increase somewhat, perhaps to about 10 per cent, though this figure is based on a relatively small body of data (Fraser, 1958a). If one parent has a cleft lip, and there are no affected children, the risk of a child being affected is about 2 per cent, and this figure rises to about 10 per cent if there is already an affected child (Fogh-Andersen, 1942). For all these situations, the probability that a sib will be born with a cleft palate, without cleft lip, does not seem to be appreciably increased, and neither is the risk that a sib will have some other major malformation. As more information accumulates it will be possible to refine these estimates still further, and possibly to identify families where the risk is markedly higher, or lower, than the average. For instance, it would be useful, for counseling, to know whether the risk of recurrence is higher in cases of bilateral than unilateral clefts, and whether the risk for sibs is really lower when the affected child has other major malformations as well.

c. *Cleft Palate, without Cleft Lip*

The frequency of cleft palate, without cleft lip, (in this section referred to as "cleft palate") is about 1/2500 births in Caucasian populations, being somewhat lower than this in American Negro, and higher in Japanese populations (Neel, 1958). More females are affected than males (Fogh-Andersen, 1942), and there does not seem to be an association with birth rank or maternal age (MacMahon and McKeown, 1953). Cleft palate is often associated with other malformations (Fogh-Andersen, 1942; Curtis, 1957).

There are a number of rare conditions showing relatively clear-cut patterns of inheritance that may include cleft palate as one feature—e.g. Marfan's syndrome (McKusick, 1955), and mandibulo-facial dysostosis (Warkany, 1958).

Apart from such conditions, cleft palate does not show a clear-cut genetic pattern, although there is a familial tendency. There does not appear to be an increase in parental consanguinity (Fogh-Andersen, 1942). When the parents are normal, and have a child with cleft palate, the probability of being similarly affected is about 3 per cent for each subsequent child. When there is an affected near relative (other than parent or sib), the risk for siblings of an affected child increases to about 6 per cent (Fraser, 1958a). More data are needed to show whether this increase is real, or a random fluctuation. When one parent has a cleft palate, the frequency of cleft palate in the children is estimated (on a small number of families) as about 7 per cent (Fogh-Andersen, 1942). When an affected parent has an affected child the risk for subsequent children is estimated (again on a small body of data) as 17 per cent.

The number of twins available for analysis in which at least one has a cleft palate is so small that no conclusions can be drawn, except that the concordance rate in monozygotic twins is probably quite low (1 in 10—Metrakos et al., 1958), which would suggest that hereditary factors are relatively unimportant in causing this malformation. Nevertheless, there is a definite increase in the frequency of affected relatives, both maternal and paternal. Further twin studies would be most helpful in clarifying this problem.

d. *Congenital Clubfoot*

Congenital *pes equinovarus* is a clinically heterogeneous diagnostic category (Stewart, 1951), and it is therefore not surprising that it is genetically heterogeneous, too. The frequency in the general population is about 1 per 1,000 births. Males are more often affected than females. There are marked racial differences in frequency (ibid.). The genetics of the condition have been reviewed by Böök (1948). The twin studies of Idelberger (cited by von Verschuer, 1957) show a higher concordance rate for monozygotic (23 per cent) than dizygotic (2 per cent) twins, suggesting (with the reservations previously discussed) that there is a genetic predisposition to clubfoot, but that environmental factors determine whether the predisposed embryo is actually malformed. A variety of genetic mechanisms have been suggested (Falls, 1953), but

all of them require postulating markedly reduced penetrance, because of the low concordance rate in monozygotic twins. This and the probable genetic heterogeneity mentioned above make it seem rather pointless to try to specify a particular mode of inheritance for this malformation.

When the parents are normal, and they have a child with a clubfoot, the probability of being similarly affected is about 3 per cent for each sibling, on the average. This risk does not seem to be increased when there is an affected near relative, other than sib or parent (Fraser, 1958a). The genetic heterogeneity of the condition is emphasized by the fact that the risk is greatly increased if the parents are first cousins, suggesting that some cases are due to recessive genes with high penetrance (Böök, 1948; Fraser, 1952). For counseling purposes, the 3 per cent average risk must therefore be used with the reservation that the true risk for an individual family may differ widely from the average.

e. *Congenital Dislocation of the Hip*

The genetics of congenital dislocation of the hip is in a very confused state. (Most "congenital" dislocations of the hip are not present at birth, but the term is so entrenched in the literature that I will bow to common usage in this connection.) The incidence is roughly 1/1500 births, females being much more frequently affected than males. There are marked racial differences in frequency (Stewart, 1951) and a very high incidence in some racial isolates (Corrigan and Segal, 1950). Record and Edwards (1958) report that congenital dislocation of the hip occurs more often in first-born children than in those of later birth-ranks, somewhat more often in children of older mothers than in those of younger mothers, and twice as often in children born in winter as in summer, suggesting that environmental factors such as methods of clothing young infants may be important. As with the other common malformations, the condition is etiologically heterogeneous. Some are secondary to other defects such as spina bifida or arthrogryposis multiplex congenita. Larsen et al. (1950) has reported a type associated with dislocations of other joints, which may be the same condition described by Mégevand (1953), and called arthrochalasis multiplex congenita by Hass and Hass (1958), which there is reason to believe has a genetic basis (Fraser, unpublished).

In the common types there is no clear-cut pattern of inheritance. The risk of recurrence in sibs of affected children (parents normal) is about 5 per cent (Record and Edwards, 1958).

f. *Congenital Heart Malformations*

Malformations of the heart may occur as a feature of several rare types of hereditary disease such as chondrodystrophia calcificans congenita (Fraser and Scriver, 1954), the Ellis-van Creveld syndrome (Metrakos and Fraser, 1954), Marfan's syndrome (McKusick, 1955), mongolism (Øster, 1953), and Turner's syndrome (Grumbach et al., 1955). In such cases the risk of recurrence depends, of course, on the probability that the syndrome itself will recur.

Apart from these rare conditions, malformations of the heart usually do not show a strong genetic tendency. Individual families with a high concentration of cases have been reported for coarctation, patent ductus arteriosus, aortic stenosis and atrial septal defects (reviewed by McKusick, 1959), but these are in the great minority, and in most families of patients with cardiac malformations there is little evidence of a genetic factor. Some workers (Lamy et al., 1957) have found a slight increase in the parental consanguinity rate (suggesting that some cases are due to rare single recessive gene pairs, or to homozygosity for a number of loci, each of which, individually, would have a negligible effect), but only in those cases with an associated malformation. Other workers did not find an increased consanguinity rate (Polani and Campbell, 1955). In twins the concordance rate is low for both monozygotic and dizygotic pairs, again suggesting the relative unimportance of genetic factors in most cases (Uchida and Rowe, 1957). The probability that a sib of a person with congenital heart disease will also be affected varies from about 0.3 per cent for interventricular septal defects to about 2 per cent for pulmonary valvular stenosis (Fraser, 1958a).

g. *Mongolism*

The discovery that mongolism is the result of an extra autosomal chromosome, probably arising by non-disjunction during gametogenesis is a great advance, but leaves many questions unanswered. The tendency for mongols to be born of older mothers is well established, but Penrose (1959) suggests that the distribution of ages in mothers of mongoloid children is heterogeneous, about one quarter of the mothers having a normal age distribution, and the rest having an abnormal proportion of old mothers. This would suggest that there may be different types of mongolism. The recent report by Ford (1960b) of a mongol child with only 46 chromosomes is of great interest in this regard. The chromosomal analysis suggested that the chromosome responsible for

mongolism was in fact present in triplicate, but that the extra member of the set was attached to one of the other autosomes, presumably following a reciprocal translocation. Ford points out that such a situation could account for the heterogeneous maternal age distribution, and also for the occasional families with a high frequency of recurrence of the condition.

Another feature that will have to be accounted for is the increased frequency of certain stigmata of mongolism reported to occur in the near relatives of mongols (Penrose, 1954; Turpin and Lejeune, 1953). A number of theories that could account for an increased tendency to chromosomal aberration in families with minor mongoloid stigmata, and for the other familial characteristics of mongolism, have been reviewed by Fraser (cited by Warkany, 1960), but so far none of the theories are supported by data.

For counseling purposes, Penrose (1956) has suggested that if a woman has had a mongoloid child, the chances that any subsequent pregnancy will result in a mongoloid child is two to three times as high as that of other women in her age group. Any woman of 25 years of age has about 1 chance in 2,000 that her baby will be mongoloid, but if she has already had an affected child the risk goes up to about 1/800. At age 45 the risk that any pregnancy will result in a mongoloid child is about 1 in 40, but for a woman that has already had one it is about 1 in 16.

Conclusion

In experimental biology great progress is being made in our understanding of developmental mechanisms and the ways in which they are controlled by genes. Practical applications to the problems of human congenital malformations will undoubtedly follow. For example, the application of cytological techniques developed for fundamental studies on radiation effects have resulted in the discovery that certain types of human malformations are caused by excesses, deficiencies or rearrangements of chromosomes.

Basic understanding of the mechanisms of abnormal development in human beings will depend, ultimately, on studies of human embryos, and although data in this field are still rather scanty, particularly as regards gene effects, the future holds great promise for this approach.

Family and epidemiological studies have begun to clarify the complicated interactions of the many genetic and environmental factors that determine whether or not a given baby will be born malformed, and

there is great need for more information of this kind, carefully collected, in adequate quantities, from a variety of populations.

The ultimate aim is, of course, prevention, and there seems at present to be little we can do in that direction except discuss with parents who are carrying genes for severe malformations the consequences of their having children. But it does not seem unreasonable to hope that by a combination of family, epidemiological and embryological studies we may succeed in learning how to protect the embryo from harmful environmental factors, to reduce the frequency of genes causing malformations, and, where this proves impossible, to prevent their effects on the developing embryo.

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Chapter 4

Blood Groups and Disease

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"It is reasonable to conclude, from what we know of polymorphism, that individuals belonging to the different blood groups are not equally viable. . . . A valuable line of enquiry which does not yet seem to have been pursued in any detail would be to study the blood group distributions in patients suffering from a wide variety of diseases. It is possible that in some conditions, infectious or otherwise, they would depart from their normal frequencies, indicating that persons of a particular blood group are unduly susceptible to the disease in question." (Ford, 1945).

"I am surprised to see how much space is being wasted in the 'Lancet' and the 'British Medical Journal' on articles dealing with blood group distribution in various diseases . . . knowledge is no substitute for understanding and mathematics is a poor substitute for accurate observations, reliable laboratory work and common sense. The hardest things to explain are those which are untrue." (Wiener, 1956).

From the evidence brought forward in this paper the reader will be able to decide whether the calm predictions of 1945 have been realized. If they have, then the uncompromising prejudices of 1956 are yet another example of the truth that "blind unbelief is sure to err."

Modern Series and Historical Note

The first efficient large-scale survey to find out if there were an association between a disease and a blood group was by Aird and his colleagues (1953).

The reason that led to this investigation was that the authors thought that in Great Britain there might be a correlation between the incidence of cancer of the stomach and the incidence of blood group O, both of which are considerably higher in the northern than in the southern part of the country. Aird was determined to collect large numbers, and because of the growing practice of the routine blood grouping of patients was able to do so from the existing records in many hospitals. The result was surprising since the anticipated correlation was not with group O but with group A, and the association was highly significant in all parts of the country. This was interpreted to mean that there are at least two influences operating in the cause of cancer of the stomach in England, one being genetic and related to the A blood group, and the other probably environmental and operating more in the north than in the south of the country. The investigation was carried out with great care and the necessary conditions for an accurate study were fulfilled. Thus, the numbers involved were large (3,632 cases), the controls were reliable, the diagnostic criteria were strict and the blood grouping techniques and statistical methods were efficient.

The following year Aird and his colleagues carried out a similar survey on peptic ulcer, and in this disease found a marked association with blood group O (Aird et al., 1954). They concluded that an individual who was group O was 35 per cent more likely to develop an ulcer than one who was not O. Two years later a team of British workers made a most interesting contribution to the subject by demonstrating a significant association between pernicious anemia and blood group A (Brit. Med. J., 1956).

Wiener (1959) thought these large surveys had no rationale and were doomed to failure. In his opinion the similar investigations carried out as long as 30 years ago were sufficiently conclusive to discredit any idea of an association between blood groups and "adult" diseases. However, if he were to look critically at the data of Buchanan and Higley (1921) from the Mayo Clinic he would see that it was only inefficient analysis which caused the association of group O with peptic ulcer and group A with pernicious anemia to be missed at that time. Buchanan and Higley recognized that their material (2,246 patients) was heterogeneous but concluded that this was due to racial differences. However, as Fraser Roberts (1957) has shown in his admirable review on blood groups and disease, if the Mayo Clinic workers had tested their individual disease groups for heterogeneity they would have found that group O was significantly in excess in peptic ulcer and group A in pernicious anemia.

Had these findings been investigated further the associations recently described would have been discovered 30 years earlier and in Rochester rather than in London. It is of interest to note that Buchanan and Higley's largest disease group (457 cases) was that of pernicious anemia. This was presumably because both doctors were pathologists and the series was collected before the days of liver therapy.

The only other historical papers which will be mentioned are those of Ugelli (1936) and Lessa and Alarcão (1949). Both showed an excess of group O in peptic ulcer but the series were small and "something more was needed; a result based on really large numbers, a significance so overwhelming that it could not be ignored" (Fraser Roberts, 1957).

Statistical Method

To obtain large enough numbers for an efficient analysis, it was essential to be able to combine figures from various centers, many of which would have different blood group frequencies. Woolf's method (1955) enables this to be done, and the following is a brief description of the procedure. The calculations are carried out in two stages. First, for any given center the disease is expressed in terms of its incidence, this being a figure which is easily understood. Thus we can say that the relative incidence in duodenal ulcer in persons of group O, for example in Liverpool, is 1.53 as compared with persons of group A. This figure is arrived at by simple cross multiplication. For example, in the Liverpool series there are 505 O's and 263 A's, while the figures for the controls are 7,536 O's and 6,013 A's. The relative incidence of O to A is:

$$\frac{505 \times 6013}{263 \times 7536} = 1.53.$$

For each center a χ^2 is calculated and these are then added together. Table I shows the results for duodenal ulceration taken from various centers and it will be seen that the departure from unity is very highly significant in 6 of these, the probability of getting so great a deviation by chance varying from one in a hundred thousand to one in a hundred million or less. It is significant at the .01 level in three others and is greater than one in all 12. A similar calculation can be made for cancer of the stomach and the results for this disease from 14 centers are shown In Table 2, the total $\chi^2_{(14)}$ being 68.29 (63.47 in Fraser, Roberts 1959 original table for 13 centers).

TABLE 1—*Duodenal Ulceration. Relative Incidence in Persons of Group O Compared with Incidence of One in Persons of Groups A, B and AB*
(From Fraser Roberts, 1959, augmented)

Center	Relative incidence	$\chi^2_{(1)}$	P
1. London	1.54	39.83	$<10^{-10}$
2. Manchester	1.21	3.76	$> .05$
3. Newcastle	1.57	22.75	$<10^{-5}$
4. Liverpool	1.53	44.26	$<10^{-10}$
5. Glasgow	1.17	7.51	<0.01
6. Copenhagen	1.48	25.06	$<10^{-6}$
7. Oslo	1.56	26.77	$<10^{-6}$
8. Vienna	1.22	9.95	<0.01
9. Iowa (whites)	1.37	26.49	$<10^{-6}$
10. Iowa (negroes)	1.32	3.21	$> .05$
11. Wilmington, Del.	1.22	2.82	$< .1 > .05$
12. Tokyo	1.66	9.46	$< .01$
TOTAL		221.87	

1-3. Aird, Bentall, Mehigan and Roberts (1954).
4. Clarke, Cowan, Edwards, Howel Evans, McConnell, Woodrow and Sheppard (1955).
5. Brown, Melrose and Wallace (1956).
6. Køster, Sindrup and Seele (1955).
7. Heistø (1956).
8. Speiser (1956).
9. Buckwalter, Wohlwend, Colter, Tidrick and Knowler (1956b).
10. Buckwalter, Turner, Raterman, Tidrick and Knowler (1957).
11. Pell, d'Alonzo, and Fleming (1957).
12. Matsunaga (1959).

Additional data

Sørensen (1957) found a definite preponderance of blood group O in patients with juxta-pyloric ulcers, i.e. ulcers in the immediate vicinity of the pylorus, both gastric and duodenal. The relative incidence is not accurately calculable on the data provided in his paper.

The second step in the calculation is quoted verbatim from Fraser Roberts (1959): "The total $\chi^2_{(13)}$ of 63.47 is divided into two parts. The first part, with 1 degree of freedom, tests the significance of the general average from 1.00. It is 48.77, or very nearly 7 times its standard error, and the probability of obtaining so great a deviation by chance is infinitesimal. The second part, a χ^2 of 14.70, with 12 degrees of freedom represents heterogeneity, that is, the departures of the individual incidences from the general average. The probability is 0.3, so there is

TABLE 2—*Cancer of the Stomach. Relative Incidence in Persons of Group A Compared with Incidence of One in Persons of Group O* (From Fraser Roberts, 1959 augmented)

Center	Relative incidence	$\chi^2_{(1)}$	P
1. London	1.20	8.89	<.01
2. Manchester	1.22	6.39	<.05
3. Newcastle	1.25	1.12	>.05
4. Liverpool	1.31	2.75	>.05
5. Birmingham	1.60	4.46	<.05
6. Leeds	1.31	3.52	>.05
7. Glasgow	1.09	0.45	>.05
8. Basel	1.36	12.16	<.001
9. Copenhagen	1.30	10.86	<.001
10. Vienna	1.00	0.00	>.05
11. Milan	1.28	6.85	<.01
12. Iowa	1.20	5.61	<.05
13. Sydney	1.06	0.41	>.05
14. New York	1.39	4.82	<.05
15. Connecticut	1.26	10.04	<.01
TOTAL		78.33	

1 & 3-6. Aird, Bentall and Roberts (1953) (two London series combined).
2. Walther, Raeburn and Case (1956).
7. Wallace (1954).
8. Dr. L. P. Holländer of Basel, cited by Aird and others (1953).
9. Køster, Sindrup, and Seele (1955); Jordal (1956).
10. Speiser (1956).
11. Beolchini, Cresseri, de Maria, Morganti, Peruzzotti, and Serra (1957).
12. Buckwalter, Wohlwend, Colter, Tidrick, and Knowler (1956a).
13. Billington (1956b).
14. Hogg and Pack (1957).
15. Eisenberg, Greenberg, and Yesner (1958).

no evidence that the individual centers are showing anything more than chance fluctuations about the general average. It should be repeated perhaps, that this procedure does not produce a 'heterogeneous' control series, or an 'artificial, unreliable point of reference'" (see Manuila, 1958). "At each center, quite independently, the question is asked: Is cancer of the stomach commoner in persons of group A than in those of Group O, and by how much? These independent estimates are then efficiently combined to give, first a general estimate, and second, a measure of departure of the individual centers from the general average."

Pernicious anemia is dealt with in the same way (Table 3).

TABLE 3—*Pernicious Anemia. Relative Incidence in Persons of Group A Compared with Incidence of One in Persons of Group O* (From Fraser Roberts, 1959)

Center	Number in disease series	Relative incidence	χ^2	P
1. London	244	1.63	12.51	0.0004
2. Oxford	258	1.09	0.41	0.5
3. Cambridge	110	1.01	0.00	——
4. Sheffield	123	1.36	2.35	0.1
5. Newcastle	109	1.03	0.02	0.9
6. Glasgow	270	1.19	1.55	0.2
7. Copenhagen	111	0.98	0.01	0.9
8. Iowa	158	1.42	3.97	0.05
9. San Francisco	115	1.70	6.96	0.008
TOTAL	1,498		27.78	

1-6. British Medical Journal (1956).
7. Køster, Sindrup and Seele (1955).
8. Buckwalter, Wohlwend, Colter, Tidrick and Knowler (1956a).
9. Creger and Sorter (1956).

(N. B.—Samples combined for London and for Glasgow.)

The combined weighted relative incidence here is 1.26 and the probability of so great a deviation from unity arising by chance is about one in sixteen thousand. The χ^2 for heterogeneity is 11 for 8 degrees of freedom, corresponding to a probability of 0.2. From these calculations it is clear that there is very strong evidence for the association in gastric carcinoma, peptic (particularly duodenal) ulcer and pernicious anemia. Henceforth these three associations are referred to as the "firm" associations.

CONTROLS

The controls on which these conclusions are based have been formed chiefly from blood donors, and in Great Britain, particularly since the establishment of the Blood Transfusion Service during the war, the mapping of the blood group distribution in different areas of the country has become increasingly efficient (Mourant, 1954). Sometimes hospital patients (excluding those with the disease being investigated) and occasionally patients with a disease known to have a normal ABO distribution have been used.

The efficiency of blood typing is high and blood group technicians

are available at all hours. Wiener's (1959) implied suggestion, therefore, that emergency ulcer cases requiring transfusion may in cases of doubt be labeled group O is quite unjustifiable in England.

Buckwalter and Knowler (1958) have made the following interesting points about blood donor controls:

1. Controls are more representative of the population from which patients are obtained when both are collected during the same period of time.

2. The inclusion of professional donors significantly increases the frequency of blood group O in the controls.

3. "Healthy" populations will in time become diseased and the blood group distribution of blood donors "reflect mean frequencies determined by multiple selective factors related to disease and blood types." Because of this, comparison of homogeneous disease groups, e.g. carcinoma of the stomach vs. peptic ulcer, may reveal larger differences than when patient and donor groups are compared. If there is no significant heterogeneity between the effect of a gene on a disease in many areas (which is, in fact, the case), then it must mean that the controls for these areas are good.

The question of control series is referred to again in the discussion on the sibship data (see p. 92).

Summary of Findings in Peptic Ulcer

Since the discovery by Aird and his colleagues a great deal of literature has accumulated, particularly on peptic ulcer. It will be seen from Table 1 that the association between duodenal ulcer and group O has been found in many parts of the world and as a general rule this association is greater than with gastric ulcer. Thus, Fraser Roberts (1957) gives the over-all incidence of duodenal ulcer in persons of group O as 1.38 compared to 1.0 in those belonging to other groups, while the figure given for gastric ulcer is 1.20 (Fraser Roberts, 1959). These differences support the clinical view that the two kinds of ulcer have distinct etiologies. The detailed results of the large series from the Mayo Clinic are awaited with interest. Regarding them, however, Berkson (1959) has allowed the following to be stated: "We have unpublished data on large numbers of cases fully corroborating the previous observations of a relatively high incidence of group A in patients with carcinoma of the stomach, and a relatively low incidence in patients with duodenal ulcer."

In the Liverpool series (Table 4) it will be seen that the association

with group O is significant only in duodenal ulcer, the figures for gastric ulcer being almost the same as the controls.

TABLE 4—*Comparison of ABO Distribution in Duodenal and Gastric Ulcer. Liverpool data, 1955,* (Clarke et al., 1955)

		O	A	B	AB
Duodenal ulcer:	860	505	263	62	30
Percentages		58.72	30.58	7.21	3.49
Gastric ulcer:	377	185	151	31	10
Percentages		49.07	40.05	8.22	2.65
Controls:	15,377	49.0 %	39.1 %	9.4 %	2.5 %

SECRETOR CHARACTER

As is well-known, when the blood group substances are in the water-soluble form they can escape into the body fluids and individuals in whom this occurs are known as secretors. Non-secretors have the antigen only in the alcohol-soluble form and none is present in the watery secretions. The control of secretion is by a pair of allelomorphs, the secretor gene (S) being dominant.

Because both saliva and gastric juice contain, in secretors, a large amount of blood group substance the Liverpool group thought it worth while to investigate the secretor character in duodenal ulcer as had been suggested for carcinoma of the stomach three years before, (Sheppard, 1953). Table 5 shows the highly significant association which was found between the disease and non-secretion (Clarke et al., 1956). The same finding was obtained in Scotland (Wallace et al., 1958), and furthermore, the incidence of non-secretion in all groups was noted to be very high in patients requiring operation. Doll (1960) has also obtained almost identical results, and moreover his preliminary figures

TABLE 5—*Percentages of Non-Secretors in 514 Duodenal Ulcer Patients and in 491 Controls* (From Clarke et al., 1956)

	Males		Females	
	controls	duodenal ulcers	controls	duodenal ulcers
Group O: non-sec.	21.55%	36.32%	28.33%	41.51%
Group A, B and AB non-sec.	22.83%	30.81%	28.12%	42.11%
Non-sec. in all groups	22.39%	33.57%	28.21%	41.76%

indicate that patients with stomal ulcer show a higher proportion of non-secretors than those with duodenal ulcer.

It was shown that in Liverpool non-secretion was not associated with gastric ulcer (Clarke et al., 1959). Since the degree of association between both duodenal and gastric ulcer and group O varies from place to place (Fraser Roberts, 1957) it is important to establish whether in other localities the degree of association between the two diseases and group O is correlated with that between the diseases and non-secretion. If a correlation be found it would suggest that both group O and non-secretion were working in the same way, but if there were no correlation it would suggest that the two factors worked in different ways. Here is an important problem that awaits solution.

The Risks to Various Phenotypes

From the above data it will be seen that there are good grounds for thinking that there is an association of both blood group O and non-secretion with duodenal ulcer. The risks to individuals of the various phenotypes can therefore now be assessed.

Table 6—*Phenotypes in Series of Duodenal Ulcer Patients and Controls, Random for Blood Group and Secretor Status* (From Clarke et al., 1959)

Phenotype	Control Series		Duodenal ulcers		Per cent increase or decrease on control
	No.	%	No.	%	
O secretor	245	36.03	186	35.70	— 0.9
A secretor	204	30.00	124	23.80	—20.7
O non-secretor	70	10.30	98	18.81	+82.6
A non-secretor	75	11.03	62	11.91	+ 7.9
B secretor	54	7.94	19	3.63	—54.3
B non-secretor	15	2.21	19	3.63	+64.25
AB secretor	14	2.06	10	1.94	— 5.88
AB non-secretor	3	0.44	3	0.58	+31.82
TOTAL	680	100.01	521	100.00	

Table 6 shows the combined blood group and secretor status of 521 consecutive duodenal ulcer patients collected in the Liverpool area between January 1, 1956, and March 31, 1958. The 680 controls were a random series of doctors, technicians, students, nurses and Liverpool Territorial Army soldiers collected between September 1, 1955, and

March 1, 1958. The same techniques were employed in scoring both patients and controls, an extract of the seeds of *Ulex europaeus* (which contain a large quantity of anti-H) being used to test group O individuals.

It will be seen that the four categories of ABH non-secretors all show an increased incidence of the disease, while the four ABH secretor phenotypes would each appear to have some degree of protection, thus indicating that the secretor locus is having a bigger effect on duodenal ulcer than the ABO locus. A fact of considerable importance from the point of view of the protection hypothesis (see p. 97) is that the excess of group O individuals with duodenal ulcer is as great in the non-secretor as in the secretor patients.

Another way of calculating the liability to ulcer of the different phenotypes is to take as a base-line the least susceptible group, i.e. the A, B and AB secretors, and Table 7 gives this information (Doll, 1959; Clarke et al., 1959). The figures for "expected duodenal ulcer" in each phenotype

TABLE 7—*Apparent Liability to Duodenal Ulcer of Different Phenotypes, Doll, R* (see Clarke et al., 1959)

Phenotype	Expected Duodenal Ulcer	Controls	"Risk"
O non-secretor	174	70	2.49 : 1
A, B, and AB non-secretor	149	93	1.60 : 1
O secretor	331	245	1.35 : 1
A, B, and AB secretor	272	272	1.00 : 1

were obtained by multiplying the number of duodenal ulcers by 272/153 so as to make equal the numbers of duodenal ulcers and controls in the A, B and AB secretor phenotypes. It can be seen that group O non-secretors would appear to be 2.49 times more liable, and the A, B and AB non-secretors 1.60 times more liable to develop duodenal ulcer than are A, B and AB secretors.

ANASTOMOTIC ULCERS

A point of great importance concerns stomal or anastomotic ulcers which may legitimately be regarded as the most extreme form of the ulcer diathesis. Brown, Melrose and Wallace (1956) found that these were particularly common in persons of group O, the incidence being almost double that in group A. Doll et al. (1960), in a very important paper, bring further confirmatory evidence. In their series of 300 patients with stomal ulcers, 181 (60.3 per cent) belong to group O, whereas

among 564 patients with duodenal ulcer the proportion is 52.8 per cent. The figure for the group O control is 45.8 per cent. The excess of group O in stomal ulcer is significantly higher than in duodenal ulcer, the relative incidence of O : A being 1.35 when comparing the two diseases. Furthermore, the authors estimate that from their data the relative incidence of stomal ulcer is 1.74 in patients of group O compared with those of group A. Table 8 gives the relevant details.

TABLE 8

A. *Distribution of ABO Blood Groups Among Stomal Ulcer and Duodenal Ulcer Patients and Among Control Subjects* (From Doll, Swynnerton and Newell, 1960)

Disease category	Number of subjects belonging to group				all subjects
	O	A	B	AB	
Total stomal ulcers from 4 London hospitals	181 (60.3%)	96 (32.0%)	18 (6.0%)	5 (1.7%)	300 (100%)
Duodenal ulcers	298 (52.8%)	214 (37.9%)	39 (6.9%)	13 (2.3%)	564 (99.9%)
Control subjects without disease	4578 (45.8%)	4219 (42.2%)	890 (8.9%)	313 (3.1%)	10,000 (100%)

B. *ABO Blood Groups Among Stomal Ulcer Patients in Collected Series in Comparison with the Distribution Among Corresponding Groups of Duodenal Ulcer Patients and Control Patients* (From Doll, et. al. 1960)

Center	No. of stomal ulcer patients		No. of duodenal ulcer patients		No. of control patients		Relative risk O : (A+B+AB)	
	O	A+B+AB	O	A+B+AB	O	A+B+AB	stomal v. duodenal ulcers	stomal ulcer v. control
1. London	181	119	298	266	4578	5422	1.36 : 1	1.86 : 1
2. London	9	2	111	86			3.49 : 1	
3. York	21	15	—	—	983	1079	—	1.54 : 1
4. Newcastle	16	11	281	186	6598	6974	0.96 : 1	1.54 : 1
5. Glasgow	55	24	947	695	3177	2721	1.68 : 1	1.96 : 1
Mean weighted relative incidence							1.41 : 1	1.82 : 1
χ^2 for difference from unity							8.518	37.397
χ^2 for homogeneity n = 1, P							0.01	0.001
χ^2 for homogeneity n = 3, P							2.918	0.559
							0.30 to 0.50	0.90 to 0.95

The findings of Doll and his colleagues strongly point towards "causality" and the findings are so striking that the blood group and secretor status are in some clinics taken into account if gastric resection is contemplated.

Family Studies in Duodenal Ulcer

The method by which the control series was collected has already been dealt with, but some criticism came from Penrose (1953). He thought it possible that without there being a causal connection between blood group O and duodenal ulcer there might be a stratum in the population (for instance, the Scots) who were both high in group O and prone to duodenal ulcer. He thought that before causality was accepted, family studies should be made in which the unaffected sibs acted as controls, thereby eliminating any possibility of stratification. Since 1954 the Liverpool team have collected over 400 sibships in which at least one individual in each family has had a proven duodenal ulcer (Clarke et al., 1956).

Method of Analysis of Sibships

The data have been analyzed by the method of Dr. C. A. B. Smith of the Galton Laboratory, London. The principle of Smith's method is to assess in each family which segregates for blood group (i.e. those in which some sibs are O and some not O), the chance of the propositus (the person with the ulcer) being O and then to compare the total "observed" results with the total "expected." To clarify the matter let us consider the simplest possible case, that of a duodenal ulcer sibship consisting of four persons, two of whom are group O and two group A. Clearly the chance of the propositus being group O is an even one, and this (the "expected") is scored as 0.5. If, in fact, the propositus is group O the "observed" score is 1, whereas if he is group A the "observed" score is zero. For each family, therefore, there is recorded an "observed" and an "expected" score which can easily be analyzed and added to from other centers (Clarke, 1959a). The only disadvantage about Smith's method is that a great number of families give no information since only about one in three sibships segregate for blood group.

The same principle can be applied to families which segregate for the secretion and non-secretion of the ABH antigens, the chance of the propositus being a non-secretor being assessed in a similar way to that

for the ABO blood group. Table 9 and 10 show the results of these investigations.

TABLE 9—*Analysis of Duodenal Ulcer Sibships (Method of Dr. C. A. B. Smith)* (Clarke et al., 1959, augmented Jan. 1960)
ABO Blood Groups

Propositus group O	
Expected	75.259
Observed	81.0
Difference	+ 5.741
Standard error	± 5.987
Probability	≏ .34

TABLE 10—*Analysis of Duodenal Ulcer Sibships (Method of Dr. C. A. B. Smith)* (Clarke et al., augmented Jan. 1960)
Secretor status
(tested with Ulex by Dr. R. B. McConnell)

Propositus non-secretor	
Expected	52.6164
Observed	61
Difference	+ 8.3836
Standard error	± 5.1392
Probability	0.1

With the ABO blood groups it will be seen (Table 9) that there is no significant difference between the "observed" and "expected" figures. Therefore, in the Liverpool series, using this method there is neither support for nor disproof of the association. Fraser Roberts (1957) suggested that it would be more accurate to pair the propositus with a sib of the same sex, but even when this is done the results are only slightly different and do not materially affect the result.

Of the total 443 sibships investigated, in those which segregate for secretion and non-secretion the association with non-secretion is significant. The great majority of these cases were tested with *Ulex* but in the first 119 families (of which 33 segregate for secretion) eel serum was used. Since this method gives a few wrong results they have been omitted (although the error is small), and when this is done the significance disappears although the deviation is still quite heavily in the expected direction (Table 10). It therefore seems important, as Levene and Smith suggest, to add to the material, although the writer still feels that it is odd that the associations noted when the general population is used as a control are very much less marked in sibships.

These results initially came as a surprise, but Howard Levene (1959) in a mathematical survey of the statistical methods used, makes the following interesting observations: "Although Clarke correctly stated in his paper, and in earlier papers based on fewer sibships, that this (i.e. the result obtained by Smith's method) neither supports nor contradicts the hypothesis of association, Clarke still tries to find some explanation of an apparent discrepancy between the sib study and the population studies; and many other workers assumed that there was 'nothing to the whole business.'" Levene goes on to say that this is unwarranted and that "far from disagreeing with the population studies, the sib study is compatible with an even greater association than is shown by the population studies; there is simply insufficient data." He continues, "Two different methods suggest that about 1,500 sibships would be needed to settle the question. This is beyond the resources of the Liverpool group, and it is to be hoped that sib studies will be undertaken by workers at other centers on both duodenal ulcer and some of the other diseases for which associations have been found."

Smith (1959) while essentially agreeing with Levene, states, "You have simply been unlucky in not having demonstrated the effect already in the families at present available; by 'unlucky' I mean that you have only been foiled by a chance fluctuation. For if the sibships had followed more closely the trend in the general population they would have given an estimate of x (i.e. the relative incidence) nearer 1.4 than 1.1, which is about 0.3 higher than the present sample. . . . This seems to mean that if 'luck had not been against you,' 400 sibships would have been enough to show the effect; as it is, because of the deviation in the sample (which is well within the bounds of reasonable fluctuation) considerably more work may be needed (supposing always the effect exists) but not, I would have thought, the 1,500 suggested by Levene." Smith goes on to say that for a really convincing demonstration, a level of significance much higher than 5 per cent is very desirable and he concludes that there may be a case for having 1,500 or more sibships but not all obtained from the same district by the same team of workers.

Because of this, and because the assembling of very large numbers of sibships is extremely slow, further data from other centers have been awaited with interest. Buckwalter et al. (1960) have been carrying out a similar investigation; and from his preliminary results it can be said that when the Iowa and the latest Liverpool data are combined, the association of group O with duodenal ulcer within families holds at about the 5 per cent level of significance ($P=0.0525$). Furthermore, the very high O non-secretor incidence in stomal ulcer argues in favor of causality and

the fact that the three "firm" associations are all with disorders of the gastrointestinal tract points in the same direction. Family studies in propositi with stomal ulcers would be of very great interest.

It still seems to the writer, however, that there is a case for thinking that the association between O and duodenal ulcer should be *more* evident in segregating sibships than in the general population. Thus in an ulcer family which segregates for O and not-O it could be argued that environmental and genetic factors (other than the ABO blood group) would tend to be rather uniform. If this were so, one would expect the predisposing action of O to ulcer to be more pronounced—i.e. that it would more readily "switch" the ulcer from not-O to O than in the general population. On the other hand, it has been suggested that the converse may occur—that is, the effect of the O gene may be diluted in families because of other genes which predispose to ulcer, and these may be more in evidence in the ulcer sibships than in the general population.

Continuing to discuss the sibship data it will be remembered that the liability of the various ABO and secretor phenotypes to develop duodenal ulcer, using the general population as a control, was given in Tables 6 and 7. When this type of analysis is applied *within* families the results are very different. Table 11 shows the distribution of the eight phenotypes in a series of duodenal ulcer patients, random for blood group and secretor status, who have also an unaffected sib whom we have studied.

TABLE 11—*Numbers of Duodenal Ulcer Propositi and Oldest Unaffected Sibs Examined, Subdivided into Phenotypes* (Clarke et al., 1959)

Phenotypes	Either sex		Same sex	
	Ulcer	Sib	Ulcer	Sib
O non-secretor	42	37	20	20
B "	5	7	4	4
AB "	4	3	2	1
A "	28	28	13	13
O secretor	90	91	47	46
AB "	6	5	5	3
A "	59	61	26	31
B "	15	17	10	9
TOTAL	249	249	127	127

Where more than one unaffected sib has been tested the oldest has been used as the control. The phenotypes are in the order of susceptibility deduced from Table 7, and by analogy one would expect to have relatively more ulcers in the top half of Table 11 and more controls in the bottom half. Even remembering the expected correlation between the

phenotypes of sibs, the almost exact equality of the numbers of the different phenotypes of the two halves of the table is remarkable. However, on examining the table one sees that the sibship propositi have a group O frequency of only 53.0 per cent whereas the general ulcer series has one of 58.72 per cent (Clarke et al., 1955). This difference may be a chance effect and account for the non-significance of the sib data in Table 11.

Furthermore, there is some evidence (Clarke et al., 1957) that the predisposition to ulcer is dependent not only on the blood group and secretor character of the affected individual but also on the type of sibship to which he belongs. Thus where the sibs segregate both for blood group and secretor status, the association which might have been expected from the general population studies is in fact found—namely, that blood group O predisposes to ulcer.

Nevertheless, the writer continues to think that the over-all sibship results are remarkable, but he agrees that the data are not extensive enough to prove a discrepancy between them and the results using the general population as a control. Because of this, and in view of the almost universal findings of the association with group O, together with the secretor data, there were thought to be legitimate grounds for investigating biochemical and serological explanations for the associations.

The Meaning of the Associations

Several hypotheses have been put forward which might explain the relationship between duodenal ulceration, group O and ABH non-secretion. Thus Køster et al., (1955) put forward the attractive view that group O individuals might have a greater potential acid output (parietal cell mass) than those of group A. However, Brown and his colleagues (1956) in Glasgow, in 276 patients with either duodenal or gastric ulcer, using the augmented histamine test meal, showed that there was no correlation between the maximum acid output, ABO blood group and ulcers at any particular site. While this is of great interest, a piece of work well worth carrying out would be the estimation of the parietal cell mass of *normal* individuals in relation to their ABO blood group.

An interesting approach to the problem has recently been made by Sievers (1959). He investigated consecutive admissions to the St. Louis Veterans Administration Hospital for plasma pepsinogen and gastric acid production (by the method of cation exchange resin). Patients with duodenal and gastric ulcer and renal disease were excluded. He found that impairment of gastric secretion was more frequent in subjects of

blood group A than in those of group O. Thus 86.1 per cent of group O patients produced "free acid" compared with 73 per cent of group A (Table 12). Furthermore, the mean value of plasma pepsinogen was higher in individuals of blood group O (564 units per ml.) than in those of blood group A (494 units per ml.) ($p < 0.01$). The blood group distribution of the sample was very similar to that of a control group (unselected blood donors).

TABLE 12—*Data Extracted from Paper by Sievers (1959)*

	Blood Group			
Acid status	B	A	O	Totals
Secretors of acid	29	111	167	307
"Achlorhydrics"	13	41	27	81
TOTALS	42	152	194	388

Aird (1955) thought that the various blood group substances might confer different degrees of protection against ulcerogenic agents, while Clarke et al., (1956) suggested that there might be a differential protection depending on the interrelationship between ABH and Lewis blood group secretions. The hypothesis that the excess of group O in duodenal ulcers is due entirely to H substance protecting less than A or B substance is refuted by the Liverpool data, since in the absence of H substance (that is, in non-secretors) there should be no difference in the liability of O and not-O people. In fact, in the data there is a significant excess of group O among the duodenal ulcer non-secretors ($\chi^2 = 4.1$ $p < 0.05$) compared with the control non-secretors. This seems an important observation (see p. 89).

The "protection" hypothesis might have either a "mechanical" or an immunological basis. Work carried out in Liverpool has added to our knowledge of both possibilities.

THE "MECHANICAL" HYPOTHESIS

Two points must first be appreciated.

1. Non-secretors of ABH usually secrete much more Le^a (the only other blood group substance to be secreted in the body fluids) than do ABH secretors, and therefore it seemed likely that there might not be much difference in the total amount of blood group substances present between secretors and non-secretors, for example in saliva.

2. The rare sugar, fucose, is found in large amounts in blood group

substances, and is a good index of the total amount of these present in any given secretion.

Therefore the amount of fucose in salivas of individuals of various blood groups and secretor status was estimated by Clarke et al., (1959), the method used being a modification of that employed by Gibbons (1955) for methyl pentose.

Three results are worth mentioning:

a. The various types of ABH secretors and non-secretors do, in fact, all have about the same total amount of blood group substances (Table 13 gives the details.)

TABLE 13–*Calculation of Total Salivary Blood Group Content Tested by D. A. P. Evans* (Clarke et al., 1959)

Phenotype	Total salivary blood group substance content μg/ml.
O, A and B non-secretor	537.5
O secretor	521.7
A "	562.6
B "	557.5

b. The mean salivary fucose concentration, and hence the total blood group substance concentration, are the same in duodenal ulcer patients as in controls, i.e. there is no effect of the disease on secretion or of the amount secreted on the liability to the disease.

c. There was little difference between the mean salivary titer of A, B and H substance in secretors with duodenal ulcer compared with their unaffected sibs.

These findings argue strongly that the protection enjoyed by ABH secretors is not due to the blood group substances protecting quantitatively. Before leaving the matter it should be mentioned that about 5 per cent of individuals in England secrete neither ABH nor Lewis blood group substances in their body fluids. Because of this an investigation is in progress at the present time in Liverpool on patients with duodenal ulcer to see what proportions possess this "double negative" phenotype. The numbers so far are very small, but if the present trend continues it would lead to the conclusion that the group O individuals who secrete neither H nor Lewis blood group substances *are* particularly at risk.

THE IMMUNOLOGICAL HYPOTHESIS

Cain (1957) suggested that the brush border of the duodenal mucosa might in non-secretors be damaged by ABH agglutinins (lectins) present in many edible plants of the *Leguminosae* family, whereas in secretors

these would be inhibited. However, this ingenious suggestion seems unlikely in view of the work of Lawler (1957), who demonstrated that most of these plant lectins are, in fact, inactivated by heating.

It seemed therefore worth while next to investigate whether the duodenal mucosal cells did, in fact, possess ABH antigens, and if so, whether these were present both in secretors and non-secretors. Coombs et al., (1956) had shown by means of the mixed cell agglutination technique that the ABO antigens are present, not only on red cells, but on many other types of epithelium as well. Furthermore, these antigens were present regardless of whether the individual was a secretor or a non-secretor.

In Liverpool, Selsnick (1959) using a suspension of duodenal cells, states that she finds ABH antigen sites on the striated border in secretors of ABH, but almost never in non-secretors. Her results on about 60 patients were obtained using both the Coombs method and the fluorescein labeled antibody technique, and the findings were similar whether the patient had been operated on for duodenal ulcer or for some other condition. Selsnick's findings may be a fundamental contribution to the solution of the problem, but the results are so unexpected that we feel that greater numbers are required, particularly of non-secretors, before we can be certain that her findings are unequivocal.*

There are, however, other immunological clues. Thus:

a. Witebsky et al., (1935) found blood group activity to be strongly displayed by the mucus of the stomach and to decrease progressively in the lower regions of the gut.

b. Barber and Dunsford (1959) demonstrated a very high concentration of A substance in the serum of a patient dying of carcinoma of the stomach. This may well have been an effect of the disease. It would be worth while to look for similar findings in patients with metastasising colloid cancers of the stomach. Conceivably the finding of a high antigen titer in the serum might prove to be a test for the presence of secondaries in some forms of this disease.

c. Gold et al., (1959) reviewed the literature on the change in group A antigen which may occur in some cases of leukemia. They described a patient in which this occurred, and where there was partial reversion towards normal during a clinical remission.

d. Glynn et al., (1957) noted that there was a high concentration of A substance in the cancer cells in carcinoma of the stomach.

e. It is well recognized that several drugs such as the steroids, aspirin and phenylbutazone, all of which may block antigen-antibody responses, can also be ulcerogenic.

* Subsequent work supports Selsnick's findings.

f. On the negative side, Dumbell (1959) found no evidence of an auto-antibody response to gastric mucosa in several patients suffering from peptic ulcer (see Bracey, 1959).

Other Less Well Authenticated Associations

A somewhat tedious list of less firm or only possible associations follows. These may be of importance, but in many of them it is difficult to see what further lines of research are indicated.

1. Lesions of the Gastrointestinal Tract or Its Appendages

Salivary tumors. Cameron (1958) found a very high incidence of group A in 206 patients with salivary gland tumors.

Cancer of the pancreas. Aird et al., (1960) found a moderately significant excess of group A in 620 patients with cancer of the pancreas. Table 14 gives the details.

Table 14—*Cancer of the Pancreas. Incidence in Group A Relative to Incidence in Group O and in Groups (O + B)* (From Aird et al., 1960)

Center	Relative incidence A : O	χ^2	Relative incidence A : (O + B)	χ^2
London	1.56	4.47	1.48	3.99
Birmingham	1.03	0.01	0.96	0.02
Bristol	1.18	0.30	1.13	0.20
Cardiff	1.39	0.99	1.37	0.99
Liverpool	1.52	5.38	1.68	8.47
Manchester	0.81	0.80	0.92	0.13
Sheffield	3.87	7.13	2.85	6.02
Leeds	0.84	0.15	0.77	0.35
Newcastle	1.36	0.83	1.26	0.52
Glasgow	0.85	0.35	0.90	0.13
Mean weighted relative incidence	1.25		1.27	
TOTAL		20.41		20.28
χ^2 Diff. from unity D of F = 1		6.57		8.36
Heterogeneity D of F = 9		13.84		12.46
P Heterogeneity		.13		.2

Cancer of the esophagus. Aird et al., (1960) reported a slight increase

of group A relative to O in 610 patients. The details are given in Table 15.

TABLE 15—*Cancer of the esophagus. Incidence in Group A Relative to Incidence in Group O* (From Aird et al., 1960)

	Center	Relative incidence A. : O	χ^2
	London	1.14	0.51
	Birmingham	1.33	1.23
	Bristol	1.24	0.25
	Cardiff	0.80	0.47
	Liverpool	1.24	1.49
	Manchester	0.86	0.27
	Sheffield	1.54	0.75
	Leeds	1.23	0.32
	Newcastle	0.86	0.11
	Glasgow	1.19	0.30
Mean weighted rel. incidence		1.14	
	TOTAL		5.70
χ^2	Diff. from unity. D of F = 1		2.17
	Heterogeneity. D of F = 9		3.53
P	Heterogeneity		.9

Cholelithiasis. Kjølbye and Neilsen (1959) report an increased frequency of blood group A in 1,369 patients with gallstone disease. Their data indicates that individuals with blood group A run a 17 per cent higher risk of developing this disease, and the more detailed analysis shows that the highest relative incidence is found in women below 50 years of age.

Portal cirrhosis. Billington (1956a) examined the records of 111 cases, mostly grouped because of bleeding from esophageal varices. There was a marked excess of group A. It must be remembered, however, that the numbers are very small.

Carcinoma of the colon and rectum. Both Aird et al., (1954) and Buckwalter et al., (1956b) found a normal distribution of the ABO blood groups in carcinoma of the colon and rectum. This may be of interest when considering the question of controls (see p. 86). Moreover, the findings form an interesting contrast to those diseases in which an association has been demonstrated.

Chromophobe adenoma of the pituitary gland. This is included here because the pituitary develops as an outgrowth of the foregut. Mayr et al., (1956) found a large excess of group O in patients suffering from chromophobe adenoma. Another series (Damon, 1957) showed the excess

to be smaller, while Aird et al., (1960) found a normal distribution in the largest series of all (408 cases) from Great Britain.

2. Diabetes Mellitus

The evidence here is fairly strong for an increase in group A but it is less marked than for pernicious anemia, although the numbers are considerably larger. McConnell, Pyke and Roberts (1956) provide a useful warning of the dangers inherent in this type of work at the stage when differences are only moderately significant. They found in the first three areas that the excess of group A was entirely confined to men. A direct comparison of the sexes was moderately significant and these matters were discussed at length. The results of Craig and Wang (1955), for Glasgow, showed the same excess of group A but in that instance it occurred in women only. Finally Mähr (1959) found a non-significant excess of A in 1,300 diabetics from Vienna (Table 16). Helmbold (per-

TABLE 16—*Diabetes Mellitus. Analysis of Incidence of Disease in Persons of Group A Relative to Its Incidence in Persons of Group O*
(From Fraser Roberts, 1957, augmented)

Center	No. in disease series	Relative incidence A : O	χ^2
1. S. W. Lancashire	634	1.12	1.71
2. West Cheshire	199	1.19	1.39
3. Oxford	500	1.11	1.49
4. Glasgow	817	1.20	5.02
5. Vienna	1300	1.10	2.02

1.-3. McConnell, Pyke and Roberts (1956)
4. Craig and Wang (1955)
5. Mähr (1959)

sonal communication and 1960) has combined the data for all five centers and finds a relative incidence of 1.14 which is significant, the χ^2 for one degree of freedom being 10.8, $p \simeq 0.001$. There was no heterogeneity between centers ($p = 0.94$). Thus the combined data show that there is nothing appreciable to contradict the hypothesis that the excess of group A occurs in both men and women, and also that it is present, and to an equal degree, over all ages at onset of the disease (see also Fraser Roberts, 1957). An interesting clinical point is that women with carcinoma of the body of the uterus not infrequently also have the "senile" type of diabetes, and furthermore, in both diseases the menopause tends to be delayed (Graham, 1954). Helmbold (1960) finds a trend towards group

A in carcinoma of the body of the uterus (number of patients not given, but several thousand), the relative incidence of A: O being 1.05.

3. *Carcinoma of the Cervix*

Helmbold (1960) found a relative incidence of 1.716 A : O in 5,818 patients with carcinoma of the cervix from ten centers. There was no significant heterogeneity between these localities and the probability of the finding occurring by chance is less than 1 in 1,000. In a further series of 2,312 women with a similar condition from five centers showing no heterogeneity, the relative incidence of A_1 to O was 1.280, giving a probability again of less than .001. However, when the ratio of A_2 to O was examined in a similar number of cases the relative incidence was 1.024 which does not differ significantly from the controls. These findings might be an indication that the strength of A substance (A_2 being weaker than A_1) is the important factor in the association.

In a small unpublished series from Liverpool, a normal ABO and secretor distribution in 219 cases of carcinoma of the cervix was found. In a very similar number of cases, however, Beolchini et al. (1957) reported an excess of group A (see Table 17).

TABLE 17—*Carcinoma of the Cervix Uteri. Relative Incidence in Persons of Group A Compared with Incidence of One in Persons of Group O*

Center	Number in disease series	Relative incidence	χ^2	P
1. 9 German cities and Vienna	5,818	1.176	27.257	<0.001
2. Liverpool	193	1.044	0.09	>0.05
3. Milan	181	1.266	2.28	>0.05

1. Helmbold (1960).
2. Liverpool data, unpublished.
3. Beolchini et al., (1957).

In an earlier paper, Helmbold (1958) investigated the ABO blood groups in genital carcinoma (all types)in 7,705 women "in 9 German cities as well as Vienna." Using Woolf's method (Woolf, 1955) he found the incidence in group A relative to that in group O to be 1.15. Since the χ^2 is 21 for 1 degree of freedom, the incidence differs from unity with high significance. It is perhaps worth noting that where there is a deviation from the normal ABO distribution in malignant disease it is nearly always in the direction of an excess of group A.

4. Rheumatic Fever

This is of more interest. The results give a clue to further work and tests can be undertaken in vitro. Glynn et al., (1956) emphasized that there must be important host factors in rheumatic fever because, although the disease usually follows a streptococcal throat infection, yet only a small proportion of cases of a streptococcal tonsillitis does, in fact, develop the complication. The authors pointed out that one important variation in the environment of the streptococcus depends on the blood group secretor status of the host, and this idea led them to investigate the secretor character in patients with rheumatic fever. In 460 school children who were used as controls they estimated an incidence of 22.6 per cent non-secretors of ABH from the results of an anti-Le^a serum and saliva test (most individuals who are non-secretors of ABH secrete large amounts of Le^a), whereas among 450 children with rheumatic fever the estimated non-secretor figure was 28.7 per cent and the difference was significant at the 5 per cent level.

The same authors have recently published further data (Glynn et al., 1959). Using the same salivary anti-Le^a technique they found a Le^a positive incidence of 21.2 per cent in 1,129 healthy school children as compared with 27.5 per cent in 611 cases of rheumatic fever (significant at the 1 per cent level). Similar results were obtained with *Ulex* extract which tests directly for secretor status by showing the presence or absence of H-substance, this being present in the saliva and other body fluids in secretors of any of the ABO blood groups. By the *Ulex* method Glynn and his colleagues found that 22.9 per cent of 669 healthy school children were non-secretors and the comparable figure in 553 rheumatic fever cases was 28.9 per cent (significant at the 2 per cent level).

As regards the ABO blood groups Glynn and his colleagues found a non-significant deficiency in group O.

A recent investigation has been made into the ABO blood groups and secretor status of 228 Liverpool patients known to be suffering from established mitral stenosis, together with 35 children who had been affected with rheumatic carditis (Clarke et al., 1960). It will be seen that in this series the non-secretor increase, though present, is not significant (Table 18), whereas the reduction in the incidence of blood group O is markedly so (Table 19). As there is no heterogeneity between the Taplow and Liverpool series the results of the investigations may be combined, and when this is done it will be seen that there is a significantly

increased incidence of the disease both in those who are not group O and those who are non-secretors.

As has been said before, it is possible to test the Glynn and Holborow hypothesis by investigating the growth and virulence of hemolytic streptococci depending on the amount of water-soluble blood group substance present in the culture media. This is being undertaken at the present time. Finally, it would also be interesting to know whether or not the blood group and secretor differences present in rheumatic fever are also found in series of patients with uncomplicated streptococcal throat infections and in those who develop acute nephritis.

5. Bronchopneumonia in Children

It has been thought that ABO iso-immunization may be responsible for the 25 per cent deficiency of A noted in the mating O♀ × A♂. Whether the children concerned die before or after birth is not known for certain. The work of Struthers (1951) on a consecutive series of 400 infants and children on which autopsy was carried out showed a highly significant deficiency of group O in the dead children, and this was particularly evident in bronchopneumonia. Carter and Heslop (1957) however, could not confirm this in a series of similar size, but in their patients the pneumonia was always "secondary" so that their results may not be exactly comparable with those of Struthers. The secretor status in a series of cases of bronchitis and asthma in Liverpool (mostly children) was normal, 23.6 per cent being non-secretors (McConnell, 1959).

6. Hypertension

Maxwell and Maxwell (1955) reported a normal blood group distribution in 2,147 patients with hypertension. However, Aird et al., (1955) pointed out that the data contained a suspicious deficiency of men who were AB. Thus in the total number of men investigated (597) the expected number of AB's was 20.111 as against the 7 observed, the χ^2 being 6.22 for 1 degree of freedom. Maxwell and Maxwell consider that the deficiency of AB's, if real, suggests an advantage in belonging to that group. Aird et al., (1955), however, point out that it is equally or more probable that the findings indicate a disadvantage, hypertensive men of group AB tending to die younger and so not appear in the series. It is to be hoped that they or other writers will be able to provide further

TABLE 18—*Numbers and Percentages of Non-Secretors in Patients with Rheumatic Carditis and Rheumatic Fever and Control Series* (Taken from Clarke et al., 1960)

	Liverpool data				Taplow data			
	263 rheumatic carditis		851 controls		553 rheumatic fever		669 controls	
	No.	%	No.	%	No.	%	No.	%
Non-sec.	73	27.8	207	24.3	160	28.9	153	22.9

Statistical analysis	Relative incidence of disease in non-secretors	χ^2 d of f = 1	p	95% fiducial limits
Liverpool	1.20	1.26	>0.2	
Taplow	1.37	5.82	<0.02	
Combined	1.30	6.63	<0.02	1.58-1.06
Heterogeneity	–	0.45	>0.3	

TABLE 19—*Numbers and Percentages of the Different Blood Groups in Patients with Rheumatic Carditis and Rheumatic Fever and Control Series* (Taken from Clarke et al., 1960)

Blood group	Liverpool data				Taplow data			
	Rheumatic carditis		Control		Rheumatic fever		Control	
	No.	%	No.	%	No.	%	No.	%
O	105	39.9	7842	48.8	255	41.9	6775	45.0
A	116	44.1	6294	39.2	256	42.0	6456	42.9
B	36	13.7	1519	9.5	64	10.5	1311	8.7
AB	6	2.3	402	2.5	34	5.6	504	3.4
TOTAL	263	100.0	16057	100.0	609	100.0	15046	100.0

Statistical Analysis

	Relative incidence of disease in not-O people	χ^2 d of f = 1	p	95% fiducial limits
Liverpool	1.44	8.15	<0.01	
Taplow	1.14	2.36	>0.1	
Combined	1.22	8.15	<0.01	1.40-1.06
Heterogeneity	–	2.36	>0.1	

data, concentrating especially on men and keeping a careful watch for any association with age.

7. *Toxemia of Pregnancy*

This is a fascinating problem and one which might well be explained on the hypothesis of an antigen-antibody reaction between mother and fetus.

Platt et al., (1958) suggested a most ingenious explanation depending on the hypothesis that mothers of toxemic women could not themselves have had the condition. Although he disproved this for a single antigenic factor, yet the possibility that several factors may be at work is not ruled out. If the hypothetical antigen were water-soluble, as it would be likely to be, ABO and secretor studies in affected mothers might be rewarding. All that is known at present about the blood groups and toxemia is that the ABO distribution is normal (Dickins et al., 1956).

The MN Blood Groups

Although no association has been found between these blood groups and disease, they are of considerable theoretical importance since the heterozygote is recognizable and very common in the population (about 50 per cent). Wiener (1943) believes that the excess of MN children from MN × MN matings found in many series has been due to technical errors. However, Morton and Chung (1959) take a different view, and are of the opinion that the evidence for mistakes appears to be untenable. These authors feel that the excess of MN children in back and intercrosses is highly significant and consistent with heterozygote advantage, though apparently limited to children of MN mothers. They suggest an interesting line of research which would clarify the matter: "The material should come, if possible from a population not practising birth control, such as the Hutterites, where early foetal death might be reflected by the interval between children. Children of MN mothers provide the critical material free from any need to exclude extramarital paternity. The S,s and other components of the MN locus should certainly be studied since they may influence MN selection. To control technical errors, several independently prepared anti-M and anti-N reagents, constant throughout the study, should be used with titration and quantitative scoring, the tests being repeated on a random sample of cells. In this way the frequency of misclassification would be both minimized and accurately known. The sample size should be of the order of thousands."

Investigation of Isolated Populations

An idea of great interest was that of Moor-Jankowski and Huser (1958) who investigated the ABO groups of a number of Swiss Walser isolates. In some of these the incidence of group O, and in others that of group A, was very high. On examining patients under treatment for all ailments they found that there were proportionately more peptic ulcers in the high O areas and more cancers of the stomach where the incidence of A was increased. The numbers of those affected were very small but the work could be extended and the paper is mentioned as an example of a stimulating type of survey that might be more rewarding on a larger scale.

Aberrant Secretion

McNeil et al., (1957) have distinguished a group of secretors which they call "aberrant" and which they claim is associated with spontaneous abortion. Beuttner-Janusch et al., (1958) have found a similar correlation with infertility. The aberrant secretor character does not seem to have been strictly defined, but it certainly includes people in whom the ratio of A to H substance in group A, or of B to H substance in group B, falls outside the normal range. Morganti et al., (1959) have gone still further and criticized the division of individuals into secretors and non-secretors. Thus they suggest that by using "refined techniques" the subdivision can be shown to be an arbitrary one, and that the presence or absence of the antigens in watery secretions other than saliva must be taken into account.

Both matters have been investigated in Liverpool (Chapter 4). From a consideration of the distribution of the ratio of A to H substance in group A secretors it is clear that many of the so-called aberrant secretors are really the arbitrarily chosen extremes of a continuous and unimodal distribution of ratios. Aberrant secretors therefore should be defined as individuals whose A : H, or B : H ratio falls more than a given number of standard deviations from the mean of the control group. If this were done it would allow people in different places, using different antisera, to compare their results.

The validity of the secretor/non-secretor hypothesis can be tested by examining family data. If, in fact, minute quantities of antigen are being secreted even by non-secretors, and the amount secreted is multifactorially controlled, then at least some non-secretor x non-secretor matings should give secretors. In the 14 non-secretor x non-secretor matings,

taken from an Rh investigation (see Clarke et al., 1959, for preliminary data), no secretors occurred among the 52 children, and this is in agreement with other similar recorded matings (see Race and Sanger, 1958). From this evidence, there seems no doubt that the particular method of scoring employed in this series *does* distinguish two phenotypic groups (differing as the result of a single pair of allelomorphs), and that the distinction between secretion and non-secretion is a real one. However, although this is so for saliva, there is no theoretical reason why there should always be a complete correspondence with other secretions. For example, Selsnick (1959) showed that one of her salivary non-secretors possessed ABH antigens on the duodenal mucosal cells. There is, therefore, a good case for investigating the degree of correspondence between secretion and non-secretion in various body fluids, particularly saliva and gastric juice, and this is at present being carried out in Liverpool. In the paper referred to above (Chapter 4) the data confirm that group O people secrete more H than group B people, and that group AB people secrete least H of all. This suggests that there may be a difference between homozygotes and heterozygotes of groups A and B with regard to the amount of H secreted, and this also is being investigated further. If it should prove to be the case that the heterozygote AO or BO can be identified by the amount of H substance secreted, it would be of interest to apply this knowledge to those individuals with duodenal ulcer who are A or B, since AO or BO might possibly be more predisposed to ulcer than AA or BB. If this were so, then light might be shed on the duodenal ulcer sibship problem, because in the analysis only segregating families were used, and therefore most (but not all) the A's and B's would be AO or BO, and it might be that it was the O gene which predisposes these people to ulcer. However, against this, Fraser Roberts (1957) gives the mean weighted relative incidence of O : A as 1.36 and O : AB as 1.35, suggesting that AB's are no less liable to ulcers than A's.

The PTC Taste Response and Thyroid Disease

The ability or inability to taste phenylthiocarbamide (PTC) is referred to as a sort of "honorary blood group" by Race and Sanger (1958), and it is their authority which accounts for its inclusion in this chapter.

Apart from the blood groups the PTC taste phenomenon is one of the few normal human characters whose mode of inheritance is known, though with less precision than was previously thought. The difference between "tasters" and "non-tasters" is controlled by a single major gene,

tasting being dominant to non-tasting; the rare contradictions to this hypothesis may be due to incomplete penetrance of the gene (Das 1957/58). In Europeans and white Americans about 30 per cent of individuals are non-tasters (genotype tt) and 70 per cent tasters (TT or Tt).

Because the thiocarbamides are goitrogenic and include the thiouracils, Harris, Kalmus and Trotter (1949) tested the taste response of groups of patients with thyroid disease. Their data suggested that non-tasters of PTC were slightly more susceptible to the development of adenomatous goiter than controls, but they found a normal taste distribution in patients with toxic diffuse goiter.

Further work on the same lines has been carried out in Liverpool (Kitchin et al., 1959). A total of 379 goiter patients, all of whom had had a partial thyroidectomy, were tested. The series is particularly valuable because in every patient the type of goiter was verified histologically. In 246 cases of adenomatous goiter, a significantly high incidence of non-tasters (genotype tt) was found.

In 133 patients with toxic diffuse goiter a significant excess of tasters was found (genotype Tt, TT), but without sex difference.

The cause of these associations is not known, but it is possible to speculate intelligently about the first of them. Thus the inability to taste PTC may be associated with a greater susceptibility to the action of goitrogens of a similar chemical type, and cows' milk could be a source of such compounds in winter months (cooking destroys the goitrogens in the vegetables which contain them). If this were so, cycles of seasonal involution and hyperplasia would occur in the thyroid, particularly in non-tasters of PTC, and it is just this pathological sequence which is known to give rise to adenomatous goiter in later life.

A notable finding in the series of adenomatous goiters is the considerably higher incidence of non-tasters of PTC in the male as compared with the female patients. The male : female ratio in sporadic goiter lies between 1 : 8 and 1 : 15, except in areas of endemic goiter where the sex ratio approaches unity. In view of the fact that in the female, cyclical changes of involution and hyperplasia are known to occur in the thyroid in relation to the menstrual cycle and childbearing, it might be anticipated that genetic influences (other than those affecting sex) would be less important in women than in the male. The Liverpool findings show this clearly, the incidence of non-tasting in the males with adenomatous goiter being 60 per cent in contrast to the female rate of 36.6 per cent.

The cause of the association of tasting with toxic diffuse goiter is quite unknown, and all that one can say generally is that the taster/non-

taster genotypes are important in determining the type of thyroid disease which a patient may develop. Clearly it would be of great interest also to know the taster status of patients with myxedema.

Experimental work with rats (Kitchin, 1959) has shown that some of these animals can also be divided into "tasters" and "non-tasters," but the division is not so clear-cut as in humans, and many rats cannot be accurately scored for the character. Another difficulty is that the drug is toxic even in dilute solution and it is thought that those that die as the result of the drug contain a high proportion of non-tasters. A point of interest is that non-taster rats, if they do not succumb, tend to develop a myxedema-like state. The appropriate matings to find out whether in rats the character is controlled by a pair of allelomorphs have so far all proved infertile, and in particular it has not been possible to get the critical mating of non-taster x non-taster. Further work is in progress and mice are now being investigated as a possible experimental animal for the same purpose.

The Role of Selection in "Adult" Associations

If the diseases discussed above are really factors in stabilizing the ABO polymorphism, then it is essential that biological fitness should be reduced in those affected. Carcinoma of the stomach, for example, is extremely rare before 40 years of age and, therefore, selection against the A gene must be very small. A similar and even more marked state of affairs would be the case in Addisonian anemia which is essentially a disease of old people. Duodenal ulcer would by contrast exercise a bigger effect than either of the above against group O since the death rate from this condition in males is 80-90 per million of the population per annum (see McConnell, 1955, and Sheppard, 1959). Some of these would be young, but in general death from peptic ulcer is much commoner in the older age groups, and selection against group O is probably only slight. However, this may not have been the case in earlier times, when treatment was not so efficient. Duodenal ulcer is generally regarded as a "new" disease, having only rarely been met with before the beginning of this century. Whether this is true is speculative because Hurst and Stewart (1929) report the occurrence of an ulcer in the duodenum as early as 1737, described by Morgagni. Furthermore, the classical symptoms of the condition were described by Abercrombie as early as 1828 and the diagnosis was made on clinical grounds by Bucquoi in 1877 (quoted by Hurst and Stewart, 1929). It seems not unlikely, therefore, that duodenal ulcer may have been commoner than is thought

before x-rays and surgery could assist in the diagnosis. However, even allowing for this it seems almost certain that the disease has increased in frequency in the last 50 years and therefore there must have been important environmental factors at work.

Diabetes mellitus, where the evidence for an association with group A is fairly strong, is, or at any rate was, in a somewhat different category. Before the discovery of insulin in 1921 diabetes in the young was nearly always rapidly fatal, and the association noted would be quite strongly selective against the A gene.

In the association between group A and carcinoma of the uterus, it must be remembered that (1) carcinoma of the cervix usually occurs in multiparae and well after the child-bearing period is over; (2) carcinoma of the body is found in middle life and more often in nulliparous than in parous women. Neither neoplasm seems likely to be playing much part in selection against group A at the present time. Nevertheless, even very slight protection from or predisposition to a disease may be important if the selection has acted over a long period of time. Again the ABO genes, in addition to the associations with the diseases described may have other and more important pleiotropic effects, their nature at present quite unknown. Haldane (1949) thinks that what is needed to throw more light on the matter is an epidemic among the young to see if those worst affected are predominantly drawn from any one serological type. In the same paper he shows that the whole problem of selection is beset with difficulties and gives examples of how the survival of the fittest can, in fact, make a population less fit. For instance, a mutant gene conferring improved warning coloration in, say, a caterpillar, may protect it from predators, but because of this it may also cause many parasites to escape destruction as well. Since these multiply more quickly than the host, and the host population is denser (because of the crypsis), more hosts will be parasitized and equilibrium is finally reached with the host population less dense than it was before the mutation arose.

In conclusion the writer is in no doubt that anyone who can assess evidence objectively must be convinced that the associations between the ABO blood groups and carcinoma of the stomach, peptic ulcer and pernicious anemia are proven, supporting the tenets of Ford (1945) and refuting the antagonisms of Wiener (1956). The findings in the other diseases are less definite, but several of them are very suggestive. Nevertheless, simply amassing correlations between blood groups and diseases is, by itself, somewhat pointless practically, and work should be so planned that biochemical or serological investigations can follow up

any associations that may be found. Moreover, investigation of associations between diseases and blood groups other than the ABO system would be well worth carrying out.

As regards the ABO groups the view that stratification can account for the associations described seems quite untenable since the same findings occur in populations where the distributions are very different from each other, the duodenal ulcer data of Matsunaga (1959) in Japan being particularly relevant in this connection. The stomal ulcer data point in the same direction.

This concludes the chapter. Critical assessment of the data must surely leave no doubt that associations (albeit small) have been shown to exist between the ABO blood groups and certain diseases. It is perhaps legitimate to remind the skeptic that the hardest things to believe are often those which they have previously insisted were untrue.

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Addendum

An ingenious suggestion has recently been made by Bateman (1960), in which he calculates the ABO percentages on the basis of trisomy occurring in the chromosome which carries the ABO locus. He shows that there would be a marked deficiency of group O in these trisomic individuals. In fact the group percentages from this theoretical calculation are very similar to those of astrocytic gliomata found in children by Yates and Pearce (1960). Clearly it would be worth while investigating children with these gliomata to see if, in fact, they were trisomic, as if so the abnormal blood group distribution would be explained.

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Chapter 5

ABO Incompatibility

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In the years following Lansteiner's discovery (1900) of the ABO blood types and of their significance to blood transfusion, various researchers suggested that differences between mother and child for the ABO blood types might produce disease in the fetus. Among those suggesting this were Dienst (1905), Ottenberg (1923), and Hirszfeld (1928). In particular, Hirszfeld and Zborowski (1925) collected a number of family studies, and found that there seemed to be a deficiency of type A offspring in matings of an A father and an O mother (A $\times$ O) as compared to the offspring of an O father with an A mother (O $\times$ A). They also found nearly complete absence of AB offspring to O mothers and considered this due to the same effect. After the general acceptance of Bernstein's (1924) triple allele theory explaining the absence of AB offspring from O mothers, Hirszfeld apparently did not consider the question further.

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However, after the discovery by Levine, Burnham, Katzin and Vogel (1941) of the causation of hemolytic disease of the newborn by Rh incompatibility, Levine returned to the subject in 1943. At that time he did not find evidence of erythroblastosis fetalis due to ABO incompatibility but he did find, on studying a series of parents who had had a number of miscarriages or stillbirths, more incompatible matings than would be expected in the general population. In considering this problem Hirszfeld compared two classes of pregnancy—homospecific where mother and child had the same blood type and heterospecific where they did not, since at that time it was not clear whether an O child with an A mother or an A child with an O mother was more likely to have difficulties. In the light of hemolytic disease, Levine was able to distinguish compatible pregnancies (or matings), where the mother could safely receive a transfusion from the child (or father), from incompatible ones where she could not. Levine also collected some additional studies published since Hirszfeld, including some of his own, and again found a deficiency of type A offspring in A $\times$ O matings as compared with O $\times$ A matings. Waterhouse and Hogben (1947) turned to this aspect and analyzed statistically a group of 12 previously published studies that met certain criteria as to serologic accuracy and completeness of the data. Comparing A $\times$ O with O $\times$ A matings and B $\times$ O with O $\times$ B matings, they found a deficiency of the incompatible matings and an even stronger deficiency of children from incompatible matings. They concluded that these differences were due almost entirely to the loss of A (or O) children from the incompatible matings, and they concluded that there was a loss of about 25 per cent of all A children from such incompatible matings. Bennett and Brandt (1954) reexamined the data used by Waterhouse and Hogben, made many criticisms of their statistical methods and concluded that there was no statistical evidence for a selective loss of A children from incompatible matings. Other more recent papers will be discussed subsequently along with a more detailed consideration of the papers of Waterhouse and Hogben, and by Bennett and Brandt.

Halbrecht (1944) described a relatively benign form of neonatal icterus which he termed "icterus praecox." Fifty-seven of 60 cases proved to have ABO types that were incompatible with the mother, whereas in 2,000 normal deliveries only 26.5 per cent of newborn were found to be incompatible. Polayes (1945) observed clinically severe cases of erythroblastosis that were seemingly due to ABO incompatibility, as early as 1942.

Further observations concerning ABO erythroblastosis began to meet

skepticism (Race and Sanger, 1950) mainly because of the antiglobulin test (Coombs, Mourant and Race, 1945) which yielded a diagnostically positive reaction in Rh hemolytic disease of the newborn but not in ABO erythroblastosis. Not until 1953 did Rosenfield obtain a consistently positive (but weak) test in this disease. Although his test was not useful to most laboratories, Rosenfield's report was sufficient to permit acceptance of the fact that there was antibody coating on the red cells of these involved newborn.

Genetics of the ABO Blood Group System

The ABO blood group system is gene-determined by at least three alleles at a single locus (Bernstein, 1924). Two of these alleles, *A* and *B*, are dominants which appear to determine corresponding antigens on a one for one basis. The third allele, *O*, is for the present considered to be an amorph (Race and Sanger, 1958) because it is not responsible for a recognizable antigen. Thus four phenotypes exist, two of which (AB and O) each correspond to single genotypes (*AB* and *OO*), whereas the other two may have either of two possible genotypes (A = *AA* or *AO*; B = *BB* or *BO*). Any phenotype lacking A and/or B antigens is expected to have a corresponding α and/or β serum antibody.

Additionally, the *A* allele may be subclassified as A^1 or A^2 (Thomsen, Friedenreich and Worsaae, 1930); a rare A^3 allele has also been described (Friedenreich, 1936). A^1 determines antigens corresponding to both α_1 and to α antibodies. A^2 determines antigens corresponding only to α and therefore appears to be recessive to A^1. The rare A^3 allele determines antigens significantly weaker in reactivity with α antibody than the A_2 antigens, and thus seems to be recessive to both A^1 and A^2 (Race and Sanger, 1958; Wiener and Wexler, 1958). For all practical purposes A^3 can be ignored (ibid.).

The subdivision of A into A_1 and A_2 permits the recognition of six phenotypes and ten genotypes.

There is as yet no means of distinguishing serologically between the genotypes which comprise a single phenotype, but family studies may permit such recognition (ibid.).

The ABO antigens on the erythrocytes are not as serologically reactive at birth as they are later in life, and in vitro serologic distinction between A_1 and A_2 is not reliable on cord blood specimens (Wiener, 1946).

The isoantibodies, α and β, are of such regular occurrence in the blood of adults that detection is part of standard blood grouping procedure

TABLE 1—*ABO Genotypes and Phenotypes, Based on Four Alleles, with Their Expected Erythrocytic Antigens and Serum Isoagglutinins*

Genotypes	Phenotypes	Erythrocytic Antigens	Serum Agglutinins*
OO	O	none	α and β
A^1B	A_1B	A_1, A and B	none
A^2B	A_2B	A and B	none
A^1A^1, A^1A^2, and A^1O	A_1	A_1 and A	β
A^2A^2 and A^2O	A_2	A	β
BB and *BO*	B	B	α

* Anti-A_1 and anti-H not shown.

(Stratton and Renton, 1959). At birth, however, the only α and β antibodies to be demonstrated in cord blood are those which can be found in the serum of the mother (Hirszfeld, 1928).

The human isoantibody, α_1, is of atypical occurrence (i.e., irregular, unexpected), but can be demonstrated as a weak cold agglutinin in the sera of some persons of A_2 or A_2B phenotype (Wiener, 1946). A more reliable source of α_1 is a saline extract of the seeds of Dolichos biflorus (Bird, 1952). Another atypical ABO isoagglutinin is anti-H, found as a weak cold agglutinin in the sera of some persons of A_1, B or A_1B phenotype; anti-H reacts to some extent with the red cells of almost all ABO phenotypes but is strongest with the red cells of type O and type A_2 persons (Wiener, 1946). A reliable reagent is obtained from the saline extract of the seeds of Ulex europeus (Cazal and Lalaurie, 1952).

The ABO alleles not only determine specific erythrocytic antigens, but specific antigens secreted as soluble mucopolysaccharides which can combine with α and β antibodies (Yamakami, 1926). Thus a person of phenotype A possesses A antigen on the erythrocytes and may have A substance in saliva, gastric juice and other sources of mucopolysaccharides. Similarly, a B person may have B in the saliva, and type AB may secrete both antigens. The phenotype O, lacking both A and B, can secrete neither antigen. The H antigen, however, may be secreted by any ABO phenotype including type AB (Sasaki, 1932). Secreted mucopolysaccharides permit no distinction between A_1 and A_2; the extract of Dolichos biflorus precipitates equally well with the soluble blood group substances that are secreted by either A_1 or A_2 phenotypes (Bird, 1959).

A, B and H antigenic structures occur on a single molecule of mucopolysaccharide (Morgan and Watkins, 1959). As yet this molecule has not been split to yield individual products (e.g., separate A and B secreted by type AB), and for the present, ABO antigenic structures appear to be products of both somatic genes.

The ABO phenotype is subject to a number of modifications, one of which is commonly encountered (Race and Sanger, 1958).

TABLE 2—*Some Modifying Conditions Affecting the ABO Phenotype*

Condition	RBC Antigens	Salivary Antigens	Serum Antibodies
sese	no change	no A, B or H	no change
xx	no (A?), B or H	no (A?), B or H	α, β and anti-H always present
yy	no A	no change	no change
some acute leukemia	no A	no change	no change

The genotypes, *sese*, *xx* and *yy*, refer to the homozygous state of recessive genes at three loci which are independent of one another and of the ABO locus. The *sese* genotype represents a modifying condition that is responsible for the non-secretor status of ABO (Schiff and Sasaki, 1932). Similarly, *xx* modifies ABO expression at both the erythrocytic and the secreted mucopolysaccharide level, and is responsible for the so-called "Bombay" blood type (Levine et al., 1955); *xx* may affect A antigens as it has been shown to affect B and H antigens, but there is no data for this as yet. The "Bombay" type (ibid.) transmits an intact *B* or *O* allele. There is only a limited amount of data concerning *yy*, but it appears to have an effect only upon the expression of A on the erythrocytes; the B antigen is not affected when the genotype is *AB*, and the A allele in an affected person is transmitted intact (Weiner et al., 1957). In some cases of acute leukemia the A (but not the B) antigen disappears from the erythrocytes (van Loghem et al., 1957; Dunsford, 1959); this change has been observed during lengthy remission of the underlying disease, and the normal erythrocytic antigen expression has been seen to return during the final relapse (Hoogstraten et al., 1960).

The distinction between A_1 and A_2 must be a fundamental one because even an extract of plant seeds can recognize it. It is entirely possible that a far greater number of distinctions exist, not only for A but for B as well, and each distinction could be gene-determined. Recent Japanese data of Ueno et al. (1959) concerning agglutination inhibition titers of human saliva with weak α and β agglutinin systems of animal origin, strongly suggest this possibility. Marked individuality, apparently ABO gene-determined, was described, and additional studies along these lines are indicated. The number of alleles at the ABO gene locus need not be any less than the number possible at the complex Rh locus.

The ABO antigens need not be determined on a one for one gene-antigen basis. When human mucopolysaccharides with A or B specific

cites were split by mild acid hydrolysis, new and different antigenic sites (P1) were described by Allen and Kabat (1958). These P1 antigens also appear to be ABO type specific and therefore determined by the same gene responsible for the normal external antigen.

Serology of the ABO Blood Group System

Significant problems exist in regard to human α and β antibodies. These isoagglutinins occur in at least two different physical forms with differing antigenic properties: γ_1 globulin antibodies with a molecular weight of about 1,000,000 and γ_2 globulin antibodies with a molecular weight of 160,000 (McDuffie et al., 1958). The γ_1 globulin antibodies, under ultracentrifugation, have a sedimentation constant (S_{19}) of about 19 Svedbergs, whereas γ_2 globulin antibodies have differing electrophoretic mobility and a sedimentation constant of only about 7 Svedbergs. The isoagglutinins of α and β specificity were separated readily into 7S and 19S fractions by cellulose ion exchange column chromatography by Abelson and Rawson (1959). Persons of type A and type B, however, appear to have very little α and β antibody activity in the 7S γ globulin fractions, in contrast to persons of type O who can have appreciable quantities of both varieties, although more in the 19S γ globulin fractions (Kochwa, Rosenfield and Tallal, 1960).

Dodd (1952) found that the serum of group O persons does not consist merely of α antibodies plus β antibodies, but a varying amount (often a very high proportion) of cross-reacting antibody, $\alpha\beta$. Wiener and Wexler (1958) have termed this anti-C (or γ). The rule that ABO isoagglutinins exist where the antigens are absent is not contradicted: persons of type O lack A and B and thus have serum antibodies of three specificities, α, β and $\alpha\beta$. This is consistent with other blood group systems, notably Rh, where the antibodies produced reflect the absence of antigens in the red cells, and cross-reactions are so frequent as to be expected phenomena (Race and Sanger, 1958). Wiener and Wexler invoke a C antigen with a distribution parallel to that of A antigen plus B antigen. In this context, the type O person, lacking A, B and C, possesses serum antibodies α, β and γ. One virtue of this hypothesis lies in the physical nature of the corresponding antibodies. While A and B antigens seem to provoke a 19S immune response, Rh usually provokes a 7S response (Abelson and Rawson, 1959). The C antigen thus differs from A and B in provoking a greater 7S response. The *OO* genotype could influence the physical nature of the A and B immune response in addition to the failure to produce A and B antigens (Lederberg, 1959).

Another possibility is that repeated antigenic insults are more likely to elicit smaller molecular weight antibodies (Kabat and Mayer, 1948). It is to be noted, though, that even early immune response to Rh seems to be of 7S character, whereas repeatedly immunized persons of type A and type B (in an effort to produce blood typing reagents of high titer) may fail to have large amounts of 7S antibody (Kochwa, Rosenfield and Tallal, 1960).

Serologic ABO Incompatibility

From the preceding discussion it can be concluded that the subject of ABO serologic incompatibility is complicated because it must be considered from a series of levels, each of which could contribute unequally to a problem under study. Some of these levels of consideration are as follows:

1. Any ABO incompatibility.
2. Incompatibility between type A and types B and AB, and between type B and types A and AB.
3. Incompatibility between type O and types that are not O.
4. Incompatibility between type O and subtype A_1, and between type O and type B.

There are, of course, many other possible combinations to be considered, but data pertaining to clinical effects are available for only these four.

Compatibility for purposes of blood transfusion is concerned with any ABO serologic differences. Since red cells are administered most frequently as whole blood, two forms of incompatibility can occur: that between the recipient serum antibodies and the donor red cells, and that between donor antibodies and the recipient's red cells. The former, or major side of the "cross-matching" test, is the more serious because the entire store of the patient's antibodies is available to react with the limited volume of infused donor red cells (Mollison, 1956).

Mollison (1956) states that the survival of small numbers of ABO incompatible red cells tagged with radioactive chromium (Cr^{51}) is usually less than 5 minutes for all common varieties of ABO serologic differences, and Jandl (1955) found the radioactive tag to be widely scattered in the body with a considerable amount in the liver. The same investigators found that Rh incompatible red cells would remain in the circulation for a longer time (ca. 20 minutes) and ultimately sequester appreciably in the spleen. This problem is reviewed by Mollison (1959).

Gershowitz et al. (1958) demonstrated α and β antibodies in the cervical sections of 17 out of 77 women tested; 15 were type O. The diffu-

sion of low molecular weight antibody of α, β and $\alpha\beta$ specifically is perhaps not unexpected, especially when type O persons are involved. It remains to be shown, however, whether these antibodies can have any effect upon sperm prior to conception, or upon the early products of an ABO incompatible conception.

The demonstration of α and β agglutinin activity in cord blood was observed by Hirszfeld (1928), but at no time were antibodies observed in the fetus which were lacking in the mother (Wiener, 1946). Rosenfield (1955) noted the regularity with which α and β antibody could be observed in the cord sera of type O infants of type O mothers. In a study of 275 specimens of ABO compatible umbilical vein blood, Rosenfield and Ohno (1955) found 169 cord sera to contain the isoagglutinin present in the mother. This material was divided into two classes: (1) O mother/O baby and (2) A or B mother/ABO compatible baby. In the first class, both α and β agglutinins were expected and were found in 90 per cent of instances, but in the second class only α *or* β agglutinins could be expected and the single specificity was observed in only 35 per cent of instances. These data are summarized in Table 3.

TABLE 3—*Maternal ABO Type and Occurrence of Maternal α and β Agglutinins in the Cord Serum of ABO Compatible Newborn* (After Rosenfield and Ohno, 1955)

Mother's ABO Type	Number of Cases	Maternal α and β Agglutinins Observed in Cord Serum
O	134	120
A or B	141	49
$\chi^2 = 87.3$; $P < 10^{-7}$		

Maternal α and β isoagglutinins activity could be encountered in the cord serum of any compatible newborn, but Rosenfield and Ohno (1955) observed it much more frequently where the mother was type O. In addition, the titer observed with cord serum only approached the titer found with the mother's serum when the mother was type O, and then only in about one-third of instances. The height of the maternal titer appeared to be unrelated to the finding of equality between maternal and fetal titration values.

This cord blood material was further tested by Rosenfield and Ohno to determine if the difference between type O mothers and mothers of types A and B, resided with a cross-reacting antibody ($\alpha\beta$) in the type O mothers. Thirty-seven cord sera of type O infants with type O mothers, each with a relatively high titer of α and β agglutinins, were divided

into two equal aliquots. One aliquot was absorbed until negative with type A_1 red cells, the other was absorbed until negative with type B red cells. In only eight of the 37 cord sera could residual separable α and β antibodies be demonstrated. This result, especially in view of the dilution entailed by repeated absorptions, is in agreement with the 49 of 141 cord sera containing α or β isoagglutinins from mothers of type A or B (Table 4). The essential differences between the newborn of type O mothers and the newborn of other mothers would thus appear to be placental passage of cross-reacting antibody ($\alpha\beta$) from type O mothers. Table 4 summarizes these results.

TABLE 4—*Separate and Cross-Reacting α and β Agglutinins in the Cord Blood Serum of Type O Infants* (After Rosenfield and Ohno, 1955)

Total cord sera with α and β agglutinating activity:	37
All isoagglutinins removed either with type A_1 or type B cells:	13
α isoagglutinins removed with type B cells	
β isoagglutinins not removed with type A_1 cells:	15
β isoagglutinins removed with type A_1 cells	
α isoagglutinins not removed with type B cells:	1
Separable α and β isoagglutinins present:	8

To determine the hematologic effects of transmitted maternal α and β isoagglutinins, umbilical vein blood specimens of almost 3000 newborn were tested by Rosenfield and Ohno for hemoglobin, reticulocytes and plasma bilirubin. Instances of atypical isoimmunization of the mother (e.g., Rh antibodies) were recognized by screening procedures and not included in the study. The infants were divided into three categories: (1) ABO compatible infants, (2) ABO incompatible infants, and (3) ABO incompatible infants with a positive direct antiglobulin test (presumably due to maternal antibody). The mean hemoglobin, reticulocyte count and plasma bilirubin values of the three classes are shown in Table 5, where it is obvious that ABO incompatibility, even in the absence of serologic evidence of antibody coating of the red cells, is associated with alteration of the cord blood hematologic values in the direction to be expected of a hemolytic syndrome in utero.

In this series, the newborn category with a positive direct antiglobulin test was found to be highly associated with mothers of type O. These data are summarized in Table 6.

The blood type of the infant was also found to play a role in the occurrence of a positive antiglobulin test, because Rosenfield and Ohno encountered a slight excess of type A (not subdivided into A_1 and A_2). This is shown in Table 7.

TABLE 5—*Effect of ABO Incompatibility on Mean Cord Blood Hematologic Values* (After Rosenfield and Ohno, 1955)

		Hemoglobin (Gm. %)	Reticulocytes (%)	Bilirubin (mg. %)
(1)	Compatible infants:			
	$\bar{x}_1$	16.05	4.34	2.11
	n_1	2256	2195	2193
(2)	Incompatible infants with negative direct antiglobulin test:			
	$\bar{x}_2$	15.82	4.53	2.22
	n_2	558	546	538
(3)	Incompatible infants with positive direct antiglobulin test:			
	$\bar{x}_3$	14.85	5.94	2.96
	n_3	89	88	82
	s $(\bar{x}_1\text{-}\bar{x}_2)$	0.08	0.087	0.037
	P $(\bar{x}_1\text{-}\bar{x}_2)$	0.004	0.029	0.003
	s $(\bar{x}_2\text{-}\bar{x}_3)$	0.203	0.23	0.112
	P $(\bar{x}_2\text{-}\bar{x}_3)$	< 0.00001	< 0.00001	< 0.00001

TABLE 6—*The Distribution of the ABO Phenotypes of Mothers of ABO Incompatible Infants, in Respect to a Positive Cord Blood Antiglobulin Test* (After Rosenfield and Ohno, 1955)

Blood Group of Mother	Direct Antiglobulin Test on Cord Red Cells				
	Negative		Positive		Total Number
	exp.	obs.	exp.	obs.	
O	359	329	57	87	416
A	115	131	18	2	133
B	84	98	14	0	98
all	—	558	—	89	647

$\chi^2 = 50.5$; $n = 2$; $P < 0.000001$

TABLE 7—*Distribution of the ABO Phenotypes of ABO Incompatible Infants of Type O Mothers, in Respect to a Positive Cord Blood Antiglobulin Test* (After Rosenfield and Ohno, 1955)

Blood Group of Infant	Direct Antiglobulin Test on Cord Red Cells				
	Negative		Positive		Total Number
	exp.	obs.	exp.	obs.	
A	238	227	63	74	301
B	91	102	24	13	115
all	—	329	—	87	416
$\chi^2 = 8.9$; $P < 0.005$					

ABO ERYTHROBLASTOSIS

The importance of cross-reacting antibody to the problem of ABO erythroblastosis was further substantiated by demonstration of this specificity in eluates from the red cells of involved infants (Haberman and Aguilar, 1957; Hubinont et al., 1960).

There are no data as yet to show that $\alpha\beta$ antibody might be a γ_2 globulin and responsible for erythroblastosis because of its small size (160,000 molecular weight) or some other unique physical feature. Franklin and Kunkel (1958) and Fudenberg et al. (1959) indicate that 7S isoagglutinins can be demonstrated in cord sera, but not 19S isoagglutinins.

Kochwa, Rosenfield, Tallal and Wasserman (1960) separated 7S γ globulins from other serum proteins, using diethyl aminoethyl (DEAE) cellulose in a modification of the column chromatographic method of Sober and Peterson (1958) and Levy and Sober (1960). In cord blood, ABO isoagglutinins were found only in 7S γ globulin fractions. Furthermore, the 7S γ globulins and the isoagglutinins in these fractions were found to be in equilibrium on both sides of the placenta when maternal and fetal sera obtained at the time of delivery were tested. Kochwa et al. (1960) did not find ABO isoagglutinins in the 7S γ globulin fractions of 28 type A mothers and nine type B mothers, but found varying quantities in 53 out of 73 type O mothers. Of the latter group, 10 type O mothers not only possessed high titer (1:20 to 1:160) 7S γ globulin ABO isoagglutinins, but delivered specifically incompatible offspring, one of which suffered from severe erythroblastosis and required exchange transfusion therapy.

When all 53 type O mothers were tested for the sensitivity of their

7S γ globulin isoagglutinins to be inhibited by specific soluble blood group substances of hog (A) and horse (B) origin (ibid.), only five were found to possess 7S γ globulin isoagglutinins resistant to inhibition. Four of these women delivered type O offspring, but one delivered a type B child, the same and only infant in the entire series to have clinical ABO erythroblastosis.

Kochwa et al. (1960) also studied six type O women with a history of having had children with severe ABO hemolytic disease; all six women were found to possess type specific 7S γ globulin isoagglutinins resistant to inhibition by soluble ABO substances. In addition, five unselected type O donors who had been immunized with soluble ABO substances of the same manufacture, were also found to have non-inhibitable 7S γ globulin isoagglutinins. Thus, not only do non-inhibitable 7S γ globulin antibodies seem to be well correlated with the occurrence of ABO disease, but they might be incited by the soluble blood group substances of the amniotic fluid at the time of delivery. This would permit some association of both disease and its severity with increasing parity.

Table 7 fails to give a true picture of the contribution of the ABO blood type of the fetus to the problem of erythroblastosis. Although Witebsky and Engasser (1949) showed that the serologic distinction between A_1 and A_2 is not clear at birth, the repeat blood typing at a later age of type A children with a history of erythroblastosis, revealed all but one of them to be subtype A_1 (Zuelzer and Kaplan, 1954a; Crawford et al., 1953; Wiener et al., 1960). In addition, the subtyping by the same investigators of the fathers of type A infants with erythroblastosis, revealed all but one to be subtype A_1. This distinction, therefore, is of considerable importance. Mollison and Cutbush (1959), in a review of ABO erythroblastosis, suggest that further subclassification when possible might furnish even more information. If A_2 were to be deducted from the data of table 7, increased heterogeneity would at least double the χ^2 value.

From our present knowledge, the risk of ABO erythoblastosis appears to reside to a very great degree with the type O mother and a fetus of subtype A_1 or type B. The frequencies of O, A^1 and B alleles differ in various populations (Mourant, 1954) but for Caucasians and American Negroes, $(\overline{O}^2) \times (\overline{A}^1 + \overline{B}) \times 100\% =$ ca 12 per cent. That is, if the frequency of type O $(\overline{O}^2)$ is multiplied by the sum of the frequencies of A^1 and B alleles, the value obtained (ca. 0.12) is very similar for Caucasians and for American Negroes. It is only slightly higher for Chinese and Japanese (ca. O.14).

Although 12 per cent of all newborn are subtype A_1 or type B, and

have type O mothers, only a small proportion have ABO erythroblastosis. Rosenfield (1955) and Hubinont et al. (1960) report up to 5 per cent of all newborn to have a weakly positive direct antiglobulin test due to ABO incompatibility and about 0.5 per cent of newborn to have clinical ABO disease. It is interesting to note that 0.5 per cent of clinical ABO disease has also been observed by Matsunaga (1959) in Japan. At The Mount Sinai Hospital for the four year period 1955-1958, 0.2 per cent of all live births received exchange transfusion therapy for this disease. Of 39 ABO infants in this period, only six required a second exchange transfusion, but of 53 Rh babies, 36 required from two to five separate exchange transfusions. ABO erythroblastosis tends to be a more self-limited disease than its Rh counterpart.

There are no data for the frequency of neonatal death or permanent neurologic sequelae due to untreated ABO disease, but the figure would probably not exceed 0.2 per cent of live births for a mixed population such as that seen in New York City. Whatever the figure, however, it might prove relatively constant.

ABO disease occurs very often in the first incompatible pregnancy, and is not necessarily associated with increasing severity in subsequent pregnancies. Wiener et al. (1960) studied 51 families where one or more children had ABO erythroblastosis; only 19 disclosed older incompatible siblings without evidence of the disease, but 28 families appeared to have erythroblastosis in the first incompatible offspring. This is quite different from the pattern of Rh disease.

Maternal antibody studies by Zuelzer and Kaplan (1954b) failed to reveal significant titer rises of α and β isoagglutinins in response to ABO incompatible pregnancy. Zuelzer and Kaplan (ibid.) also studied post-partum maternal titers. In one-third of the cases, where the newborn was not only ABO incompatible but also a secretor of ABO soluble blood group substances, significant increases in maternal titer were observed. No changes in maternal titer were noted, during or after pregnancy, when the incompatible infant was a non-secretor of soluble ABO blood group substances.

Passage of red cells from fetus to mother is a common event, both during pregnancy and at the time of delivery. Wiener et al. (1951) have estimated that anamnestic increase in Rh antibodies occurs in one-third of Rh incompatible pregnancies, and in an additional one-third of instances after the delivery of an Rh incompatible child. Therefore mothers of ABO incompatible infants must be exposed to fetal red cells, but an α and β anamnestic response is not elicited. However, at delivery, embolization of mothers with varying quantities of amniotic fluid could,

and evidently does, provide antigenic challenges when the offspring are secretors of soluble ABO blood group substances. Since maternal titer increases are observed only in the post-partum period, it is not likely that sufficient amounts of fetal ABO mucopolysaccharides traverse the placental barrier during gestation to constitute an antigenic challenge to the mother.

ABO incompatibility is strongly protective against other varieties of isoimmunization, notably Rh (Levine, 1958). The mechanism of this protective effect may explain the failure of Zuelzer and Kaplan to observe α and β titer increases during the course of an ABO incompatible gestation. If ABO incompatible red cells are rapidly sequestered (Mollison, 1959) by reticuloendothelial structures which cannot synthesize antibody protein (e.g., Kupfer cells of the normal liver), such fetal red cells would fail to elicit either α, β or Rh antibodies.

Increasing severity of hemolytic disease in successive ABO incompatible newborn is observed but not as regularly as with Rh incompatibility. From the data of Wiener et al. (1960), the clinical severity of hemolytic disease seemed to remain unchanged in 10 families, but to increase in severity in 20 families. This irregularity in the occurrence of increasing severeity in ABO hemolytic disease might be due in part to restriction of some anamnestic post-partum responses to antibodies which would not diffuse across the placenta (Franklin and Kunkel, 1958), or which could be neutralized readily by non-erythrocytic antigens in the fetus.

ABO erythroblastosis is often marked by striking hematologic changes (Levine et al., 1953). In infants at The Mount Sinai Hospital who require only a single exchange transfusion for adequate management of hemolytic disease, the cord blood values in ABO erythroblastosis show greater anemia, more reticulocytes, and higher plasma bilirubin than is to be observed in Rh disease. This is shown in Table 8.

TABLE 8—*Mean Cord Blood Hematologic Values of Infants Adequately Treated by Single Exchange Transfusion; Comparison of the Findings in ABO and Rh Erythroblastosis*

	Hemoglobin (Gm. %)	Bilirubin (mg. %)	Reticulocytes (%)
ABO hemolytic disease	11.10	5.02	16.4
Rh hemolytic disease	13.42	4.23	12.5
P (Rh-AB)	.026	.4	.24

Soluble A and B substances do not react equally well with all kinds of human α and β agglutinins, and insufficient inhibition of 7S γ globu-

lin agglutinins seems to be associated with ABO erythroblastosis (Kochwa et al., 1960). Some low molecular weight isoagglutinins from type O mothers not only traverse the placenta freely but agglutinate erythrocytes in the presence of large amounts of soluble ABO substances. Since soluble blood group substances may incite these same antibodies (ibid.), the mechanism of non-inhibition may be related to the formation of soluble reversible complexes which liberate small amounts of antibody to agglutinate specific erythrocytes.

In addition, ABO erythroblastosis is characterized by marked spherocytosis (Grumbach and Gasser, 1948). Altered erythrocyte morphology may be observed under a variety of conditions, but one cause was shown by Bessis (1956) to be agglutination followed by mechanical disruption of the agglutinates into discrete cells. Isoagglutinins of α, β and $\alpha\beta$ specificity are agglutinating antibodies under a wide variety of circumstances, including conditions existing in the fetal circulation. Rh antibodies, especially those encountered in the newborn, are not agglutinating in a fetal plasma menstruum, and rarely produce spherocytes (Levine et al., 1953).

A final distinction between ABO and Rh erythroblastosis is the increased tendency of the former to be self-limited. This may be related to the distribution of blood group substances in the body. Whereas Rh substance is restricted to the erythrocyte, ABO substances occur in mucopolysaccharides either secreted or non-secreted (Hartmann, 1941). Thus maternal Rh antibody can be bound only by an antigen-antibody reaction occurring at the Rh positive erythrocyte, but α and β maternal antibody can encounter antigen under a variety of circumstances in the incompatible fetus.

Soluble A and B substances need not react equally well with all kinds of human α and β agglutinins. Described distinctions have been discussed by Kabat (1956), and these may play a role in the course of ABO disease. Hypothetically, 7S $\alpha\beta$ antibody might be responsible for a much more active hemolytic syndrome if these antibodies reacted preferentially with erythrocytic A or B substance rather than the water soluble non-erythrocytic variety.

Three studies have revealed the following distribution of secretor status in infants with ABO erythroblastosis:

	Secretor	Non-secretor	Total
Zuelzer and Kaplan (1954a)	25	3	28
Crawford et al. (1953)	11	0	11
Wiener et al. (1960)	51	7	58
Total	87	10	97

This indicates an excess over the expected 80 per cent secretor frequency. Wiener's studies included secretor status of the parents of involved infants:

	Secretor	Non-secretor	Total
Mothers	31	12	43
Fathers	41	5	46
Both	72	17	89

The incidence of secretors among mothers of involved infants seems to be normal or only slightly low, but there are too many secretor fathers and offspring. The non-homogeneity of secretor status between mothers and fathers is at the 4 per cent level of significance. A larger study will be required, however, before any firm conclusions may be drawn.

Fetal Loss in ABO Incompatibility

Hemolytic disease of the newborn due to ABO incompatibility is thus seen to be a serious medical problem, second only to that due to Rh incompatibility. It is also a social problem in terms of the anguish of parents of seriously affected, and particularly of untreated, children. However, from a biological point of view, it appears to be of minor significance. The incidence of death or serious disability due to ABO erythroblastosis is low, being perhaps two per 1000, as we have seen above. The effect of natural selection of this order of magnitude on the frequency of the A, B and O genes will be small. On the other hand, there is some evidence for a loss of potentially incompatible children at an earlier stage than that involved in hemolytic disease. This loss may involve stillbirth, late or early abortion or even failure of fertilization or implantation. From the social point of view, since most of this presumed loss is early, it is much less serious than erythroblastosis unless it results in sterility. But from the biological point of view this loss is probably much more frequent and hence more important. The evidence for the earlier losses is quite diverse and sometimes contradictory. It will now be reviewed in some detail.

Blood Types of Children in Reciprocal Matings

The most unequivocal evidence concerns the deficiency of A and B children relative to O children in matings of A and B fathers and O mothers versus reciprocal matings of O fathers and A and B mothers. In the theoretical discussion, only A and O will be considered to save circumlocution. Everything said applies equally to B.

Table 9 represents the expected numbers of children in reciprocal matings.

TABLE 9—*Expected Numbers of Children in Reciprocal Matings*

	Number of children		
Mating M F	O	A	Total
A x O	*cn*	*racn*	$(1 + ra)\ cn$
O x A	*n*	*an*	$(1 + a)\ n$

Here n depends on the number of incompatible families and their basic fertility and cn depends on the number of compatible families and their basic fertility. In addition to the difference in number of families, c is affected by differences of fertility and fecundity between reciprocal matings due to genotype of mother and of father, as well as to incompatibility per se; a represents the ratio of A to O children in compatible matings and depends on the relative frequency at conception and possible differential survival of the two genotypes, and r represents the additional effect of the incompatible matings on A offspring.

$$\frac{\text{The cross ratio}}{\frac{(\text{A incompatible}) \times (\text{O compatible})}{(\text{A compatible}) \times (\text{O incompatible})}} = \frac{(\text{rcan}) \times (\text{n})}{(\text{cn}) \times (\text{an})} = \frac{\text{rcan}^2}{\text{can}^2} = \text{r},$$

so that r is the ratio of the ratio of A to O in incompatible matings to the ratio of A to O in compatible matings. The cross ratio for the actual data is an estimate, $\hat{r}$, of r. The variance of $\log_{10} \hat{r} = z$ is

$$\sigma_z^2 = 2.3026 \left(\frac{1}{\text{O compatible}} + \frac{1}{\text{O incompatible}} + \frac{1}{\text{A compatible}} + \frac{1}{\text{A incompatible}} \right).$$

Confidence limits for z can then be obtained, for example as $z \pm 1.96\ \sigma_z$ for 95 per cent confidence coefficient, and corresponding confidence limits for r by taking the antilogarithm of the confidence limits for z. The method is discussed in more detail by Woolf (1955), and Haldane (1956). (Haldane erroneously gives the variance of his modified z under the null hypothesis as $\frac{(H+h)(K+k)}{Nn(N+n)}$ in his notation. The value should be $\frac{(N+n)^3}{nN(H+h)(K+k)}$).

The combined data of Waterhouse and Hogben (1947), with a minor correction by Bennett and Brandt (1954), are given in table 10.

TABLE 10—*Waterhouse and Hogben data. All Families* (After Bennett and Brandt)

	Number of children			Number of matings
Mating M F	O	A	Total	
A x O	302	386	688	210
O x A	320	515	835	244

The cross ratio is .794, representing a loss of 1–.794 = .206 or 20.6 per cent of all A children in A ♂ × O ♀ matings. The 95 per cent confidence interval for r is .65 to .98, and for the percentage loss is 35 to 2. That is, we estimate that this loss is 20.6 per cent with 95 per cent confidence that its true value lies between 2 per cent and 35 per cent. Since these limits do not include zero, we reject the hypothesis that there is no differential loss at the 5 per cent level of significance. This hypothesis could also be tested by the usual χ^2 test for independence in a fourfold table with a closely similar result.

The above statistical analysis would be completely valid if table 10 represented homogeneous data. Actually it represents the combination of 12 studies, with possible different gene frequencies and fertilities. On the assumption that the desired parameter r is constant, or that we want to estimate its average value, the cross ratio for the combined table is correct if *either* c is constant for all studies *or* a is constant for all studies. Waterhouse and Hogben suggested confining attention only to those matings that segregated (that is, have both O and A offspring), thus proving that the A parent was heterozygous, *AO*. For such matings, making the reasonable assumption that differential mortality is absent or slight in compatible matings, a will equal ½ and the analysis will be valid. Table 11 gives the data for the segregating matings. The cross ratio

TABLE 11—*Waterhouse and Hogben data. Segregating Families* (After Bennett and Brandt)

	Number of children			Number of matings
Mating M F	O	A	Total	
A x O	254	209	463	114
O x A	270	278	548	134

is now .799, giving a loss of 21 per cent with 95 per cent confidence limits of -8 per cent (a loss in *compatible* matings) to 31 per cent, so that there is no significant difference from zero. Waterhouse and Hogben did not compute the cross ratio directly and did not notice that Table 11 is seriously biased.

Our last analysis would be correct if we chose *a priori* those matings where the A parent was heterozygous. In fact we chose those matings that segregated. Each such mating necessarily contains one A and one O child, and it is only the remaining children who give information about r. Bennett and Brandt noted the fact but did not pursue it directly. It has the effect of diluting the estimated r by making the number of A and O children artificially close. A completely valid analysis can be made by subtracting from each family the one A child and one O child that proved it segregated and considering only the remainder. This is most easily done by subtracting as many A children and as many O children from the compatible families as there are matings of this type, and doing the same for the incompatible matings. The result is Table 12.

TABLE 12—*Waterhouse and Hogben data. Segregating Families. With One A and One O Child Removed from Each Family*

	Number of children			Number of matings
Mating M F	O	A	Total	
A x O	140	95	235	114
O x A	136	144	280	134

For this table the cross ratio is .641. The estimated loss of A children from incompatible matings is then 35.9 per cent with 95 per cent confidence limits of 9 per cent and 55 per cent, and is statistically significant. Incidentally it is mathematically necessary that the loss estimated after removal of the children that proved segregation should be more extreme than before, but it is an accidental property of these data that the estimate of r for segregating families including all children should be so close to that for all families. In fact, if the data were homogeneous the estimates from Tables 10 and 12 should be in general agreement and different from that from Table 11. However, where heterogeneity is likely, as is true here, the estimate from Table 12 is preferable.

Bennett and Brandt introduced a measure of the deficiency for segregating families, θ, equal to the proportion of O children minus the proportion of A children in a given kind of mating. The odds for A are then $x = (1-\theta)/(1+\theta)$. If x_c is the value for compatible and x_i for incom-

patible matings, $r = x_i/x_c$, while $1-x_i$ is the deficiency of A children in incompatible matings, including both the loss due to incompatibility and any differential loss of A children per se (which would be compensated for in $1-r$). For the Waterhouse and Hogben combined data x_c is nearly one, so that x_i is close to r, and we will use the letter r for x_i in what follows to facilitate comparison with the other results. Bennett and Brandt developed an efficient test of the hypotheses that there is no selective loss, by testing whether $\theta = 0$, but their method does not give an efficient estimate of θ (or of r) when r is not zero. For the combined data they found r significant but found significant heterogeneity in values of r and so concluded that the χ^2 test of significance of r is invalid. They then use an F test that is not significant. However, Bennett and Brandt load the dice two ways. First, it can be shown that the F test gives an (inefficient) estimate of the loss, $1-r$, of 16.7 per cent with confidence limits of –53 per cent and + 56 per cent, so that this test neither confirms nor denies the existence of an effect. They also remark that if source 9 (with 5 families) and source 10 (with 11 families), together accounting for about 10 per cent of the information, are removed, the remaining families are homogeneous and do not give a significant r. This is true, but the only apparent reason for omitting these two specific families is that they give this result. If two sources are to be removed, it is more reasonable to remove sources 9 and 7 (with 7 families) since these have the most extreme θ's (+.89 and –.89 respectively) and contribute most to the heterogeneity χ^2. The heterogeneity χ^2 is then 10.3 with 9 degrees of freedom and the estimated loss is 25 per cent and significant. Even more reasonable is to retain only sources giving more than 10 units of information in Table 5 of Bennett and Brandt. This retains sources 1, 2, 3, 6, 8, 10 and 12, containing 88 per cent of the total information. The heterogeneity χ^2 is then 7.08 with 6 degrees of freedom and not significant, while the estimated loss is 33 per cent with 95 per cent confidence limits of 10 per cent and 46 per cent, in quite good agreement with our direct results from Table 12 using the combined data.

Since the paper of Bennett and Brandt two further studies have appeared by Matsunaga (1953, 1955) and Johnstone (1954). From the published data it is not possible to separate the segregating families; on the other hand these studies are more homogeneous and hence the total data may be treated. Matsunaga's paper reports on a series of studies, mostly done on hospital deliveries by Furuhata (1932; cited by Matsunaga, 1959) over a period of years. Johnstone's paper also deals with a random series of deliveries in a hospital. All three authors consider matings A ♂ x O ♀ vs. O ♂ x A ♀ and B ♂ x O ♀ vs. O ♂ x ♀ separately. Waterhouse

and Hogben did not divide the B families into segregating and non-segregating, presumably because of the small numbers involved; because of possible heterogeneity their B data are not included here. The data of Matsunaga and of Johnstone are given in Table 13.

TABLE 13—*Data of Matsunaga and of Johnstone*

	Matsunaga				Johnstone			
Mating M F	Number of children			Number of families	Number of children			Number of families
	O	not-O	Total		O	not-O	Total	
A x O	320	369	689	313				
B x O	190	204	394	165	258	331	589	583
O x A	282	430	712	301				
O x B	142	214	356	158	229	355	584	584

Because of the small number of matings involving B in Johnstone's data his two series are combined. Table 14 gives the estimated loss, with lower and upper 95 per cent confidence limits, for each survey separately, as well as weighted averages for the Japanese data and for all the data. The weighted average can be used validly to combine even heterogeneous data as long as the true values of r are comparable. Matsunaga's data on B are barely significant because of small numbers, but they are in agreement with the values for A. Johnstone's data are not significant because of small numbers and an apparently smaller loss, but the estimate of r is well within the bounds of chance variation from the general average. The estimates of r for the other studies and the weighted average of r are significant. Taking all the studies together it appears that there is a loss of between 14 per cent and 32 per cent of all A or B children from matings of an A, B (and presumably AB) father and an O mother, as compared to the reciprocal mating, and that the most likely value for this loss is about 25 per cent.

The beauty of the above method of analysis is that it is completely self contained. In the absence of incompatibility effects, the number of A ♂ x O ♀ and O ♂ x A ♀ matings should be equal, they should have equal numbers of children, and the relative frequency of A children should be the same in both classes of matings. If other types of matings such as compatible A x A and incompatible A x B are considered, the expected number of such matings and the relative frequencies of the different ABO types in the offspring will depend on the gene frequencies in the population. Heterogeneity of gene frequency will now be quite serious. Johnstone (1954) has calculated expected numbers for each type of offspring in

TABLE 14—*Deficiency of Incompatible Children Born to O Mothers Compared with the Number Derived from the Reciprocal Matings* (Estimated Deficiency in Bold Face Type)

Data	No. of children	z	σ_z	r	Percentage deficiency lower 95% limit	estimate	upper 95% limit
Waterhouse and Hogben[1]	515	−.4449	.1778	.641	9	**36**	55
Johnstone[2]	1173	−.1893	.1186	.828	−5	**17**	34
Matsunaga[3]	1401	−.2794	.1082	.756	6	**24**	39
Matsunaga[4]	750	−.3391	.1640	.712	2	**29**	48
Matsunaga weighted average[5]	—	−.2975	.0903	.749	11	**25**	38
All data weighted average[5]	—	−.2838	.0667	.757	14.2	**24.7**	32.4

Note: The father is listed first in each mating.

[1] (A x O) vs. (O x A) segregating matings with one A and one O child removed from each mating.

[2] [(A x O) + (B x O)] vs. [(O x A) + (O x B)]

[3] (A x O) vs. (O x A)

[4] (B x O) vs. (O x B)

[5] Average of z values weighted by $1/\sigma_z^2$

all possible matings and compared them with the numbers actually observed. He does not state the source of the gene frequencies for this calculation. Considering only A ♂ x O ♀ versus O ♂ x A ♀ matings he found close agreement between observed and expected for the incompatible mating, and a deficiency of O relative to A in the compatible mating. He confirmed this surprising result for the data of Waterhouse and Hogben, and of Hirszfeld (1928) (largely included in Waterhouse and Hogben). On the other hand the data of Matsunaga, which were unknown to Johnstone, show agreement for compatible matings and a deficiency of A (and B) in the corresponding incompatible matings. Furthermore the data of Waterhouse and Hogben for *segregating* matings (see discussion above and Table 12) also show approximate equality of A and O children for compatible matings and a deficiency of A children in incompatible matings. Other types of matings are not published by Matsunaga or Waterhouse and Hogben, and Johnstone's series is the smallest of the three in number of children. However, examining Johnstone's data shows that other types of incompatible matings capable of producing O and not-O children (A x B and B x A) give 40 O observed to 38 expected, while other compatible matings giving O and not-O (A x A and B x B) give 80 O observed to 92 expected. These numbers are too small to be decisive, and several conclusions are possible. First the results may be due to chance. Second they may be due to heterogeneity and incorrect values for the gene frequencies used in calculating the expected values. Finally it is possible that O children have a lowered viability that may appear in compatible matings, but be masked or reversed by selective loss of A and B children in incompatible matings.

Fertility and Fecundity

If there actually is a loss of incompatible children as indicated above, it might be expected to cause lower fertility or fecundity of incompatible compared with compatible matings. Let us first consider fertility in the sense of presence of children. There are a few small and largely unpublished studies suggesting a higher frequency of incompatible matings among couples sterile for no apparent medical cause, but more must be done before this can be considered. If incompatible matings are more often sterile or produce fewer children, then in a sample of matings chosen because they had at least one child we could expect to find a deficiency of incompatible matings. To avoid problems about gene frequencies, only the reciprocal matings X x O and O x X will be con-

sidered, where X may stand for A, B, or AB. The available large studies are Waterhouse and Hogben (1947), Matsunaga (1953, 1955), Johnstone (1954), Matsunaga and Itoh (1953, 1958), and Reepmaker (1955). The data are given in Table 15.

Combining all the data we estimate that $(2237 - 2087)/2237 = .067$ of all incompatible matings are unrepresented. There is no statistically significant heterogeneity between the studies ($\chi^2 = 10.6$ with 5 degrees of freedom, $P = .06$). The deficiency of incompatible matings is significant ($t = 2.28$, $P = .023$) and 95 per cent confidence limits are .010 and .121. It should be noted that this deficiency, even if real, exceeds the amount of sterility due to incompatibility, because it represents both sterile matings, and less fecund matings which have less chance to be represented in the sample since they may have no children during the period under study.

Turning our attention to fecundity as measured by number of live births per family, we find contradictory data. Matsunaga and Itoh (1953), as reported in Matsunaga (1955), studied about 2000 clinic multiparae delivered at a university hospital. They obtained histories of number of pregnancies, number of miscarriages and number of "normal deliveries," (possibly including stillbirths). Matsunaga and Itoh (1958) studied 1429 random couples in two mining towns, testing them for ABO and other blood types, and obtained histories of number of pregnancies, miscarriages, and "living children." It is not clear whether "living children" means live births or children living at the time of the investigation; the total figures are 1429 matings with 4567 pregnancies, 568 miscarriages, and 3449 "living children." By subtraction there are 550 pregnancies unaccounted for, representing stillbirths and possible infant and childhood deaths. Reed and Kelly (1958), in a follow-up study of 161 couples who had essentially reached the end of their reproductive period, reported parental blood groups, number of pregnancies, abortions, stillbirths, and live births. Reed and Ahronheim (1959) reported on 558 couples of known blood groups, and the number of children living at the same address as determined by a city directory. The figures from this study are obviously of little value for most purposes; however there should be no bias in the comparison of compatible and incompatible matings.

Up to this point it has been advisable to restrict ourselves to the reciprocal matings X x O and O x X. This avoided problems about gene frequency involved in expected numbers of different types of matings and the distribution of blood types of the children within a mating. However in studying fecundity, abortion rate, etc., this restriction is unnecessary, since we compare total number of live births for a particular

TABLE 15—*Comparative Number of X x O and O x X Fertile Matings from Various Sources*

Source	Waterhouse and Hogben	Matsunaga	Matsunaga and Itoh 1953	Matsunaga and Itoh 1958	Johnstone	Reepmaker	Total
Nature of X	A	A+B	A+B	A+B+AB	A+B+AB	A+B+AB	A+B+AB
Number of X♂ x O♀ matings	210	478	290	246	616	247	2087
Number of O♂ x X♀ matings	244	459	368	264	615	287	2237
Total	454	937	658	510	1231	534	4324
% X♂ x O♀	46.2	51.0	44.1	48.2	50.0	46.3	48.27
% loss of incompatible matings	13.9	−4.1	21.2	6.8	−0.2	13.9	6.7

mating class, with the actual number of matings of that type. Furthermore, whereas ABO hemolytic disease seems to involve mainly O mothers, the available evidence indicates that whenever other types of loss of offspring occur, they do so for all types of incompatibility. Thus we will for the most part now consider all incompatible matings versus all compatible matings whenever the figures are available. Table 16 summarizes the fecundity data. Usually the variance of family size is greater than the mean, reflecting more than Poisson variation. Reed and Kelly have a smaller value, but a small sample, Reed and Ahronheim a very large value as would be expected from their method of ascertainment, and Matsunaga and Itoh (1958) with a large sample have the variance 1.4 times the mean. In the absence of a better value, the same ratio of 1.4 is used for Matsunaga and Itoh (1953) and Waterhouse and Hogben.

The complete data of Matsunaga and Itoh (1953 and 1958) show a highly significant deficiency of children in incompatible matings, Waterhouse and Hogben's data show no difference, Reed and Kelly's data show a non-significant excess, and Reed and Ahronheim's data show a significant excess for incompatible matings. The confidence limits show that all but the somewhat suspect data of Reed and Ahronheim are compatible with a considerable deficiency in incompatible matings. On the other hand there is apparent heterogeneity, with only the Japanese data giving unequivocal evidence of this deficiency. It may be noted that most of the studies show a small family size. Reed and Kelly state that all but 10 of their couples used contraception. If family size is consciously limited, a considerable fetal loss could be compensated easily and the desired final family size achieved. It is possible that such compensation might be less common in Japan, or not yet be complete, if the couples were younger in the Japanese studies. Accordingly, we will take as a temporary working hypothesis a loss of about 0.5 children per incompatible mating. This supposed loss may be further checked by considering the incidence of miscarriages.

Three studies give the incidence of miscarriages in random matings classified as to mating type. These are Matsunaga and Itoh (1953), Matsunaga and Itoh (1958), and Reed and Kelly (1958). Abortion rate can be given either as average number of abortions per mating or average number of abortions per pregnancy. It is not clear which is preferable. The number per pregnancy gives the force of selection in purer form; on the other hand if couples with many abortions should as a consequence have more pregnancies, until a given number of live births is achieved, the average per pregnancy may show less striking effects than

TABLE 16—*Number of Offspring (as Defined in the Text for the Various Studies) for Incompatible and Compatible Matings*

Author	Average No. per family	Variance per family	Average incomp.	Average comp.	Difference incomp.-comp.	Standard error of difference	95% Confidence limits of difference	
Matsunaga and Itoh 1953[1]	2.61	3.65[5]	2.34	2.88	−.54	.15	−.83	−.25
Matsunaga and Itoh 1958[2]	2.38[6]	3.20[6]	2.21	2.44	−.23	.15	−.53	+.07
Matsunaga and Itoh 1958[3]	2.38	3.20	2.17	2.60	−.43	.095	−.62	−.24
Waterhouse and Hogben[4]	3.34	4.68[5]	3.33	3.34	−.01	.13	−.17	+.15
Reed and Kelly[3]	2.47	1.65	2.54	2.39	+.15	.23	−.31	+.61
Reed and Ahronheim[3]	1.79	4.72	1.96	1.69	+.27	.12	+.03	+.51

Note: The father is listed first in each mating.

[1] [(A x O) + (B x O)] vs. [(O x A) + (O x B)]

[2] [(A x O) + (B x O) + (AB x O)] vs. [(O x A) + (O x B) + (O x AB)]

[3] All incompatible vs. all compatible

[4] (A x O) vs. (O x A)

[5] Variance calculated as 1.4 times mean (see text).

[6] Taken from the total data (line 3).

TABLE 17—*Number of Miscarriages for Incompatible and Compatible Matings*

Author	No. per preg. incomp.	No. per preg. compat	No. per mating incomp.	No. per mating comp.	Difference per mating inc.-comp.	Standard error of difference	95% Confidence limits of difference	
Matsunaga and Itoh 1953[1]	.176	.111	.500	.361	+.139	.074[4]	−.00	+.28
Matsunaga and Itoh 1958[2]	.162	.103	.527	.309	+.218	.075[4]	+.07	+.37
Matsunaga and Itoh 1958[3]	.153	.103	.478	.336	+.142	.049[4]	+.05	+.24
Reed and Kelly[3]	.198	.161	.659	.510	+.149	.201	−.42	+.72

Note: The father is listed first in each mating.

[1] [(A x O) + (B x O)] vs. [(O x A) + (O x B)]

[2] [(A x O) + (B x O) + (AB x O)] vs. [(O x A) + (O x B) + (O x AB)]

[3] All incompatible vs. all compatible

[4] Variance per family calculated as 2 times mean (see text).

the average per mating. Since matings may show habitual abortion for many causes other than ABO incompatibility, the mating is the most natural unit for statistical treatment. The variance of number of abortions per mating may then be calculated directly from the data. This was done by Reed and Kelly, who found the variance to be two times the mean, compared to a variance of live births in their data that was less than its mean. Matsunaga and Itoh (1958) use χ^2 tests and variances based on the binomial distribution for the proportion of abortions per pregnancy. This is not valid when there is heterogeneity between matings. Since they do not give the variance per mating, it has been arbitrarily taken here as twice the mean. Matsunaga (1955), quoting Matsunaga and Itoh (1953), gives a standard error for the mean number of miscarriages per mating, but it is not clear how it is calculated, so it has been recalculated here on the same basis. Table 17 gives the data and their statistical analysis.

Both the Japanese and American studies show more miscarriages in incompatible matings, although small numbers make the Reed and Kelly study compatible with the actual existence of either a large deficiency or a large excess. Comparison of Tables 16 and 17 shows that the more informative Japanese data might permit the loss of live births or live children to be due to abortion, but that it is more likely that abortion explains only part of the loss. The remaining loss might then be due to perinatal death or to abortion so early it was not recognized. In fact Matsunaga and Itoh (1958) show perinatal death (by subtraction) to be .473 for incompatible and .318 for compatible. Nevertheless the excess of abortions in incompatible matings, both those involving and not involving O mothers, reinforces the general conclusion of selective fetal loss.

In addition to the direct studies of frequency of abortion in unselected populations, there are a number of indirect studies on the percentage of incompatible matings in a group of matings where there has been frequent abortion or fetal death. Although such indirect studies are never as satisfactory as direct ones, they are frequently useful, and often the only way of obtaining evidence on some issue. A number of these studies are collected in Table 18.

Combining all of them, there are 878 matings of which 42.6 per cent are incompatible, compared with an expectation, in random European matings, of about 35 per cent. This excess of incompatible matings with abortion would be highly significant if the data were homogeneous ($\chi^2_1 = 22.3$). In fact the figures for individual studies range from 25.6 per cent to 58 per cent incompatible, and in spite of the small size of some of the series, the heterogeneity is highly significant ($\chi^2_8 = 41.4$).

TABLE 18—*Percentage of Incompatibility in Series of Habitual Aborters*

Study number	1	2	3	4	5	6	7	8	9	Total	Random
Number of Cases	133	43	269	41	60	89	115	43	85	878	—
% Incompatible	25.6	28	37.9	41	45	53	54	56	58	42.6	35

1. Van Loghem (1951) more than 2 abortions (quoted by Reepmaker 1955).
2. Race and Sanger (1950) 2 or more abortions.
3. Grubb and Sjöstedt (1955) 2 or more abortions.
4. Levine (1943) (from Paroli, and Toronquilli-Leali) 2 abortions or stillbirths.
5. Levine, Buxton, Steer and others, unpublished, habitual aborters.
6. Wiener, Wexler, and Hurst (1949) 2 or more abortions.
7. Levine (1943) 2 or more abortions.
8. Levine (1943) 2 abortions or stillbirths.
9. McNeil et al. (1954) 2 or more abortions.

By far the most important contribution to the heterogeneity is a value of 15.7 for the study of van Loghem, the next largest value being 7.9 for McNeil et al. If the van Loghem study is omitted, the over-all incompatibility is 47.4 per cent, ($\chi^2_1 = 47.6$). Heterogeneity is still significant ($\chi^2_7 = 24.8$, $.0005 < P < .001$) but is now smaller, and the F ratio of the two χ^2 values is 13.4 and significant at the 1 per cent level. It appears that the four studies with the lowest frequency of incompatible matings are from Europe, and the remaining five studies are from the United States, but these are all populations of European origin (except possibly for some Negroes in studies 6, 7 and 8), and this difference must be presumed to be accidental. In the presence of so much heterogeneity from unknown causes we may only say that, taken with the previous findings, these studies may indicate some over-all tendency for ABO incompatibility to increase the risk of abortion.

OTHER TYPES OF EVIDENCE

There have been several studies involving the ABO blood types of random mothers and infants, without the blood types of the fathers. Since O mothers may be expected to be involved in incompatible matings more often than other types, the fertility and fecundity of O mothers may be expected to be somewhat lower for this cause than that of mothers of other types. However, the effects found in incompatible matings were small, and since nearly half of the O mothers will be mated compatibly, the effect would be small and hard to measure. On the other hand the putative loss of 25 per cent of all non-O children of O mothers estimated above, is large enough to be noticed in the present studies. One

of the largest of such published studies is that of Kirk et al. (1955). From O mothers they observed 5334 O children and 2251 not-O children. Taking the gene frequency of *O* as .68 for a largely British population, the frequency of not-*O* is 1–.68 = .32. Thus 32 per cent of all children of O mothers should be not-O, or there should be .32/.68 = .47 not-O children for each O child. Now .47 (5334) = 2507, giving an observed deficiency of 2507 – 2251 = 256, or a loss of 256/2507 = .10 of all not-O children of O mothers. This calculation is exceedingly sensitive to the gene frequency assumed; for a frequency of .67 the loss would be .14 of all not-O children.

Another feature of the data of Kirk et al. (1955) is a deficiency of AB children compared to AB mothers. This could easily be due to misclassification of AB infants. Reed (1956) using more sophisticated mathematical techniques assumed that fetal loss was confined to O mothers and that some AB children were misclassified. Using all of the data of Kirk et al. he estimated the loss of not-O fetuses as 6.4 per cent and the misclassification of AB infants as 16 per cent. Reed also considered a model where there was an identical loss rate of all incompatible fetuses regardless of maternal blood type, and found a poorer fit, although the difference is not as striking as claimed. Reed also mentions an unpublished study of Haberman et al., with 37,814 mother-child pairs, with a calculated 9 per cent selection against A fetuses in O mothers. Smaller studies of Boorman (1950) and of Bennett and Walker (1956) show no significant effect. The study of Bennett and Walker does not give the blood type of the fathers or of the infants, and may be criticized on other grounds.

Finally it may be mentioned that several studies have shown peculiarities in sex distribution of children classified by mother-child blood type combinations. This material is still too contradictory, and possible explanations too nebulous, to make discussion in this review profitable. Nevertheless this may eventually prove to be an important problem. A review of most of this work is given by Edwards (1957).

Interaction of ABO and Rh Incompatibility

Levine (1943) noted a lower rate of ABO incompatibility in matings showing hemolytic disease due to Rh. A number of further studies are summarized by Levine (1958) and there is also a paper by Clarke et al. (1958). The general picture is that immunization to Rh (D) occurs only in pregnancies where the ABO group of the fetus is compatible with that of the mother. Race and Sanger (1950, p. 235) suggested that moth-

ers are sensitized to Rh by fetal erythrocytes which leak through gaps in the placental villi, and that ABO incompatible red cells do not survive long enough in the maternal circulation to have this effect. The most direct evidence comes from reciprocal matings AB ♂ x O ♀ and O ♂ x AB ♀, since all offspring of the first mating are incompatible. Out of 3123 matings with Rh hemolytic disease collected by Levine (1958) from the literature, there were 59 O x AB matings and only 2 AB x O matings, in one of which the mother might have been immunized by a previous transfusion. Clarke et al. (1958) report that in 14 cases where the immunizing fetus could be determined with certainty, it was always compatible. Thus it seems clear that *immunization* to Rh, with rare exceptions, occurs only when the fetus is ABO compatible.

Once the mother is immunized to Rh however, virtually all subsequent Rh incompatible infants are affected, regardless of their ABO type. Thus ABO incompatibility has no further protective effect.

Discussion and Summary

ABO erythroblastosis is highly restricted in its distribution to the incompatible offspring of type O mothers. The etiology seems to be diffusible isoagglutinins in the serum of many type O individuals. The disease is more self-limited than erythroblastosis due to Rh incompatibility, perhaps because of the wider distribution of ABO antigens in the body. The incidence of ABO disease may be relatively constant in a number of different populations, since the incidence of A^1 or B children of O mothers is $(\bar{O})^2 \times (\bar{A}^1 + \bar{B})$, which is remarkably constant for Europeans, Africans, Chinese, and Japanese.

Although serious as a health problem, ABO erythroblastosis does not represent a serious fetal loss from a biological point of view. On the other hand, evidence of many kinds shows a much larger and biologically important loss of possible incompatible children. The loss is estimated to be of the order of 8 per cent to 35 per cent, with 25 per cent a likely value. Such a loss, if not otherwise compensated, would cause an increase in the *O* gene frequency of the order of .005 per generation. Since this process would presumably have been going on for some time, and since large studies have shown no differences in gene frequencies in age groups spanning two generations, there must presumably be some counter selection in favor of *A* and *B*. Little progress is likely to be made in elucidating this point until there is some means to distinguish between A and B homozygotes and heterozygotes.

On balance it seems that, unlike ABO hemolytic disease, the more

important early loss occurs in non-O as well as O mothers, although the whole mass of data suggest that the loss may well be greater in O mothers.

The loss of prospective incompatible children has been shown, with varying degrees of assurance, to be due to infertility or relative infertility of some incompatible matings, to abortions, to perinatal deaths, and possibly to very early loss of incompatible offspring due to failure of ABO incompatible sperm to fertilize eggs, or to miscarriage so early as not to be recognized as such. A good part of the early loss of incompatible offspring can be made up by later compatible offspring, so that reduction of family size is much smaller than the actual loss. This is particularly true in those reports having the smallest average family size, and hence the greatest opportunity for compensation and the bringing of the family to the desired size.

Some of the evidence can be discounted because of misclassification of type AB, and possibly other infants, and illegitimacy (which would tend to increase the number of O children from supposedly A ♂ x O ♀ matings but not from O ♂ x A ♀ matings), but these factors can have only a very minor influence on the conclusions reached.

A possible connection between the ABO blood groups and the sex ratio at birth requires further investigation; no conclusions can be reached at present.

Finally there is strong evidence that fetuses that are incompatible with the mother with respect to ABO are rarely able to immunize her to the Rh factor. Although this tends to give incompatible infants an advantage, Rh hemolytic disease is so infrequent (ca. 0.5 per cent) that this effect is negligible in comparison with the estimated deleterious effects of ABO incompatibility (ca. 5 per cent).

Addendum in Proof

Professor Newton E. Morton has kindly shown us several manuscripts by himself, Dr. C. S. Chung, and Dr. E. Matsunaga, to appear in the *American Journal of Human Genetics* and the *Japanese Journal of Human Genetics*. They have used different statistical methods, and, in part, have used different series of data. Their conclusions agree in the main with ours on the selective effect of incompatibility. The main differences are that they estimate only about 11-12 per cent selection against incompatible children, and they also estimate about an 8 per cent deficiency of compatible *OO*, *AA* and *BB* children, where we doubted the evidence for this deficiency of *OO*. Combining these two effects would give a loss of 1-(0.88/1.08)=1-0.81 or 19 per cent of A children from

A ♂ x O ♀ matings as compared with O ♂ x A ♀ matings compared with our weighted average estimate of 25 per cent for the same ratio, attributed by us mainly to the incompatible mating. Finally, they find a parity effect in incompatibility for O mothers and for homozygous compatible children, whereas we did not examine the parity effect because of a belief that the data were inadequate. Aside from differences in the selection of the data, the main difference in statistical analysis is our greater reliance on methods which are less efficient in the sense of size of standard error for a given size of sample, but which we believe to be less sensitive to departures of the data from certain basic statistical assumptions.

Dr. Morton has also called our attention to Haga's "Studies on natural selection in ABO blood groups with special reference to the influence of environmental changes upon the selective pressure due to maternal-fetal incompatibility."[23a] Working with a population under more favorable living conditions than that of Matsunaga and Itoh (1958), he finds smaller fetal losses and less evidence of selection.

A. J. Rawson and Neva M. Abelson (1960), discovered antibodies of $\alpha\beta$ specificity in the 7S fractions but not in the 19S fractions of two type O sera which contained both α and β isoagglutinin activity in each fraction.

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Chapter 6

The Hemoglobinopathies

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The explosive development of knowledge regarding inherited abnormalities of hemoglobin synthesis has been reviewed numerous times in recent years.[28, 76, 141, 151, 158, 174, 181, 182, 185, 201, 231, 232, 257, 278, 283, 285, 352, 404] The tempo of investigation and discovery shows no signs of abating—1958 and 1959 witnessed the publication of at least 400 papers in this field. The present review makes no attempt at a complete summary. Rather, after an introductory section designed to orient the reader unaware of recent developments regarding hemoglobin chemistry, we shall record what seem to us the outstanding recent developments with respect to eight of the more active areas currently under study.

I. The Nature of Hemoglobin

The enormous amount of basic information available regarding the physicochemical state and physiology of hemoglobin has been summarized in a number of recent reviews.[28, 76, 141] Hemoglobin has now been sufficiently dissected to allow us to reconstruct the molecule, by no means completely, but nevertheless in sufficient detail to permit the integra-

tion of a number of different facets of its behavior. Nineteen different amino acids are found in hemoglobin. The entire molecule is composed of approximately 560 amino acid residues.[176, 366] The amino acids are united to form polypeptides, portions of which are arranged in a stable tight helix as a consequence of hydrogen bonding between amino acids on adjacent turns of the helix.[293] Each helical polypeptide chain is further coiled and folded into a compact mass to which is attached the heme group, composed of a protoporphyrin ring with an iron atom in the center. It is this latter group which is responsible for the affinity for oxygen of hemoglobin. The hemoglobin molecule is composed of four such polypeptide chains,[316, 317] associated in an ellipsoid-shaped mass with a dyad axis of symmetry,[54, 209] measuring approximately $55 \times 55 \times 70$ Angstrom units and now believed to have a molecular weight approximating 66,000.[89] As a consequence of the amphoteric nature of proteins, hemoglobin has a net charge which is a function of the difference between the pH of the solvent and the isoelectric point of the protein (the pH at which the net charge on the molecule is zero). In solutions of pH greater than its isoelectric point hemoglobin molecules behave as anions and, conversely, as cations at a pH acid to the isoelectric point. Differences in net charge are the basis of the characteristic rates of electrophoretic migration which have been the major means of identifying the variants of normal hemoglobin.

The action of urea, acid, or alkali dissociates hemoglobin into half molecules of approximately equal molecular weight.[113, 144] N-terminal amino acid analyses established that at least two different terminal sequences were present, in equal proportions, leading to the postulate that the molecule contained two different types of polypeptide chains, called α and β-chains.[316, 317] The manner of association of these chains in the molecule was investigated by the ingenious device of labeling normal hemoglobin (termed A) either isotopically, or by converting to methemoglobin, and then dissociating and recombining a mixture of appropriately labeled A and a second, abnormal hemoglobin, termed S.[188, 358, 393] In the hemoglobin A and S then isolated from the recombined mixture, the distribution of label was such as to indicate that the units of molecular dissociation and recombination are pairs of either α or β-chains, termed α_2 and β_2 dimers. The structural differences between hemoglobins A and S are confined to one of the two dimers,[188] since identified as the β_2 dimer.[173, 393] The structure of hemoglobin A can then be designated as $\alpha_2^A\beta_2^A$, and the hemoglobin S as $\alpha_2^A\beta_2^S$.

The statement that hemoglobin exists in the erythrocyte in a relatively pure state must now be qualified by pointing out that even in the red

blood cells of normal individuals a number of minor hemoglobin fractions are present. One of these is fetal hemoglobin, so named because of its role as the major component during embryonic life. Fetal hemoglobin, (hemoglobin F), which comprises about 1 per cent of the hemoglobin of the adult, is different from hemoglobin A in the following respects: ultra-violet absorption curve,[29] electrophoretic mobility,[29, 321] chromatographic behavior,[159, 161] immunologic specificity,[64] sulfhydryl group titration,[156, 272] resistance to alkali denaturation,[202, 212, 356, 357] and amino acid composition.[366] All of these suggest rather large differences between hemoglobins A and F. By means of N-terminal amino acid analysis,[339] recombination experiments,[198] and "fingerprinting,"[163] to be discussed at length later, it has been established that fetal hemoglobin contains α chains apparently identical to those of hemoglobins A and S, the other dimer being unique for hemoglobin F; hence, the symbol $\alpha_2^A\gamma_2^F$. Fetal hemoglobin concentration is increased in blood under various physiologic and abnormal circumstances and we shall return to this later.

In addition to fetal hemoglobin, the other minor components of hemoglobin are also under scrutiny. Hemoglobin A_2, migrating much slower than hemoglobin A at alkaline pH, constitutes approximately 2.5 per cent of the normal adult erythrocyte pigment, being absent at birth. Unpublished recombination experiments indicate that it consists of a pair of α-chains as in hemoglobins A and F,[336] but the other two polypeptide chains are different from those in either A or F and have been designated ∂-chains.[176] Symbolically this is then $\alpha_2^A\partial_2^A$. Hemoglobin A_3, a minor component of the order of 8 per cent of the total hemoglobin, appears as a component preceding hemoglobin A when electrophoresis is carried out at pH 8.6.[214] Its nature is uncertain at present but it is believed to be a "senescent" form of the normal molecule.[214] Salting out experiments[85] and column chromatography[12, 44, 72, 267, 268] have raised the possibility of a number of other components, but it remains to be proven whether these are distinct, genetically determined proteins, artifacts of preparation or analytic technique, or "hybrid" molecules, as a consequence of the various forms of hemoglobin normally present in the erythrocyte. Thus, approximately 15 per cent of the red cell hemoglobin must be considered as a group of proteins, consisting of biochemically specific fractions in various combinations. Whether these are isomers of normal hemoglobin in the same sense as the various porphyrin isomers present in the erythrocyte, ontogenetic precursors, or whether these are decomposition products of hemoglobin are challenging questions. The answers may well provide fundamental knowledge regarding mechanisms of erythrocyte formation and function.

II. Techniques Currently Available for the Characterization of Hemoglobin

In view of the very complete compilation of the methodology of hemoglobin analysis by Jonxis and Huisman,[203] we will not undertake an extensive discussion of this subject but rather a general orientation, primarily for those unfamiliar with these techniques who wish an introduction to the complexity which the procedures for describing and characterizing hemoglobin have attained.

The most commonly used methods for the quantification of fetal hemoglobin are based upon the relative resistance of the latter to denaturation by strong alkali. Although the method of Jonxis and Huisman,[202] whereby the change in absorption spectrum is followed during the course of the reaction, is said to be more accurate for fetal hemoglobin concentrations in excess of 10 per cent, the convenience of measuring the degree of spectral change at the end of one minute is such that the method of Singer, Chernoff, and Singer[356] remains more widely used. The latter method has now been modified to allow its use on whole blood rather than washed cell hemolyzates.[65]

As noted in the introduction, the basis of electrophoretic separation is a charge difference between proteins at a given pH. This charge effect is modified, however, by interaction between protein, solvent system, and the supporting medium, allowing the investigator a choice of media for specific purposes. Tiselius moving boundary electrophoresis, the application of which set the stage for the current interest in hemoglobin, is a sensitive method of separation of proteins,[181] but its usefulness is limited by the expense of the equipment and the relatively large amount of labor involved in a single determination. Whereas earlier Tiselius electrophoretic techniques were unable to separate hemoglobin F from A, resolution has been increased by use of more dilute buffer solutions.[29] The use of filter paper as a supporting medium in zone electrophoresis has greatly facilitated progress in this field. The chief asset of this method lies in simplicity and adaptability to the mass production necessary for routine clinical use and for extensive field surveys. The two basic techniques employed consist either of the use of vertical paper strips suspended in a humidity chamber[43] or the use of a "closed" system in which the paper is pressed between two silicon-treated glass plates during electrophoresis.[203, 359] Although the former technique does not permit a large migration distance, resolution is adequate and it has found extensive use, especially when quantification by densitometry is contemplated. A technique which employs tris(hydroxymethyl)aminomethane instead of the

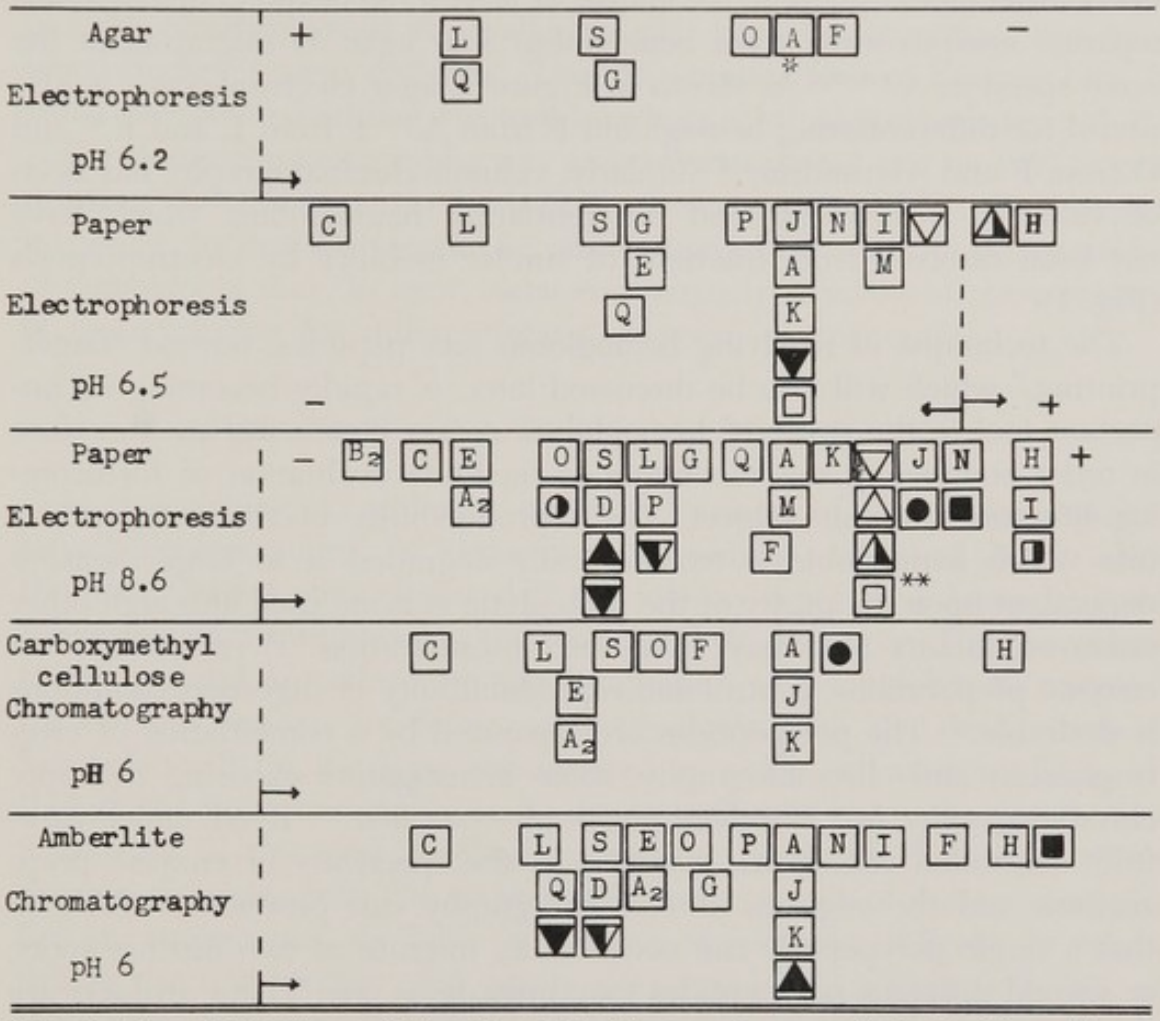
usual veronal buffer, followed by the application of a protein stain to the preparation, has been employed as a method for quantification of the A_2 fraction;[78, 79, 110] however, this alternative compromises accuracy for facility.[110] Although we are cognizant of differences in opinion, we have found the glass plate technique particularly valuable for examining large numbers of samples, and are partial to it because of the greater distance of hemoglobin migration, allowing better separation of poorly resolved fractions. Even more rapid screening is possible by using a microhematocrit-tube technique for applying the hemoglobin without prior washing of cells.[68] Electrophoresis in acid as well as alkaline buffers is useful for differentiating various hemoglobins because of changes in relative mobilities as demonstrated in Figure 1.

Starch block electrophoresis, using sedimented washed starch granules, is utilized for visualization or quantification of minor hemoglobin fractions such as A_2, or for preparatory procedures. The thick block allows insertion of larger amounts of concentrated hemoglobin solution, thus permitting the detection of components as small as 1 per cent of the applied hemoglobin. The enclosure of the block in a humidity chamber[124, 297] results in very photogenic separations, but we are partial to the original technique of Kunkel and Wallenius[216, 217] of covering the block with a thin cellophane cover, again resulting in longer migrations and wider separations. The values yielded by the two methods are comparable, however. Starch block electrophoresis is not ideal for routine or field investigations because of the labor required for washing the starch, preparation of stroma free hemoglobin solutions and elution of the hemoglobin for quantification. The development of a simpler medium as a substitute for the starch block would greatly facilitate the detection of individuals with increased amounts of A_2 and so render the thalassemia complex more amenable to population studies.

Starch gel electrophoresis,[363] utilizing hydrolyzed "cooked" potato starch as the supporting medium, has not been used extensively for hemoglobin electrophoresis, despite its demonstrated resolving power in the separation of diverse proteins. It does allow sharper resolution of minor components, especially when electrophoresis is performed with the slab in the horizontal position. Although the relative rates of migrations of the more common hemoglobins are not altered on starch gel electrophoresis, this medium in addition to a recently introduced acrylamide resin[312] offers some promise with the increasing demand for more sensitive methods.

The important effect that the supporting medium can have on the electrophoretic pattern is well demonstrated by the results of employing

METHOD



Dotted line indicates point of application. Arrow points in direction of migration.

The symbols refer to the following hemoglobins which have not been designated alphabetically: ◭ = Fessas and Papaspyrou; □ = Liberian-2; △ = Hopkins-2; ■ = Bart's; ● = Norfolk; ▲ = Lepore; ◨ = Hopkins-1; ▽ = Durham-1; ▼ = Stanleyville-1; ▼ = Stanleyville-2; and ◑ = Alexandria. Hemoglobin Liberian-1 has been designated hemoglobin N.

FIG. 1—The relative mobilities of the hemoglobin variants under different technical conditions.

* Denotes the following hemoglobins which do not separate from A on agar: D, I, J, P, Alexandria, Hopkins-1 and -2, and Durham-1.

** In Table 1, Liberian-2 has been indicated as probably identical with hemoglobin K. However, the possibility exists that at least some specimens of so-called K have a slightly different mobility from Liberian-2 at pH 8.6 on paper, as indicated in this diagram.

an agar gel; although hemoglobins D and S migrate at identical rates by every other electrophoretic technique, at least some of the D hemoglobins separate unequivocally from hemoglobin S or agar, D migrating at the same speed as A.[258, 321] As shown in Figure 1, agar electrophoresis is also useful for differentiating hemoglobin F from A,[321] P from L and F,[86] and O from F and Alexandria.[111] Similarly, column chromatography has been of value in quantifying and differentiating hemoglobins which have not been resolved from fractions of similar mobility by electrophoresis (Fig. 1).

The technique of resolving hemoglobin into peptides, termed "fingerprinting," which will also be discussed later, is rapidly becoming an important tool in the study of hemoglobin. A few comments are therefore in order concerning this technique to facilitate evaluation of forthcoming literature on this subject. The reproducibility of the polypeptides into which hemoglobin is enzymatically degraded is in large measure dependent upon the purity of the proteolytic enzyme used although other unknown factors also may enter into consideration. Even using ideal enzyme preparations, control and reproductibility of digestion conditions is desirable.[171] The polypeptides are separated by a combination of electrophoresis and chromatography, some investigators choosing to apply chromatography first,[206] others electrophoresis first.[171] Even under carefully controlled conditions, variation in the specificity of enzyme preparations and the vagaries of chromatography can produce artifacts in that a single polypeptide can occasionally migrate as two distinct spots, or several different polypeptides may migrate as one. These artifacts are usually inconsistent but they may also be detected by elution of the peptides in question, hydrolysis and qualitative amino acid analyses; in some cases even quantitative determinations may be required. On the other hand, two peptides may have the same qualitative and quantitative composition and yet be dissimilar in the sequence in which the constituent amino acids are arranged. Inversions of a peptide segment may be undetectable unless the inversion spans a tryptic cleavage point or unless sequential amino acid analyses are performed on the peptide involved. These qualifications of the fingerprinting technique are not major obstacles to its use, and, indeed, this method of protein analysis opens the door to exciting opportunities for more precise definition of a number of inherited protein anomalies.

This leads us into the question of what are the minimum number of techniques required to establish the identity of a new hemoglobin. In retracing the history of developments in this field in the past 10 years it is obvious that the minimal criteria have depended upon the nature

of the hemoglobin mutant to a certain extent, but in general, criteria have been extended as more hemoglobins have made their appearance. In 1954, electrophoresis at an alkaline pH, alkali denaturation, and solubility studies[179] were sufficient. The characterizations of hemoglobins H and I necessitated adding electrophoresis at acid pH to the battery.[328] By 1957 column chromatography was a necessary adjunct and by 1958 fingerprinting was added on the basis of the heterogeneity of D.[34] Techniques involving the dissociation and recombination of the α and β polypeptides of hemoglobin may, in some instances, partially circumvent the necessity of fingerprinting for differentiating unknown components.

III. The Formal Genetics of Hemoglobin

At this writing, 17 different inherited variants of normal hemoglobin have been recognized. By virtue of an agreement arrived at shortly after the first variants were detected,[365] the various types have been assigned the letters of the alphabet in the order of their discovery. The exceptions to this general procedure are the designation of hemoglobin F, reserved for obvious reasons for fetal-type hemoglobin, and the designation of hemoglobin S for the type encountered in sickle cell anemia. This, although the first abnormality to be discovered, was given the designation S because of the long-standing recognition of its physiological consequence, the sickle cell. The original references to each of these abnormalities are summarized in Table 1. Because of frequent difficulty in establishing the non-identity of a newly discovered hemoglobin with one of those previously described, the current trend in cases of doubt is to apply temporary designations based on place or personal names.

Each of these variants behaves as if its presence were controlled by a single gene. As a rule, heterozygosity for such a gene results in some 30-40 per cent of the hemoglobin being of the abnormal type, whereas homozygosity results in all of the major component of hemoglobin being abnormal. In many homozygotes, the proportion of fetal hemoglobin is significantly elevated. In general, the chief cytological manifestation of the heterozygous condition is a small increase in the number of "target" cells, whereas the homozygote (when known) is usually characterized by many target cells, microcytosis, and a mild hemolytic process with a slight to moderate degree of anemia. Hemoglobin S is unusual in the very low solubility of the deoxygenated form of the hemoglobin; this is the basis for the sickling phenomenon. Hemoglobin H differs from the others in its instability and the fact that when encountered, it is always in individuals who in addition have thalassemia.[271] Recent developments,

TABLE 1—*The Human Hemoglobins in the Order of Their Discovery*

Year of identification	Letter designation*	Investigators
1949	S	Pauling, Itano, Singer, and Wells[294]
1950	C	Itano and Neel[186]
1951	D	Itano[178]
1954	E	Itano, Bergren, and Sturgeon[184] Chernoff, Minnich, and Chongchareonsuk[66]
	G	Edington and Lehmann[99] Schwartz and Spaet[341]
1955	H	Rigas, Koler, and Osgood[314] Gouttas, Fessas, Tsevrenis, and Xefteri[130]
	I	Jensen, Page, and Rucknagel[193] Cabannes, Sendra, and Dalaut[53]
	M	Hörlein and Weber[157] Singer[355]
1956	J	Thorup, Itano, Wheaby, and Leavell[380]
	K	Cabannes and Buhr[51] Robinson, Zuelzer, Neel, Livingstone, and Miller (Liberian 2)[323]
	N	Robinson, Zuelzer, Neel, Livingstone, and Miller (Liberian 1)[323]
	O	Eng[104]
1957	L	Ager and Lehmann[4]
	P	Schneider and Haggard[337]
1958	Q	Vella, Wells, Ager, and Lehmann[392]

* Still undesignated are: (1) the "fast" hemoglobin of Fessas and Papaspyrou;[112] (2) the congenital sulfhemoglobinemia of Miller;[260] (3) Bart's hemoglobin of Ager and Lehmann;[5] (4) Durham-1 of Chernoff;[64] (5) Lepore hemoglobin of Gerald and Diamond;[124] (6) Hopkins-1 and-2 of Smith and Torbert;[362] (7) Norfolk of Ager, Lehmann, and Vandepitte;[6] (8) Alexandria of Fessas, Mastrokalos, and Fostiropoulos[111] and Vella, Ager, and Lehmann;[391] (9) Stanleyville-1 of Dherte, Vandepitte, Ager, and Lehmann[87] and Schneider and Haggard;[338] and (10) Stanleyville-2 of Dherte, Vandepitte, Ager, and Lehmann.[87]

to be discussed later, place this hemoglobin in a separate category from the others. Contrary to the general finding, hemoglobin J was the major component of the hemoglobin of heterozygous individuals in the family in which it was first described.[380] More recently, however, hemoglobins which seem to migrate as hemoglobin J have been described which are minor components in the blood of heterozygotes.[160, 311]

Marriages Where One Partner Possesses Two Hemoglobin Abnormalities

The elucidation of the chromosomal relationships of the genes responsible for these various abnormalities has proceeded slowly, because of the relative rarity with which marriages providing critical information have been encountered. The most suitable marriages for the study of this problem are those where one partner possesses two hemoglobin abnormalities and the other none. The production in such marriages of either normal children or those with both abnormalities suggests that the genes responsible for the abnormalities are not alleles. Hemoglobins S and C, which are present in some 9 and 2 per cent of American Negroes respectively, have frequently been encountered together. The report of Ranney in 1954 that from marriages of normal individuals with persons possessing both these hemoglobins there result no children like the parents, but only children with either the sickle cell trait or the hemoglobin C trait, has not been contradicted.[305] The genetic evidence in this case therefore suggests either allelism or close linkage of the two genes concerned.

Exclusive of hemoglobins S and C, only some five marriages have thus far been reported in which one marital partner possessed two abnormal hemoglobins and the other was certainly or presumably normal.* Each of these warrants a brief description. The most controversial of these reports is that by Schwartz et al.,[342] on a family in which hemoglobins S and G, as well as thalassemia, were present. The original interpretation of the observation that the marriage of a man who appeared to possess the genes for hemoglobins S, G, and thalassemia with a normal woman resulted in a child with neither hemoglobins S or G was that the genes responsible for hemoglobins S and G were segregating independently.[282, 342] The subsequent report, to be discussed later, that the biochemical alteration in hemoglobin G involves an amino acid immediately ad-

* A marriage involving an individual with hemoglobins "Bart's," H, and A will be discussed later because this apparently involves a different type of situation.

jacent to the amino acid involved in hemoglobins S and C[154] has raised questions concerning the correctness of that interpretation, since under most current concepts of the mode of gene action, non-allelic genes should not be involved in the specification of adjacent amino acids in a polypeptide chain. A discussion of alternate interpretations will be found in Neel[284] and Ingram;[175] some of these will be referred to later.

Smith and Torbert[362] have described a three-generation pedigree involving hemoglobin S and a second abnormal hemoglobin (Hopkins-2) which on paper electrophoresis at pH 8.6 migrates more rapidly than normal and has not yet been definitely differentiated from hemoglobins J, K, or N. The critical marriage for the genetic interpretation occurs in the second generation, where the union of a woman possessing both these hemoglobins with a normal male results in seven children, six of whom were studied. Of the six studied, one possessed both abnormalities, while three had the sickle cell trait and two the "fast hemoglobin" trait. The appearance of a child with both abnormalities attests to the non-allelism of the genes responsible for these two hemoglobins. Should the re-examination now in progress of the family described in the preceding paragraph result in an alternative interpretation, then the family investigated by Smith and Torbert would provide the chief evidence for the existence of two different loci controlling hemoglobin characteristics. It is noteworthy that in this family, the presumed dihybrids also produced hemoglobin A, whereas this was not the case in the preceding family, although the interpretation of the latter is complicated by the fact that the presumed dihybrid also possessed the thalassemia gene. The significance of these observations will become apparent in the section on biochemical genetics.

Aksoy and Lehmann[10] reported the results of a marriage between a normal woman and a man with hemoglobins S and E. All four of the children showed the sickle cell trait. The chance of producing no normal children, or children with both hemoglobins S and E, among four offspring, if the genes are segregating independently, is 1 in 16. However, the father with both hemoglobins S and E is the offspring of a woman with the same blood picture, a finding explained by the authors by the assumption that his deceased father had the sickle cell trait. The probability of this among the Eti-Turks, from whom this family is drawn, is about 1 in 8. From the genetic standpoint, then, the data can be interpreted as providing evidence for allelism at the .06 probability level only if one first assumes an event that has a .12 probability of occurring. No hemoglobin A was present in the individuals who possessed S and E, a finding which from the biochemical standpoint reinforces the concept of allelism.

Cabannes and Portier[52] have reported a mother whose hemoglobin was composed of three components, A, D, and K, with three children, one showing an AD pattern and two an AK pattern. Unfortunately, the fact that the father could not be examined limits the conclusions which can be drawn.

Dherte, Lehmann, and Vandepitte[86] have described a family in which both hemoglobins P and S are present. The critical marriage in this family involves a male with both the abnormal hemoglobins (who also possesses some hemoglobin A, as well as 4 per cent hemoglobin F) with a normal woman. Of the resulting six children, three exhibited the sickle cell trait and three the hemoglobin P trait. The finding of hemoglobin A in association with S and P would, on the current concepts of gene action to be discussed later, suggest that these two genes were not alleles, and yet the probability, if they are not alleles, of six children showing only hemoglobin S or hemoglobin P, but not both or neither, is $(\frac{1}{2})^6$, i.e., below the 5 per cent significance level.

In summary, only five marriages capable of shedding critical light on the genetic relationships of the "hemoglobin genes" other than S and C have been reported. These are marriages where one parent is known to possess two abnormal hemoglobins, and the other certainly or probably none. Such marriages yield decisive information in two ways. If they produce children who are either normal or exhibit both traits, this is positive evidence for non-allelism. Two of these marriages appear to have each produced a "critical child," but in one the parallel biochemical findings are such as to challenge current concepts of biochemical genetics and so force one to look very hard for alternatives. There is thus really only one "critical child," in the Hopkins-2 family, to supply the evidence for two "hemoglobin loci." The second decisive type of data comes from the accumulation from such marriages of a sufficient consecutive series of children exhibiting either the one or the other hemoglobin abnormality that on a statistical basis non-allelism becomes unlikely. Here again the thinness of the evidence is all too apparent; aside from the S-C hemoglobins, the only other combination (S-P) beyond the 5 per cent level is one where on biochemical grounds one might be led to postulate non-allelism. The need for the location and intensive study of these critical families is obvious.

Abnormalities in Fetal Hemoglobin

In the normal individual the level of fetal hemoglobin has fallen to about 1 per cent of the total by the end of the first year of life, and remains in this range thereafter. Elevations of the amount of fetal hemo-

globin are often encountered in the hemoglobinopathies, as already noted, as well as in association with certain diseases.[219, 356] But an elevation of fetal hemoglobin is not always a manifestation of disease. Rarely apparently normal individuals are encountered in whom 10 to 20 per cent of the hemoglobin is of the fetal type.[100, 101, 286] Several studies have now shown that this tendency is inherited as a simple dominant trait.[191, 396] To what extent this elevation should be regarded as primary (i.e. a failure in function of the regulating mechanism normally responsible for the decrease in fetal hemoglobin production after birth) and to what extent the elevation is secondary, as may be the increase in hemoglobin A_2 in thalassemia to be discussed later, is not clear; nor can one be certain that all of these are a homogeneous group.

The study of qualitative, genetically-controlled variations in fetal hemoglobin presents certain obvious difficulties, since for the most part these can be considered for so limited a portion of the life cycle. Fessas and Papaspyrou[112] have reported on an abnormal hemoglobin, relatively sensitive to alkali denaturation, which comprised 14 per cent of the total at birth, the remainder consisting of 60 per cent F and 26 per cent A. During the next three months this component decreased to 4 per cent, while hemoglobin F fell to 20 per cent. The child exhibiting this component also had the thalassemia trait. Ager and Lehmann[5] have described in a newborn child with the thalassemia trait, an abnormal component intermediate between F and A in its sensitivity to alkali denaturation. In both instances, this abnormality has now been shown to be of a special type which does not involve fetal hemoglobin in the strict sense (see p. 174). Fessas, Mastrokalos, and Fostiropoulos[111] and Vella, Ager, and Lehmann[391] have also reported variants of fetal hemoglobin sensitive to alkali denaturation (the former applying the term "Alexandria" to the component described by them). Finally, abnormal hemoglobin components have been decribed in the blood of premature infants[17, 90, 136] and in association with multiple congenital anomalies of the newborn.[137] These have not been universally accepted because of technical criticisms. The genetic significance of all of these observations is unclear.

Abnormalities in Hemoglobin A_2

Survey studies have led to the finding that occasionally the A_2 component may consist of two fractions, approximately equal in amount.[58-60, 215] In the American Negro this abnormality was observed in 1.4 per cent of a series of 507 hospital patients.[264] This "split A_2" trait behaves as if due to a single gene in its inheritance. The new component which occurs in

persons with "split A_2" has been designated B_2 by Ceppellini, who has studied the result of a marriage in which a man with the sickle cell trait and a "split A_2" married a normal woman.[59] All six children showed either the sickle cell trait or the "split A_2" but not both nor neither. On this basis Ceppellini has quite properly raised the possibility of linkage of the genes concerned, a possibility to which we will return later.

In summary, evidence exists for variations in each of the clearly defined components of normal hemoglobin, namely, A, A_2, and F. With the exception of F, all the variations have been shown to have a simple genetic basis, but the genetically urgent task of defining the formal relationships of the responsible genes to one another is scarcely under way.

IV. The Biochemical Genetics of Hemoglobin

In 1956 Ingram,[170] extending techniques earlier evolved by Sanger,[332] developed a method of "fingerprinting" hemoglobin which has been widely adopted. The heat denatured hemoglobin molecule is enzymatically digested with trypsin, which splits the peptide bonds formed from the carboxyl groups of lysine or arginine. The resultant polypeptide fragments are then separated by electrophoresis along one edge of a sheet of filter paper and then by chromatography perpendicular to the line of electrophoretic separation. Staining the fragments by spraying the chromatogram with ninhydrin results in a peptide map or fingerprint of some 26 spots, each a polypeptide containing lysine or arginine at the carboxyl end. The fingerprint of sickle cell hemoglobin differed from that of A only by the cathodal displacement of one particular polypeptide, arbitrarily labeled the number 4 polypeptide.[170-172] A step-wise degradation of this peptide showed that among the eight amino acid residues comprising it, the corresponding hemoglobin S peptide differed from that of hemoglobin A only in the substitution of an uncharged valine molecule for a negatively charged glutamic acid in the sixth position (Fig. 2), accounting for the net charge difference of plus two for the intact molecule as determined by differences in electrophoretic mobility.[294, 335] Approximately 30 per cent of the hemoglobin molecule—the core—was resistant to trypsin degradation. Further digestion of this residue with chymotrypsin failed to detect any other differences between hemoglobins S and A.[164] Hemoglobin C, which it will be recalled results from a gene apparently allelic to that responsible for S, was found to differ from A by the substitution of a positively charged lysine for the very same glutamic acid, resulting in a net charge difference of two per β-chain or plus four for the entire hemoglobin C molecule.[165] As would be expected, this

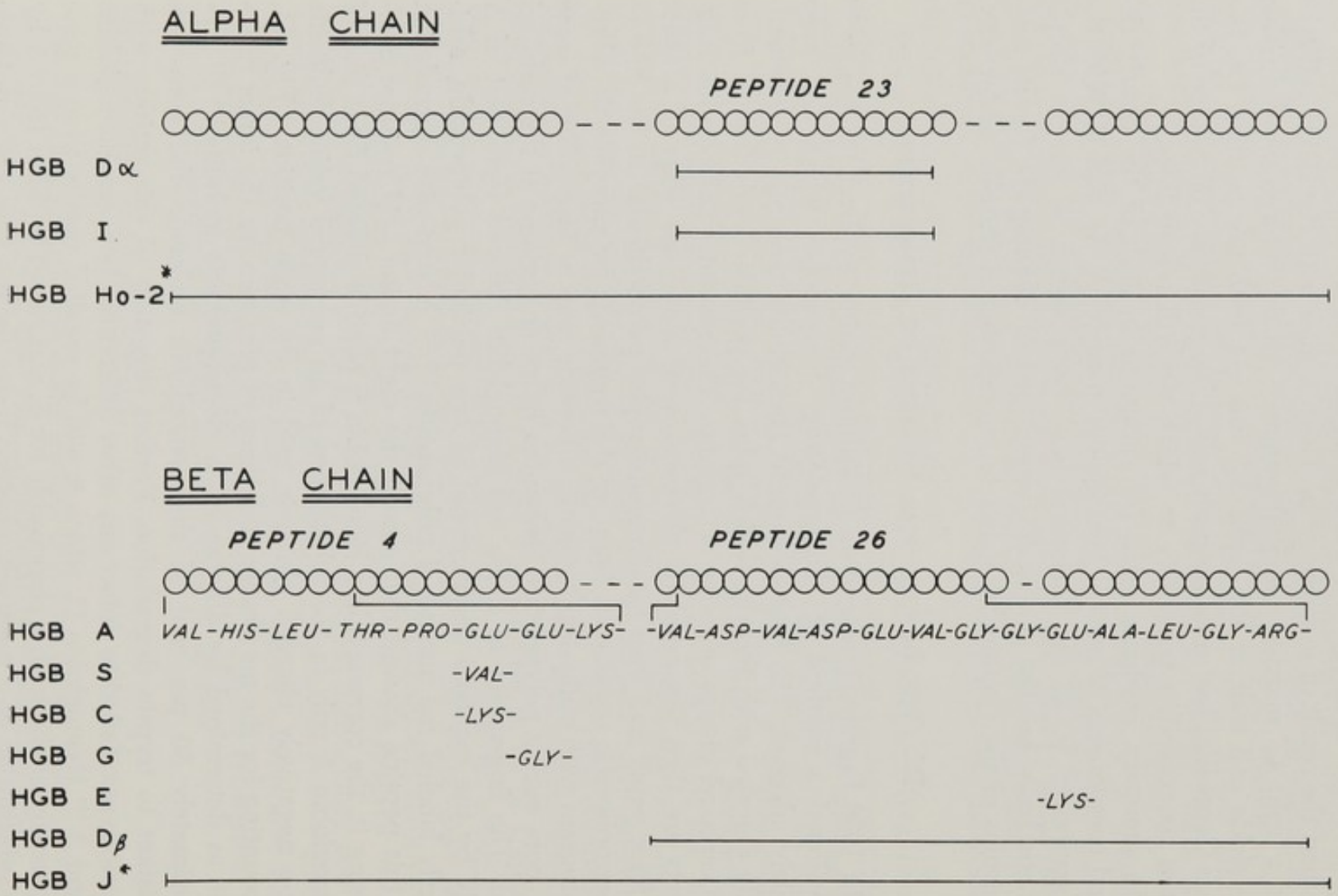


FIG. 2—A summary of present knowledge concerning the nature of the biochemical alterations in the hemoglobin variants. Where an asterik (*) has been employed, the amino acid substitution has not been located on a specific peptide of the corresponding polypeptide chain to date.

change was manifested by the appearance of two new peptides as a consequence of the tryptic action on the lysyl-glutamyl bond within the hemoglobin C number 4 peptide (Fig. 2). The number 4 peptide was then shown to reside in the β-chain,[173, 393] and the evidence linking the action of a gene to a specific amino acid substitution in a polypeptide chain was complete. This dramatic demonstration has touched off a flurry of activity in this area which insures that this writing is undoubtedly obsolete at its inception.

To date four abnormal hemoglobins in addition to S and C have been examined in this fashion, although in some instances the nature of the defect has not been established as succinctly as for hemoglobins S and C. Hemoglobin E also is due to the substitution of a lysine for a glutamic acid, with a net charge difference of two per half molecule as is the case for hemoglobin C.[167] In this case, however, the substitution occurs in peptide number 26, which is in the β-chain.[167, 173] Although both hemoglobins C and E entail substitutions of lysine for glutamic acid, different ionization constants as a consequence of varying relationships with neighboring amino acids are apparently sufficient to account for the differences in electrophoretic mobility.

The results of fingerprinting hemoglobin D have been especially instructive. The three very first hemoglobin D's to be analyzed yielded different fingerprints.[34] Hemoglobin D_α, found in a Turkish Cypriot, is due to an amino acid substitution involving peptide number 23, in the α-polypeptide chain.[173] In hemoglobin D_β, from a Gujerati Indian, there is a substitution of unknown type in peptide number 26, which, as noted above, is in the β-chain. Hemoglobin D_γ, found in a Sikh, appears different from both of the previous D sub-types but has not been characterized further. The heterogeneity found in hemoglobin D has rather far-reaching implications in that it raises the possibility that similar heterogeneities may exist with respect to the other hemoglobins; this possibility had already been raised as an explanation for the presence of intraerythrocytic crystals in some but not all patients with hemoglobin C disease.[181]

A hemoglobin with the properties of hemoglobin I[340] has also been partially characterized; by these techniques the number 23 peptide of the α-chain—the peptide containing the D_α change—appears as an altered fragment and contains tryptophan, an amino acid not normally found in this peptide.[273] A "hybrid" molecule can be formed by the dissociation and recombination of hemoglobins S and I, a finding in agreement with the concept of the α-chain as the site of the hemoglobin I substitution.[187, 273]

Finally, there is preliminary evidence that individuals heterozygous for the genes resulting in hemoglobins S and Hopkins-2 normally manufacture an $\alpha_2^{Ho\text{-}2}\beta_2^S$ molecule.[183] Such a "hybrid" molecule has also been formed artificially in dissociation-reassociation experiments.[187, 189] The hemoglobin J of Thorup et al.[380] appears to have abnormal β-chains.[189]

It is noteworthy that the six abnormal hemoglobins characterized thus far are the result of substitutions in only three different peptides or regions of the hemoglobin polypeptide chains. This raises the possibility of a non-random distribution of mutational change on the genetic template, but might also reflect a screening process by natural selection, in consequence of which only certain changes have been retained in the population.

The study of hemoglobin H has been limited by the fact that it is found only in individuals who also have thalassemia (although, of course, not all individuals with thalassemia exhibit hemoglobin H).[263, 271] Its concentration in certain sibships suggests a genetic basis. Recombination studies suggest that the probable polypeptide structure of this hemoglobin is β_4^A.[197] This perhaps accounts for its marked instability. In view of the hypothesis to be discussed later, that one type of the thalassemia gene may partially block α-chain production, the role of the gene responsible for hemoglobin H would appear to be to facilitate the association of (an excess of) β_2^A dimers into a functional hemoglobin molecule when α-chain production is blocked. In a parallel development, hemoglobin "Bart's" has been shown to be a γ_4^A polymer,[168] and presumably also owes its origin to a partial block in α-chain production in the presence of a mechanism facilitating an abnormal γ-chain association.

The appellation of hemoglobin M has been applied to what is almost certainly a heterogeneous entity,[123, 125, 145, 157, 210, 299] so designated because of the similarity of the hemoglobins concerned to methemoglobin.[355] Appropriate studies have demonstrated that the defect is not in the methemoglobin reductase enzyme system responsible for the maintenance of ferrohemoglobin. Whereas in some of the cases studied the heme group reacts with ligands and reducing agents in a fashion similar to methemoglobin,[123, 299] in others the heme reactivity is unique, suggesting a different mode of attachment of approximately half of the heme groups to the globin moiety.[125] In two instances the defect has been localized to the globin moiety since substitution of heme from normal hemoglobin did not alter the spectral characteristics of the hemoglobin M.[145, 157] The nature of the globin defect responsible for alteration of the stability of the heme iron is still unknown. An inherited abnormality of the heme ring per se has been postulated on the basis of studies on a large Japa-

nese kindred with so-called "black blood disease."[376, 377] More intensive study of these hemoglobins offers promise of contributing to the basic understanding of the mode of heme attachment and reactivity.

We are now ready to consider certain problems involving genetic concepts and terminology. The evidence cited in Section II to the effect that two independently segregating loci were involved in the control of the characteristics of the hemoglobin molecule led Neel[281] to suggest a locus designation of Hb_1 and Hb_2, with the precise gene present at either locus to be designated by a letter superscript corresponding to the type of hemoglobin associated with the gene, e.g., Hb_1^S or Hb_1^C, the normal allele being designated as Hb_1^A. With the demonstration of α and β polypeptides in the hemoglobin molecule came the suggestion that one of the postulated two loci controlled α-chain production and the other β, the two loci then being redesignated as Hb_α and Hb_β, with superscripts as before.[166] We shall employ this terminology hereafter. Obviously, should future work reveal that more than one genetic locus contributed to the specificity of either of these polypeptides, then the terminology would be forced to evolve still further.

By inference, the γ chain of fetal hemoglobin and the δ chain of A_2 are under the control of two additional, separate loci. Implicit in this suggestion is of course a basic concept of gene action, wherein earlier "one gene-one enzyme" concepts are modified to "one gene-one polypeptide"—with, in some instances at least, several polypeptides then associating, in a manner yet unknown, into a single protein molecule. The genotype of a person who forms only normal hemoglobin would be written Hb_α^A/Hb_α^A Hb_β^A/Hb_β^A Hb_γ^F/Hb_γ^F $Hb_\delta^A{}_2/Hb_\delta^A{}_2$. The use of the superscripts F and A_2 for the normal allele at the Hb_γ and Hb_δ locus indicates merely the normal hemoglobin component with which these loci are associated. The localizations just described of hemoglobin abnormalities to either the α or β chain would on the basis of the foregoing hypothesis suggest that the genes responsible for hemoglobins D_α, I, and Hopkins-2 form one group of alleles and those responsible for hemoglobins S, C, D_β, E, and J another.

Individuals heterozygous for the Hb_β^S gene possess no hybrid molecules, such as $\alpha_2^A\beta^A\beta^S$.[188] These might be expected if β chains following their synthesis passed into a common "β-chain pool," in which they then associated as β_2 dimers. The mechanism ensuring that only like β chains associate, i.e. β^S with β^S, and β^A with β^A, is not understood. However, the finding is suggestive of the existence of two kinds of intracellular β-chain templates in Hb_α^A/Hb_α^A Hb_β^A/Hb_β^S individuals, with dimer association occurring in proximity to the template.[188]

If this concept of gene action is correct, an individual heterozygous for an abnormal gene at both the α and β locus should still be able to form some normal (A) hemoglobin, since the single Hb^{A}_{α} and Hb^{A}_{β} genes present should control the production of normal α and β chains which, forming dimers, should then associate into normal $\alpha_2\beta_2$ molecules. There should also result in such individuals hemoglobin molecules in which only the α or only the β-chain is abnormal and, finally, some molecules in which both α and β-chains are abnormal. Conversely, if an individual possesses a pair of alleles responsible for a change in either the α or β-chain, he should on this concept no longer form any normal hemoglobin, since the capacity for normal α or β-chain production is now lacking. The absence of hemoglobin A would thus be a biochemical test for allelism.

Data for a critical test of this very attractive hypothesis are still extremely scanty. Several question marks stand out. Firstly, in the preceding section we have referred to some minor discrepancies between the biochemical evidence and the segregation data. These are not critical discrepancies, since they are based on negative evidence (the failure of a certain type of child to occur) rather than positive evidence (appearance of a child incompatible with the hypothesis). Nevertheless, it is noteworthy that among the 16 children born to marriages where one parent has two abnormal hemoglobins plus hemoglobin A (i.e. ADK, APS, ASHopkins-2) and the other parent is definitely or presumably normal, 15 children exhibit one or the other of the abnormalities whereas only one child shows both. The biochemical evidence in these three families suggests non-allelism of the two hemoglobinopathy genes concerned. However, this is a significant departure from the ratio of 1:1:1:1 expected in the absence of linkage. A similar discrepancy between the segregation data and the findings to be expected on the basis of the hypothesis of independent inheritance of the two loci concerned has already been commented on in the case of hemoglobins S and B_2. Should further data confirm this trend, then either the postulate of linkage between the loci regulating hemoglobin synthesis becomes necessary, or else some more basic modification of the current hypothesis is in order. Although linkage between loci with related functions is known in bacteria, it appears to be unusual in higher organisms, and hence should not be invoked over-easily as an explanation of the unusual segregation ratios.

Secondly, evidence for the postulated "hybrid" molecule which should be observed in dihybrids remains scanty. However, Itano[183] has recently chromatographically isolated a second component from the hemoglobin

A fraction of an individual possessing in addition hemoglobins S and Hopkins-2 which may be the $Hb_{\alpha}^{Ho\text{-}2}$. Hb_{β}^{S} hybrid; there may often be technical difficulties in the detection of such hybrid molecules.

For the third piece of evidence which raises questions for the hypothesis, we return to a consideration of the family mentioned in the preceding section in which hemoglobins S and G were present. In this family there occurred an individual who was in a position to inherit the genes responsible for both hemoglobins G and S and for thalassemia, and who, on the basis of hematological and biochemical studies, appeared to have done just that. It was from the failure of this individual to transmit either the gene responsible for hemoglobin S or G to his son (who did however exhibit thalassemia minor) that it was concluded that these two hemoglobins resulted from gene substitutions at different loci. Now, however, it has been reported that fingerprinting and sequential amino acid analysis of the hemoglobin G of one member of this family (who also possessed the thalassemia gene) demonstrates the substitution of glycine for glutamic acid in the number 4 peptide, in the position immediately adjacent to that involved in the hemoglobin S-C substitution.[154] This finding raises profound questions. Blood group studies on the family provide no evidence of non-paternity in the critical father-son pair, and indeed the fact that the son exhibited the stigmata of thalassemia present in the father could be construed as evidence in favor of paternity. Technical error in the identification of hemoglobin G in the father cannot be excluded, and in particular the possibility raised by Ingram[175] bears consideration, namely, that the so-called G was really an α_4 molecule, comparable to the β_4 recently demonstrated in the case of hemoglobin H.[197] An intensive re-examination of this family is now in progress. Should the earlier findings be confirmed, this evidence plus the segregation data referred to above would lead to the concept of a more complex control of polypeptide structure than currently envisioned, such as multiple loci responsible for α and β-chain production, or the contribution of several loci to the characteristics of one polypeptide.*

* Since this manuscript was submitted for publication, there have been several important developments as regards the "one gene-one polypeptide" hypothesis. R. Hill (Proc. Symposium on Methodology in Human Genetics, in press) has reported confirmation of the original electrophoretic and solubility findings in the individual referred to above as probably possessing the genes responsible for hemoglobins S, G, and thalassemia, but has been unable to isolate the G polypeptide from this individual, and has suggested that what appeared to be hemoglobin G may be a collection of "minor" components occurring in increased proportions. If this explanation proves correct, the apparent objections to the hypothesis raised by this family would no longer prove valid. Secondly, additional evidence has been forthcoming for the occurrence of a

In conclusion, it should be pointed out that with the techniques of electrophoresis only those abnormalities are detected which result in changes in net molecular charge. One can readily envision changes from which no alteration in charge would result or, where two amino acids in different peptide fragments participated, wherein the alterations in charge were in opposite directions and so compensatory. The latter might be detected by carrying the "fingerprinting" no further than the use of trypsin digests, but detection of the former would involve the even more laborious techniques of peptide degradation. The "invisible mutations"[283] responsible for these changes might under certain critical circumstances be of profound biological significance. Indeed, it has been suggested that changes of this nature may provide the basis for the spectrum of effects associated with the thalassemia genes,[177] to a consideration of which we now turn.

V. The Nature of Thalassemia

Although thalassemia (Mediterranean or Cooley's anemia) has not generally been classified as a hemoglobinopathy, in that a specific alteration of the hemoglobin molecule has not been demonstrated, no discussion of the abnormal hemoglobins can avoid involvement with this entity. Briefly, thalassemia is characterized by a microcytosis and leptocytosis, often accompanied by an apparently compensatory polycythemia; in some cases hypochromia is also present. Although the appearance of

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"hybrid substance" in individuals simultaneously heterozygous for a gene involving the α chain and a gene involving the β chain (C. Baglioni and V. M. Ingram, unpublished; P. S. Gerald, H. O. Pearson, and P. R. McCurdy, presented at Soc. for Pediatric Research, 1960). Finally, J. Atwater, I. Schwartz and L. Tocantins (Blood, *15:* 701, 1960.) have described two sisters with four hemoglobin components, namely, A, C, a G-like fraction, and an unidentified fraction, one of whom has a child exhibiting only hemoglobin A, the other of whom has two children, one exhibiting only A and the second A and the G-like component. Raper, Gammack, Huehns, and Shooter (Brit. M. J. 1960. *2:* 1257-1261) report a family in which mother and daughter exhibit four hemoglobins, namely, A, G_{Br}, C and a previously undescribed fraction. The G_{Br} is shown to involve an α-chain abnormality, while C, in keeping with the findings noted above, is shown to be due to a β-chain alteration. The new hemoglobin is a hybrid molecule exhibiting both the α and β-chain abnormalities and has been designated G_{Br}/C. The mother also has two children with the C trait. In addition, T. Bradley and S. Boyer (personal communication) have studied a new child in the ASHopkins-2 family reported by Smith and Torbert[362]: the child shows only hemoglobin A. On the assumption that the husbands of the women reported by Atwater et al. were hematologically normal, this alters the segregation ratio to 19:8. All of these developments strengthen the "one gene-one polypeptide" hypothesis.

the blood film in thalassemia may mimic iron deficiency anemia, in thalassemia the serum iron concentration is normal or high, iron stores are abundant, and the anemia is iron refractory. The microcytosis and leptocytosis result in an increased resistance to hypotonic saline, an important diagnostic point. Two main categories of the disease can be recognized. Thalassemia minor is characterized by mild morphologic abnormalities, a rate of erythrocyte destruction slightly if at all above normal,[204, 295, 345] and normal to slightly elevated levels of hemoglobin F.[356] Hemoglobin levels average approximately two grams below normal.[383] Thalassemia major is characterized by extreme morphologic abnormalities of the erythrocyte, erythroblastemia, and blatant evidence of hemolysis.[23, 106, 138, 204, 370] Marrow hyperplasia may be so intense as to erode bone to the point of pathologic fracture; extramedullary hematopoiesis is often encountered. The production of large amounts of hemoglobin F is characteristic. Transfusions are necessary to sustain life and affected individuals seldom survive to adulthood.

Extensive family studies in Italy and the United States indicate that thalassemia minor and major are related to one another as heterozygote to homozygote, i.e. that the same gene which produces the minor form when heterozygous produces the major form when homozygous. The occurrence of forms of thalassemia intermediate between major and minor has been recognized by the term thalassemia intermedia.[69] However, most of these cases were described before the more recent developments with respect to the abnormal hemoglobins, and in the light of the findings to be discussed shortly, there seems little doubt that most of these intermediate cases find their explanation in the combination of a single thalassemia gene with a gene responsible for a hemoglobin abnormality, with other such cases reflecting the impact of an unusual environmental stress on the thalassemia minor genotype.

Hypochromia and microcytosis, despite abundance of iron stores, have suggested to many investigators a block in the synthesis of hemoglobin as a major factor in the pathogenesis of this disease. This hypothesis is supported by the finding of diminished Fe^{59} incorporation into hemoglobin,[23, 295, 370] increased erythrocyte free uroporphyrin,[369] in vitro evidence for diminished rates of heme synthesis,[25] and increased intraerythrocytic non-heme iron.[38, 205] None of these findings provides definitive evidence as to the nature of the abnormality, however.

The introduction of starch block electrophoresis and the discovery that the minor A_2 hemoglobin fraction is present in excess of 3 per cent in most heterozygous individuals has facilitated the diagnosis of thalassemia and placed it on a more objective basis.[124, 216, 217] The cause of the

A_2 elevation in the so-called "classical" thalassemia minor[124] is unknown, but it has been proposed[214] that it is a compensatory increase analogous to that postulated by Rich[318] to account for the large amount of fetal hemoglobin in thalassemia. Nevertheless, in some instances of thalassemia minor the A_2 component is not elevated, suggesting that either heterogeneity of thalassemia exists or that the elevation of A_2 is a secondary phenomenon and responds, perhaps, to other variables. Evidence favoring the former interpretation stems from intra-family similarities in the proportion of A_2 in individuals with thalassemia minor,[124] as well as the fact that thalassemia minor as encountered in the Negro is less often associated with an A_2 elevation.[73] In thalassemia major the amount of hemoglobin A_2 is usually relatively low, especially in those having a large amount of fetal hemoglobin.[217] For this reason it has been suggested that in thalassemia major it would be more appropriate to relate the A_2 level to the amount of hemoglobin A rather than the total amount of hemoglobin.[216] If the δ chains of hemoglobin A_2 are under independent genetic control as are the γ chains of fetal hemoglobin, the validity of such comparison is questionable and the mechanism of this interaction would appear obscure. Elevation of the A_2 levels in the absence of cytological abnormalities has been reported.[353] The likelihood that this represents the extreme of the thalassemia spectrum is enhanced by the report of a child with thalassemia major resulting from a marriage in which one parent had thalassemia minor and the other only the A_2 abnormality.[60, 353]

The intense interest in the abnormal hemoglobins has contributed considerably to the understanding of thalassemia. Particularly instructive have been studies on the results of simultaneous heterozygosity for the genes for thalassemia and an abnormal hemoglobin. The resulting syndromes, in general, are characterized by a moderately severe microcytic hemolytic anemia, although the range in variability is at least as great as that seen in thalassemia itself. These syndromes, encountered most frequently in Caucasians of eastern Mediterranean ancestry and more recently described in Negroes and in Indians, may vary from a completely asymptomatic state to a severe disease mimicking sickle cell anemia in every respect. Splenomegaly is quite variable and hemoglobin levels from 5 to 13 grams per cent have been reported. The most consistent characteristics are microcytosis and target cells; hypochromia is less constant. In cases where S and C are the abnormal hemoglobins involved, electrophoretic observations have revealed in a minority of cases the presence of hemoglobins S or C and A in proportions not unlike those encountered in the individual simply heterozygous for one of the

abnormal hemoglobins, i.e. greater than 50 per cent A and the balance hemoglobin C or S. In the majority of instances, however, hemoglobin A constitutes only 10 to 20 per cent of the hemoglobin synthesized, and in about half of these it is lacking entirely; fetal hemoglobin ranges between the normal level (2 per cent) and 20 per cent, varying approximately inversely with A. Clinical manifestations cannot be closely correlated with these variations, suggesting that factors other than the amount of abnormal hemoglobin may be involved in the pathophysiology of these diseases.

Because of their relative frequency and widespread distribution, there are numerous reports of the combination of a thalassemia and an Hb_β^S or Hb_β^C gene in a single individual. The analysis of the results of marriages of such an individual with a normal person has led to the suggestion that there are at least two types of thalassemia, one resulting from a gene allelic to the Hb_β locus, the other due to an alteration at a separate locus.[282, 283] Table 2 brings up to date the available data on this point. Among the 42 children resulting from 12 hemoglobin S-thalassemia disease x normal marriages, 6 + 1? are of a type which indicate non-allelism of the genes concerned. The findings can be explained by the assumption of a single thalassemia locus linked to the Hb_β locus or, more likely, of two thalassemia loci, one identical with the Hb_β locus and the other not, the latter being found on the same or a different chromosome. On the assumption of two thalassemia loci, one identical

TABLE 2—*A Summary of the Types of Children Resulting from Marriages of Normal Individuals with Persons with Sickle Cell-Thalassemia Disease*

Authors	Ethnic origin	Sickle cell-thalassemia	Normal	Thal. Minor	Sickle cell trait	Σ
Silvestroni and Bianco, 1952	Italian	1	0	0	0	1
Powell, Rodarte, and Neel, 1950	Italian	0	0	2	0	2
Neel, Lawrence, and Itano, 1953	Greek	0	0	0	1	1
Aksoy and Lehmann, 1957	Turk	0	1	1	2	4
Cohen, Zuelzer, Neel, and Robinson, 1959	Negro	1	3	1	3	8
Ceppellini, 1959	Negro	0	0	3	4	7
	Greek	0	0	1	2	3
	Italian	0	0	2	1	3
	Afro-It.	0	0	0	1	1
	Negro	0	0	2	1	3
	Nego	0	1 (?)	3	1	5
	Italian	0	0	2	2	4
		2	4 + 1?	17	18	42

with the Hb_β locus and the other segregating independently, the findings would indicate that about one-third of thalassemia resulted from a gene non-allelic with the Hb_β locus. If the postulated second thalassemia locus were linked with the first, then, since the genes concerned would probably be more likely to be in the repulsion than the coupling phase, the proportion of non-allelic thalassemia would be even higher, since evidence for non-allelism could only arise when crossing-over was superimposed. However, inasmuch as Ceppellini,[59] whose data constitute so important a contribution to the table, recognized as hemoglobin S-thalassemia combinations only individuals with more than 65 per cent hemoglobin S, there has been a selection for the "interacting" type of thalassemia. It will be noted that in two of the three families providing evidence for non-allelism, the thalassemia is of the "non-interacting" type. Accordingly, the proportion of thalassemia due to a gene non-allelic to the Hb_β locus may well be higher than the table would indicate—and also may vary from locality to locality.

Aksoy[7] has recently described several Turks thought to have thalassemia minor accompanied by concentrations of fetal hemoglobin in excess of 20 per cent. Unfortunately, in each of the three families where this occurred, he was able to study only one of the parents of the affected individuals. The similarity of these cases to those earlier encountered by Olesen et al.[291] in Liberia is striking. The latter investigators felt that the most likely explanation of such cases was the coincidence of a single thalassemia gene with the gene responsible for elevated concentrations of fetal hemoglobin (see p. 222).

Several investigators have emphasized the extent to which qualitative alterations in the structure of hemoglobin in the cases of hemoglobins S and C are also apparently accompanied by alterations in the rate of hemoglobin synthesis.[180, 288] With this observation as a primary point of departure Ingram and Stretton[177] have recently advanced the hypothesis that the thalassemias are due to amino acid substitutions similar to those of the other hemoglobinopathies but in which the substitution results in no discernible difference in net charge of the protein and hence in a normal electrophoretic rate. Such a scheme allows for a large number of different types of thalassemia. We have already considered the possibility of two different thalassemia loci, one of which appears to be identical with the Hb_β locus. Ingram and Stretton speculate that a second thalassemia locus is in fact identical with the postulated Hb_α locus. The existence of still other genetic loci capable of regulating the thalassemia phenotype cannot at this point be excluded.

Current terminology for a thalassemia gene involving the Hb_α or Hb_β

locus is Hb^{Th}_{α} and Hb^{Th}_{β} Inherent in Ingram and Stretton's concept is the possibility of a series of thalassemia alleles for each locus, in which case the terminology will soon require an extension. Such can be done by appending to the "Th" superscript a descriptive designation. This concept provides a logical basis for some of the aforementioned evidence for heterogeneity of thalassemia. Thus, thalassemia resulting from an α-chain alteration, combined with a Hb^{S}_{β} gene, (genotypically $Hb^{Th}_{\alpha}/Hb^{A}_{\alpha} \cdot Hb^{S}_{\beta}/Hb^{A}_{\beta}$) should result in the production of normal α and β chains, sickle cell type β chains, and a biochemically abnormal yet apparently normal type of α chain which for the sake of convenience we shall refer to as a "pseudo-alpha chain." In the extreme case, where the block in pseudo-alpha chain production is complete, then the normal alpha chains which the patient might synthesize at perhaps 50 per cent of normal capacity—since only one gene would be "operational" under these circumstances—might be expected to be distributed among hemoglobins A, S, F, and A_2. The relative proportions of these four hemoglobins would be dependent upon the relative rates of synthesis of the two types of β chains and the γ and δ chains, respectively, but in general these four components should retain the proportions seen in the sickle cell trait. Such is, indeed, the phenotype of the so-called non-interacting sickle cell-thalassemia. One can readily see how a number of extraneous factors could influence these proportions, such as age of the patient, splenomegaly and severity of hemolysis, nutrition, inherited limitations on β-chain synthesis, etc. That factors such as blood loss or iron deficiency in goats[162] and man[404] can alter the relative proportion of hemoglobins in heterozygous states might be cited in support of these modifying factors. Variations in the completeness of the block in pseudo-alpha chain synthesis among individuals would complicate the picture even further and they might also impose limitations on the hematopoietic reserve.

On the other hand, a β-chain thalassemia mutant of genotype $Hb^{A}_{\alpha}/Hb^{A}_{\alpha} \cdot Hb^{S}_{\beta}/Hb^{Th}_{\beta}$ resulting in complete loss of function of one β-chain gene would result in the synthesis only of hemoglobin S type β chains. Synthesis of α chains would be unimpaired; these would be associated with β^{S}, γ and δ chains expressed as an $S + F + A_2$ phenotype, as is the case in the "interacting" type of sickle cell-thalassemia. Since the thalassemia mutation would probably result in some synthesis of altered beta chains, one might envision the production of some apparently normal hemoglobin even in some cases of the interacting type of thalassemia.

Recently Ramot et al.[304] have described the simultaneous occurrence of small amounts of "Bart's" and H hemoglobin in a woman with the thalassemia gene, whose daughter shows the same picture. It will be

recalled that structurally "Bart's" is γ_4 and H is β_4. This situation is thus different from those other cases in whom two abnormal hemoglobins are present. In these individuals, it may be postulated that a partial block in α-chain production is accompanied by a mechanism that permits the conjugation of excess β_2 to β_4 and γ_2 to γ_4. As the authors point out, such an association might be expected to occur relatively frequently; they feel that in part technical problems may have impeded recognition of the association.

An alternative hypothesis concerning the nature of the thalassemia gene, also advanced by Ingram and Stretton,[177] is the so-called "tap" hypothesis, which postulates that specialized areas of the DNA chain which constitute the chromosomal backbone are devoted to the determination of the rates of the reactions associated with adjacent determinants on the DNA chain. Mutations involving the "tap" in the proximity of the α or β polypeptide genes could thus alter their respective synthetic rates without altering their primary structure per se. The former hypothesis is more amenable to testing, by fingerprinting the apparently normal hemoglobin component of various thalassemic syndrome bloods, in addition to being more compatible with current knowledge regarding the nature of the hemoglobinopathies, and hence is more attractive at the moment. At the time of this writing, therefore, we are perhaps on the threshold of at least a partial solution to the "thalassemia problem," and the answer may well be available by the time this appears in print.

As matters stand at present, then, it is possible to erect a conceptual framework wherein four genetic loci can control all the known variations in human hemoglobin, including the thalassemias. At least three readily envisioned developments would provide a real challenge to this hypothesis. The first would be the demonstration that independently segregating genes were responsible for substitutions on the same α or β-chain. The second would be the discovery of a marriage of a normal person to a spouse who indubitably possessed two abnormalities of hemoglobin A as well as thalassemia, from which there issued a normal child. The third would be the demonstration of an abnormality of hemoglobin in one of the very rare sex-linked hypochromic anemias, such as have been reported by Rundles and Falls[331] or Lukl, Wiedermann, and Barbořik.[251]*

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* Note added in proof: During the past several months a number of reports have appeared on the children resulting from marriages where one parent possessed the "high-F" gene plus either the Hb^{S}_{β} or Hb^{C}_{β} gene, and the other parent was normal: Herman and Conley[149a], 1960; Motulsky[271a], 1960; MacIver, Went, and Irvine[254a], in press; Kraus, Koch, and Burckett[212a], in press. See also Edington and Lehmann.[101]

VI. Physiologic Aspects of the Hemoglobinopathies

Much of our knowledge concerning the structure of normal hemoglobin stems from interest generated by perplexing aspects of the role of hemoglobin in mammalian physiology. In the wake of the wealth of recent new information contributing to an understanding of the primary structure of hemoglobin, consideration of the possible physiologic effects of the abnormal hemoglobins may prove fruitful for extending our understanding of the function of normal hemoglobin and for approaching possible mechanisms of natural selection.

The first bit of evidence that indicated various hemoglobins might function differently was provided by the demonstration of a greater affinity of fetal blood than of maternal blood for oxygen;[26, 143] later this same difference was observed in solutions of fetal and adult hemoglobin.[253] Although some contradictions exist in the older literature, this greater affinity for oxygen of fetal whole blood compared with adult blood has been adequately verified.[2, 81] The demonstration that dialysis of fetal and maternal hemoglobin against the same glucose solution will shift the fetal curve to the right (decreased affinity for oxygen) to coincide with the mother's[13] has implicated intracorpuscular non-hemoglobin factors rather than hemoglobin structural differences per se as the cause of this dissociation curve shift.

Equally obscure are the factors regulating fetal hemoglobin produc-

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Of 19 children born to such marriges, *none* was normal or possessed both of the abnormalities. As several of the authors concerned have pointed out, this is genetic evidence either for allelism or linkage. Individuals simultaneously heterozygous for these two genes do not possess detectable hemoglobin A, i.e., they meet the biochemical criteria for allelism. In view of the several chemical differences between hemoglobins A and F, plus the fact that hemoglobins A and F (i.e., $\alpha_2^A \beta_2^A$ and $\alpha_2^A \gamma_2^F$) may be formed simultaneously, it is difficult to conceive of the "high-F" gene as an allele of the Hb_β locus.

One of us (Neel, 1960)[280a], has suggested that the basic defect in the hereditary elevations of fetal hemoglobin is in the mechanism responsible for the normal switch-over from γ to β-chain production, with the genetic unit responsible for this switchover adjacent to the β-locus (i.e., pseudo-allelism with a cis-trans effect). Alternatively, the high-F gene can be regarded as a suppressor of β_2^A (but not β_2^S) production which is linked to the Hb_β locus, with the continued production of γ_2^F chains at fetal levels due to failure of β_2^A chains to exert a normal modulating effect on γ_2^F production. For various reasons neither of these explanations is completely satisfactory. As noted earlier, Ceppellini has presented evidence for the linkage (or allelism) of the "split-A_2" trait with the Hb_β locus. The possibility thus arises of a striking genetic linkage for related biochemical functions. The explanation of these findings constitutes at the moment the outstanding problem in hemoglobin genetics.

tion in the development of the embryo. Although the shift of the dissociation curve would seem to facilitate oxygen transport from maternal to fetal circulation, adult hemoglobin is found in fetal blood relatively early, between the ninth and thirteenth weeks of gestation,[199, 379, 394] and constitutes 15 to 35 per cent of neonatal hemoglobin.[1, 67, 199] Conversely, fetal hemoglobin production continues for variable periods of time after birth.[67, 70, 134, 199] Tissue culture of fetal liver, spleen and bone marrow in isotopically labeled media suggests that fetal hemoglobin is not synthesized selectively in the spleen or liver, the sites of most active fetal erythropoiesis.[379] Cytologic studies on fetal blood have shown that adult and fetal hemoglobin may coexist in fetal red cells,[211] also militating against separate populations of fetal and adult hemoglobin-containing cells. The evidence, therefore, that fetal hemoglobin is an adaptive mechanism for intrauterine existence is more inferential than direct.

The mechanism of the control of the amount of fetal hemoglobin synthesized in the hemoglobinopathies presents another problem. In general, most of the hemoglobinopathies due to homozygosity or heterozygosity for two different mutations involving the same polypeptide chain (except where thalassemia is involved) are accompanied by fetal hemoglobin production of less than 10 per cent, in contrast to thalassemia major where the amount of hemoglobin F produced varies between 10 and 90 per cent among patients and is reasonably constant within a given patient. Patients with apparent sickle cell anemia or hemoglobin C disease in whom more than 15 per cent hemoglobin F is found are to be suspected of heterozygosity for a thalassemia and an Hb^{S}_{β} or Hb^{C}_{β} gene until family studies prove otherwise, although sickle cell-thalassemia disease frequently may also demonstrate little or no increase of hemoglobin F. The continued synthesis of fetal hemoglobin in hemoglobinopathies has been considered to be a consequence of the stress of chronic anemia,[318] but the level of fetal hemoglobin does not correlate with the severity of anemia. Although considerable opposition had been voiced to equating the hemoglobin F of the newborn to the alkali resistant fraction present in the various hemoglobinopathies, most investigators are now convinced of their identity.[29, 64, 397] A slight increase in fetal hemoglobin is encountered in pregnant women, explained in part by the fact that variable amounts of fetal cells may leak across the placenta into the maternal circulation.[71, 75, 92, 127, 349] The possibility that physiologic factors also may play a role in the amount of fetal hemoglobin present in maternal blood was suggested by the finding of an abnormally increased production of hemoglobin F by women at a stage of pregnancy too early to have resulted from fetal-maternal transfer.[326, 327] The postulate that

chorionic gonadotropin (or other products of trophoblastic tissue) may stimulate fetal hemoglobin production[327] has been further strengthened by the finding of significantly increased amounts of hemoglobin F in women with hydatid moles[48] and in eight out of 40 men and women with trophoblastic tumors in which enormous amounts of chorionic gonadotropin were secreted.[326] Although abnormal amounts of hemoglobin F have occasionally been found in patients with carcinoma,[356] in the situation just cited myelophthisis secondary to carcinomatosis was not a factor since the bone marrow was not invaded in those cases and seldom is in trophoblastic tumors.[150] Further observations upon such factors are needed.

Physiologically as well as biochemically the sickle cell remains a challenge and offers prospects of contributing still more to an understanding of basic erythrocyte physiology. In addition to describing the role of oxygen in the sickling process, earlier investigators noted that sickling was inhibited in alkaline media despite deoxygenation and also noted that sickling requires approximately 5 minutes when carbon dioxide (which also lowers pH) is passed under a hanging drop suspension of sickle cell blood.[135, 348] The phenomenon was quantified, using sickle cell anemia blood, by the demonstration that the relationship between the percentage of sickled forms and oxygen tension is a sigmoid curve, with sickle cell formation (and increased mechanical fragility) beginning as the partial pressure of oxygen was lowered below 45 mm. Hg.[225, 348] It was also noted that pressures below 15 mm. Hg were required to induce sickling in sickle cell trait erythrocytes. These studies were further extended by Harris et al.[142] with the demonstration that blood viscosity changes and osmotic fragility parallel the amount of sickling, and by Griggs and Harris[133] who also showed that sickle cell-hemoglobin C disease and sickle cell-thalassemia erythrocytes were intermediate in their propensity to sickle, the latter being closer to sickle cell anemia erythrocytes. Thus, the primary pathogenesis of sickle cell anemia has been explained as a progressive process of deoxygenation of blood in vivo giving rise to sickling and increased viscosity with impairment of capillary blood flow. The probable further increase in viscosity and sickling, potentiated by tissue acidosis, would ultimately be expected to result in peripheral thrombosis.

In vivo studies of the sickling phenomenon, however, have given cause to re-examine this scheme and call for its modification as a true picture of the pathologic physiology of sickle cell anemia. In an attempt to evaluate the relationship in vivo of oxyhemoglobin saturation to sickle cell count, blood was taken from various venous and right heart sites by

venous catheterization.[194, 195] Despite saturations ranging from 20 to 60 per cent in venous blood, the sickle cell count was surprisingly low and constant in the venous samples from any one patient. In addition, more sickled forms were present in arterial blood than anticipated on the basis of simultaneous in vitro studies (Fig. 3). In further investigations into the mechanism of this difference it has been observed that sickle cell blood placed in oscillating tonometers through which various oxygen-nitrogen mixtures plus 5 per cent carbon dioxide are passed, comes to saturation equilibrium within 20 minutes, whereas the sickle cell count increases another 50 to 75 per cent in an additional 25 minutes of equilibration.[326] Although other investigators have indicated that only two to five minutes are required for cells to sickle,[19, 135, 301, 348] there are technical differences between their methods and those just described. Although more refined data on this point are desirable, on the basis of these observations one can then view the frequency with which sickled erythrocytes are encountered in vivo as a dynamic equilibrium of rapidly circulating blood, as a consequence of anemia, whereby erythrocytes traverse pulmonary capillaries too rapidly to revert completely to the discoid form at ambient oxygen tension; similarly erythrocytes may traverse peripheral capillaries either too rapidly or with too little trauma to sickle

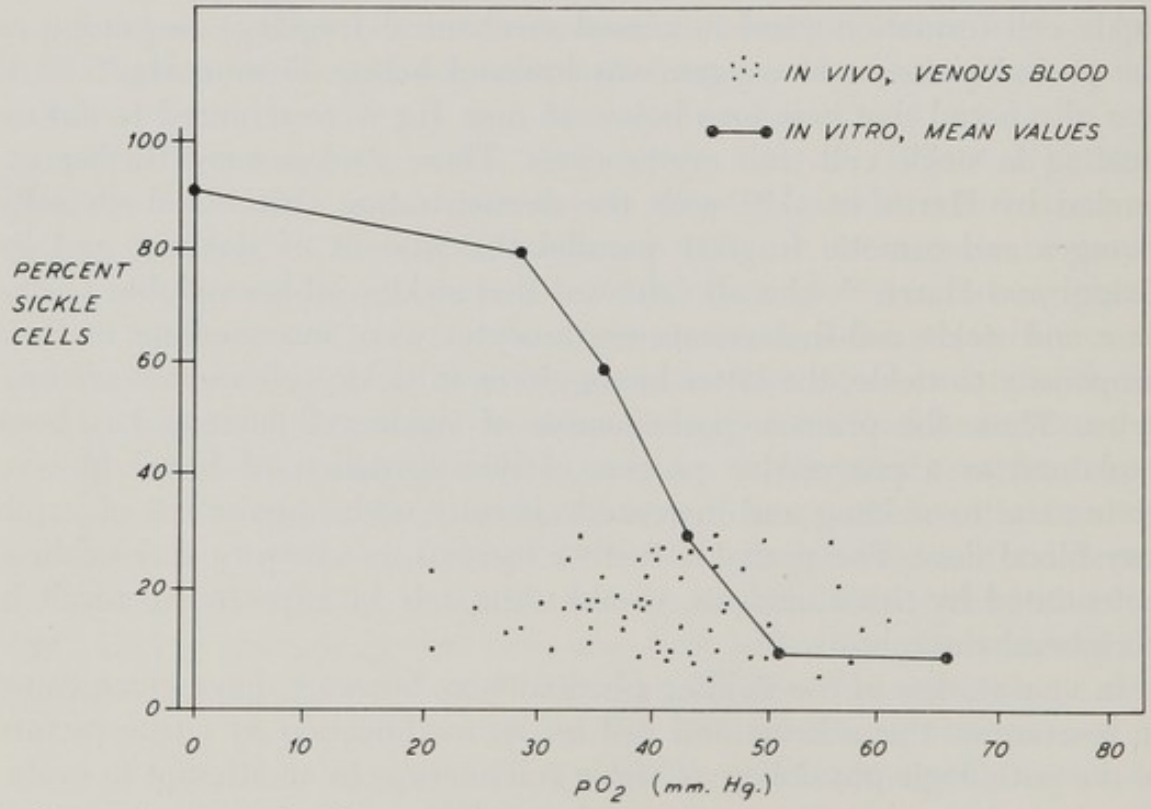

FIG. 3—A comparison between the amount of *in vivo* and *in vitro* sickling in 12 patients with sickle cell anemia. Further explanation in text.

as extensively as anticipated from in vitro studies. Thus, with respect to sickling, oxygen tension may play a permissive role in vivo. The amount of intravascular sickling, therefore, may be a statistical mean of all of these factors, and the fluctuation of the sickle count due to oxygenation and reduction of the cells in pulmonary and peripheral capillary beds, respectively, may be relatively small. The disappearance of sickle cells from arterial blood when the subject breaths 100 per cent oxygen suggests that trauma may play a significant role in the formation of the "irreversible" sickled cells.[347] On the other hand, the evidence for thrombosis and infarction in bone marrow and spleen in sickle cell anemia appears incontrovertible; for slow sinusoidal capillary beds the classical pathogenic scheme may be valid.

Despite the fact that the combination of other hemoglobin types or thalassemia with hemoglobin S generally results in a hemolytic anemia of less severity than homozygous sickle cell anemia, pathologic evidence for extensive intravascular thrombosis in the sickle cell variants is striking. Unfortunately, in much of the literature lack of electrophoretic documentation and family studies make an estimate of the relative frequency of these complications in the various sickle cell variants impossible. Nevertheless, sufficient evidence is available to suggest that intravascular thrombosis is encountered at least as commonly, if not more so, in sickle cell-hemoglobin C disease and sickle cell-thalassemia disease as in the type due to homozygosity for Hb^{S}_{β}. This applies in particular to the retina,[128, 139, 149] femoral head,[56, 360, 378] intramedullary bone,[290, 346] and lungs.[227, 269] In contradistinction, liver function impairment occurs more frequently and is of greater severity in sickle cell anemia than in the variants.[109, 131, 360] Pregnancy represents a very real threat to women with sickle cell-hemoglobin C disease.[3, 80, 93, 103, 360] These observations can be reconciled with the decreased tendency for sickling by the sickle cell anemia variant erythrocytes[133] by considering that the higher hematocrits attained in the sickle cell variants may permit slower, or even the normally intermittent, capillary flow and thus allow time for more extensive intravascular sickling than is seen in homozygous sickle cell anemia in which capillary blood flow would be expected to be much faster.

One of the more intriguing peripheral manifestations of sickle cell anemia is the loss of renal concentrating ability[129, 208, 218, 254, 403] and predominantly unilateral hematuria.[61, 147, 270, 344] Pathologically the kidneys show evidence for tissue destruction in the renal medulla and similar alterations with perhaps increased vascularity in the corticomedullary area.[37, 270] Renal function abnormalities are limited to impairment at low urine flow of tubular reabsorption of water[146, 208, 241] which is apparently

reversible with intensive transfusion of normal blood up to the age of about 10 years. Under conditions of osmotic diuresis the maximum tubular reabsorption of water was normal, however.[398] Numerous explanations for this have been offered, ranging from "toxicity" of sickle hemoglobin to renal tubular cells to pleiotropic effects of the sickle cell gene. At first glance it would appear that this could be simply the result of thrombosis as noted in other capillary beds, and perhaps contributed to by the inefficient oxygen transfer to be discussed next. Acceptance of this has been thwarted, however, because the phenomena are also observed in sickle cell trait, where deoxygenation must be nearly complete before extensive sickling occurs and consequently it has been difficult to conceive of sickling per se as a factor. Although similar microscopic findings to those encountered in sickle cell anemia have been described in the kidney in sickle cell trait,[270] these findings have not been a consistent observation.[307] That sickle cell trait cells will sickle in vivo under sufficiently reduced oxygen tension has been amply demonstrated by the well known splenic thromboses attendant with high altitude flying[77, 148, 325, 361, 373] and by the splenic sequestration of Cr^{51} labeled sickle cell trait erythrocytes transfused into patients with arterial oxygen unsaturation due to chronic cardiopulmonary disease.[213]

Although direct evidence is lacking, a way out of this impasse is now provided by intriguing new developments in renal physiology and anatomy. Pappenheimer and Kinter[292] have postulated that the juxtamedullary area and renal medulla are perfused by blood of much lower hematocrit than the remaining cortex, due to a "skimming off" of red cells in the renal arteriolar circulation. Another unusual feature of the renal circulation, due to the juxtaposition of the peritubular arterioles and venules,[249] is a counter-current diffusion of dissolved substances from the medullary afferent arterioles into the adjacent efferent medullary venules.[36, 140] Thus, rapid diffusion of dissolved oxygen from the arterioles entering the medulla into the venules leaving the medulla, in which oxygen tension would be expected to be low, results in the formation of an oxygen tension gradient diminishing toward the tip of the renal papilla. Physiologic studies have indicated that the partial pressure of oxygen in canine renal pelvic urine is much lower than renal vein oxygen tensions.[315] This latter difference is felt to be due to equilibration of urine in the collecting ducts with peritubular fluid of correspondingly low oxygen tension. Although critical evidence for the existence of such a circulation in humans is lacking, in such a system a sickle trait erythrocyte could be brought into the range of hypoxia and stasis sufficient to induce sickling. Since urea and sodium gradients in the reverse direction

(highest concentrations in the renal papilla) are also believed to be present,[36, 140, 240] relative acidosis or other ionic changes may also facilitate sickling. This hypothesis is also compatible with the predominance of left hematuria in sickle cell syndromes[129, 270] since pressure on the left renal vein by the aorta might contribute to relatively more stasis in the left kidney. Whether the "skimming effect" is greater for sickle cells is unknown. While this concept provides a basis for a tendency for sickling to occur more frequently in the renal circulation than elsewhere in the body, the steps whereby this might lead to hyposthenuria remain unclear.

Although the first report of abnormal respiratory gas exchange in sickle cell anemia was recorded by Scriver and Waugh,[343] who in 1930 mentioned a diminished affinity of sickle cell anemia blood for oxygen, little attention was paid to this until the "renaissance" of sickle cell anemia research in 1949; their observations have since been verified several times.[30, 114, 120, 324, 329] In the meantime numerous instances of arterial oxygen unsaturation have been noted in sickle cell anemia patients.[114, 120, 194, 225, 238, 324, 364, 381] Although sickle cell anemia patients are believed to have extensive pulmonary thrombotic disease, most pulmonary function and cardiopulmonary dynamics studies do not support the contention that impaired pulmonary function plays a major role in this unsaturation.[114, 238, 324] The shift of the in vitro dissociation curve to the right, indicating a diminished affinity for oxygen of sickle cell anemia erythrocytes, is of sufficient magnitude to account for this unsaturation. The fact that the oxygen dissociation curve for cell free hemoglobin S solutions is normal[102] suggests that the intraerythrocytic environment of the hemoglobin is responsible for the shift, as is believed to be true in the case of fetal erythrocytes. One of us (DLR) has demonstrated the shift in washed sickle cell erythrocytes and verified the identity of the dissociation curves for solutions of hemoglobins A and S. These results are difficult to evaluate, however, because the oxygen dissociation curve of even normal hemoglobin solutions reveals a greater affinity for oxygen than that of normal whole blood;[155] the nature of this change in affinity for oxygen upon removal of hemoglobin from the cell has not been determined experimentally. A study of sickle cell anemia patients in a tri-racial isolate showed considerable variation among patients in the magnitude of the in vitro dissociation curve shift which correlated well with variations in the degree of arterial oxygen unsaturation and severity of disease.[329] Since genetic studies eliminated thalassemia in these patients, the cause of the variation in the behavior of the sickle cells between individuals was not apparent. This did not appear to be due to differences in glutathione instability, in agreement with similar studies of Naylor et al.,[275] nor did

it appear to be due to variants of hemoglobin S on the basis of fingerprinting of hemoglobin of patients at either extreme.[207]

The full physiologic implications of these changes are unclear at the moment. Although the shift to the right of the sickle cell dissociation curve limits the amount of oxygen that can be absorbed in the pulmonary capillary, this effect may be compensated for by the maintenance of a high tissue oxygen tension as a consequence of the shift. Whether the compensation is adequate under conditions of circulatory stress, such as heavy exercise and fever, or in metabolically unusual capillary beds as found in the renal medulla, remains to be determined.

Since little is known regarding similar physiologic effects of the remaining hemoglobinopathies, due in large part to the rareness of homozygous individuals and the sporadic nature of the data, they will not be considered here.

From the foregoing discussion it is obvious that many factors are operating in the attainment of the mission of hemoglobin and much more data are necessary for completion of the picture before the mechanism of action of the abnormal hemoglobins can be profitably discussed. It is imperative that future research and observations in this area exploit the proper genetic criteria for defining the genotype of the patients upon whom observations are made; simple paper electrophoresis of the hemoglobin is insufficient in this regard.

VII. Mutation at the Loci Controlling Hemoglobin Synthesis

The almost unique problems in population genetics presented by the abnormal hemoglobins were at once apparent following their discovery and the elucidation of their genetic control.[276] In particular, with reference to the Hb^{s}_{β} gene, the early demonstration that in a broad belt extending across most of central Africa the frequency of trait carriers approximated 20 per cent raised many questions. If the results of homozygosity for this gene were the same in Africa as in the United States, then on the average 1 in 100 children would be expected to die of sickle cell anemia. If the populations concerned were at or near equilibrium, how was this gene loss compensated for? More specifically, to what extent were the gene frequencies in Africa the result of mutation pressure and to what extent of positive selection? Furthermore, in view of the occurrence of the Hb^{s}_{β} gene in such diverse groups as the "aboriginal" tribes of India, most of the tribes of Africa, and the inhabitants of certain areas in Greece, was there sufficient evidence for mutation to permit one to postulate multiple foci of origin for the gene, or was the mutation

in question to be regarded as one of great rarity, with the corollary of a very limited number of independent origins?

For a brief period, the possibility received consideration that the results of homozygosity for the Hb_{β}^{S} gene were much more serious in the United States than in the African populations, presumably because of the disruption of systems of genetic modifiers through hybridization in the former country.[229, 306] However, due primarily to the careful clinical studies of the Lambotte-Legrands,[220, 222] Foy, Kondi, and Brass,[117] and Vandepitte,[384] it quickly became apparent that in Africa the consequences of homozygosity were even more lethal than in the United States, while in the United States, no evidence could be found that sickle cell anemia was more severe in those Negroes with the greatest apparent degree of Caucasian admixture.[277] Attention was thus focused on the evidences for mutation and selection in favor of the heterozygote.

The collection of valid data on mutation presents many difficulties. Although there are several types of parent-offspring combinations which provide evidence bearing on this question, by far the most reliable data stem from families in which a child has sickle cell anemia and the father has the sickle cell trait, but the mother does not. If on careful study of the mother, she cannot be shown to possess any genetically determined hematological abnormality (e.g. thalassemia) which when combined with a single Hb_{β}^{S} gene may result in the clinical picture of sickle cell anemia, this constitutes presumptive evidence for mutation. For an entirely convincing demonstration, serological studies should be performed, to exclude the remote possibility of child exchange or concealed adoption. Finally, although there is no evidence for the existence of "suppressor" genes with respect to the abnormal hemoglobins, a completely satisfactory demonstration of mutation should include a study of the mother's family in search of evidence for suppressor effects.

To the best of our knowledge, there exist only four series of observations in which the responsible investigators have examined routinely where possible both parents of children with sickle cell anemia apparently due to homozygosity for the Hb_{β}^{S} gene. Banks, Scott, and Simmons[24] found no evidence of mutation in 14 American Negro mothers tested, the one exceptional, non-sickling mother whom they reported having evidences of thalassemia minor. In the other three series, although findings interpreted at the time as compatible with mutation were encountered, these must now be reconsidered in the light of subsequent developments. Neel[277] reported that in 49 American Negro families where both parents of one or more children with sickle cell anemia were tested, there were three instances (two fathers, one mother) in which one parent failed

to sickle and in which there were no serological grounds for parentage exclusion or clear hematological evidence of other abnormality involving the erythrocyte. With particular reference to the one exceptional mother, although the MCV was at the lower limits of normal, her hemoglobin was normal to moving boundary electrophoresis, and no clear cytological stigmata of thalassemia minor were seen. However, a re-evaluation of this family some five years later revealed that the patient, whose blood had not previously been subjected to electrophoresis, had 84 per cent S, 15 per cent A, and 1 per cent fetal, while the mother was on this occasion definitely microcytic, with an increased erythrocyte resistance to hypotonic saline, although her proportion of hemoglobin A_2 was within normal limits (2.5 per cent).[322] Despite this latter finding, it is felt that the results are best explained by the occurrence in this family of a thalassemia allele with minimal effects.

Vandepitte et al.[389] found in the Belgian Congo that among a total of 233 mothers of patients with sickle cell anemia tested for the sickle cell trait, 231 were positive but two negative. Among 206 tested fathers, 190 were positive. In the remaining 16, paternity could be excluded for 10 on the basis of history or serological determinations, leaving only six apparent exceptions. The two families in which the mother was the exceptional parent were examined as carefully as circumstances permitted at the time. In both instances, the affected children on electrophoresis appeared to possess hemoglobin all of which was type S. Routine blood studies of the mothers revealed no significant abnormalities, and electrophoresis disclosed only hemoglobin A. The possibility of thalassemia, which at that time had not been reported in the Belgian Congo, was carefully considered but no supporting evidence could be adduced. However, Vandepitte,[387] without publishing the detailed findings, had subsequently expressed the opinion that these two mothers were in fact carriers of the thalassemia gene. The Lambotte-Legrands,[220] also examining natives of the Belgian Congo, observed that among 87 mothers of infants with sickle cell disease, three failed to sickle, while among 83 tested fathers, there were seven exceptions. Further hematological findings were not presented, but it again seems that the presence of thalassemia is a more likely explanation than mutation.

There is, then, no clear example to date of mutation resulting in the Hb_β^S gene. Vandepitte et al.[389] calculated that *if* the exceptional cases they reported were both the result of mutation, then in a population such as the one studied, with 25 per cent of the sickle cell trait, this would correspond to a mutation rate of 1.7×10^{-3}. Even this *maximal* estimate corresponded to only one-tenth the rate necessary to maintain

the observed frequency of the sickle cell trait. In view of the fact that no probable cases of mutation exist at present, a *maximal* mutation rate cannot be calculated. However, from a formula derived by these investigators we can readily calculate the expected proportion of non-sickling mothers if the mutation rate were 1×10^{-5}/gene/generation, i.e., of the order of magnitude commonly encountered in mutation rate studies. In the Belgian Congo, with the above-mentioned frequency of the sickle cell trait, this proportion would be .0001, i.e. only 1 in each 10,000 mothers tested would be "exceptional." It is apparent that a prohibitive amount of labor would be required to establish a mutation rate of 1×10^{-5} by this approach, which is actually the approach involving the fewest possibilities for error. Conversely, the total data available at present are so limited as to be of little aid in estimating a mutation rate for the Hb_{β}^{S} gene. Certainly, even though, as we shall see, on "epidemiological" grounds there are tempting reasons to postulate a very limited number of centers of origin of the Hb_{β}^{S} gene, the actual data on mutation rates are simply too scanty to be helpful.

It is important to recognize that on theoretical grounds alone, we may expect the rate of mutation resulting in any specific abnormality of hemoglobin to be well below the usual estimates. Thus, where we measure the rate with which mutation results in the absence of activity on the part of a particular enzyme, or in a specific phenotype whose biochemical basis is still imperfectly understood, we may be observing the end result of a change in any one of several hundred amino acids. But in studying the abnormal hemoglobins we are recognizing *specific* amino acid changes, which introduces an entirely different order of magnitude into the studies. Thus a mutation rate estimate of 1×10^{-5}/gene/generation which rests on *any* change in a 100-amino acid protein implies, if all the constituent amino acids are equally subject to genetic variation, a rate of 1×10^{-7} at the level of amino acid substitutions at any specific position in the polypeptide sequence. The existing evidence suggests that not all of the constituent elements of a gene are equally subject to mutation.[33] With respect to that element involved in the Hb_{β}^{S}-Hb_{β}^{C} mutation, then, it is readily conceivable that for this reason as well as the specificity of the change, the mutation rate is of the order of 1×10^{-8}—in which case the responsible mutation might be expected to occur approximately once each generation in all Africa. The fact that two changes (the S and C) have been localized to precisely the same point in a polypeptide sequence cannot be used as an argument for a "genetic hot spot," since in sampling a population the findings reflect both mutation *and*

selection, and we have abundant evidence that both these changes are subject to positive selection.

Essentially the same considerations as the foregoing apply to the two other hemoglobinopathy genes which have achieved relatively high frequencies in certain populations, namely, Hb_{β}^{C} and Hb_{β}^{E} In both instances, the homozygote suffers from a mild hemolytic anemia which almost certainly confers some selective disadvantage, the magnitude of which cannot be estimated with accuracy at present. The genes also have deleterious effects when combined with some other members of the Hb_{α} and Hb_{β} series. To what extent does mutation pressure offset this selective disadvantage? There are simply no data on this point at this writing. Similar considerations apply to the thalassemia complex. Here the identification of the carrier state on hematological grounds alone is well known to present occasional difficulties. Where only one parent of a child with thalassemia major can be demonstrated to possess thalassemia minor, non-penetrance of the gene in the exceptional parent thus stands as a clear alternative to the postulate of mutation. The recent demonstration that in such instances the exceptional parent may, although cytologically quite normal, possess an elevated A_2 proportion,[55, 353] with the thalassemia major in the child presumably the result of the combination of the gene responsible for this trait with a single thalassemia gene, illustrates the kinds of special studies which must be performed prior to conclusion regarding the occurrence of mutation in this system.

Although it is now clear that the answer to the relatively high frequencies of certain of the hemoglobinopathy genes is to be sought in selective mechanisms, any definitive understanding of the apparently restricted distribution of so many of the hemoglobinopathies would be greatly facilitated by mutation rate data. The considerations of the present section render it probable that barring technical breakthroughs, such as the application to the problem of the methods of somatic cell genetics, it will probably be extraordinarily difficult to arrive at a reasonable estimate of the mutation rates concerned.

VIII. The Evidence that the Trait Forms of the Hemoglobinopathies Are Subject to Positive Selection

The considerations of the preceding section indicate that positive selection for the heterozygote must be the primary reason for the relatively high frequency of the Hb_{β}^{S} gene, and, by inference, for some of the other *Hb* genes as well. Now, four of the inherited hemoglobinopathies—namely, hemoglobins S, C, E, and the thalassemia complex—occur with suffi-

ciently high frequencies that studies on the manner in which the heterozygote is acted on by selection should be feasible. For one of these, hemoglobin E, no studies on a significant scale are yet available. The studies to date on the thalassemia complex have been inconclusive.[278] Most of the studies with respect to the population dynamics of this trait have involved populations where the frequency of the trait is so low that significant data can only be amassed with great difficulty. The initiation of studies in regions where 15 per cent of the population shows thalassemia minor raises the possibility of important advances in the near future.[57, 265] But for hemoglobins S and C, especially the former, there is a considerable amount of interesting and recent data available, to which we now turn.

Evidence for Positive Selection for the Sickle Cell Trait in Africa

A two-allele system in which the frequency of a given allele is primarily maintained by a selective advantage of the heterozygote over either homozygote is the simplest example of a balanced polymorphic system. The gene frequencies attained at population equilibrium in such systems are of course primarily a function of the selective values of the three genotypes concerned. In the case of the Hb^{S}_{β} gene, we know that the reproductive expectancy of homozygotes for this gene in Africa is virtually nil.[222, 386] We further know that in a number of different African tribes, trait (heterozygote) frequencies of 40 per cent have been encountered.[15, 118, 236] If we assume that these are tribes in which the Hb^{S}_{β} gene has its greatest selective advantage and in which the gene has been present long enough to attain equilibrium values, we can readily calculate the selective advantage the trait bearer must have over normal to offset the loss of Hb^{S}_{β} genes through the early death of the homozygotes. This selective advantage may in essence be achieved by either one or some combination of two events, namely, differential survival and/or differential reproduction.

Let us consider first what is required if differential survival is responsible for the maintenance of the polymorphism. In a population in which at equilibrium 35 per cent of adults have the sickle cell trait with no homozygotes surviving to adulthood and reproducing (i.e., a conservative estimate of the equilibrium frequency), the Hb^{S}_{β} gene has a frequency, p, of $\frac{35}{2} = .175$, and on the assumption of equal fertility of the two genotypes concerned, the zygote proportions in the next generation of the three genotypes, $Hb^{A}_{\beta}/Hb^{A}_{\beta}$, $Hb^{A}_{\beta}/Hb^{S}_{\beta}$, and $Hb^{S}_{\beta}/Hb^{S}_{\beta}$, are given by an expansion of the expression $(p+q)^2$, where $q = 1-p$. The actual

predicted values are shown in Table 3. If all Hb^S_β/Hb^S_β individuals die prior to the age of reproduction and if selection operates entirely through differential mortality, then in order for the gene frequency to remain constant from generation to generation, during each generation the relative frequency of heterozygotes must increase from 0.2888 at birth to 0.3500 at adulthood. If fitness be defined as the ratio of genotype frequency in the adult to that in the newborn, then the fitness of the Hb^A_β/Hb^A_β genotype is 0.955 and of the Hb^A_β/Hb^S_β, 1.212. The relative fitness of the heterozygote with respect to normal is 1.27.

TABLE 3—*Expected Proportions of Normal, Sickle Cell Trait, and Sickle Cell Anemia Individuals at Birth and at the Age of Reproduction, on the Assumption that Equilibrium is Maintained through Differential Survival Alone*

Genotype	Zygote proportions	Adult proportions	Fitness
Hb^A/Hb^A	.680625	.650000	0.955
Hb^A/Hb^S	.288750	.350000	1.212
Hb^S/Hb^S	.030625	.0	.0

The situation is somewhat different if equilibrium is maintained wholly by differential fertility. A basic question with respect to fertility is the extent to which the individual fertilities of two individuals who marry are to be regarded as additive, to what extent as multiplicative, and to what extent a modification of one or the other, or a combination of both. In the extreme case, when one spouse is infertile, the concept of additive fertilities breaks down, but yet it may apply to intermediate fertilities. Further, to what extent does inherent female fertility overshadow male, i.e., is there a weighting factor? The issues involved are complex; to the authors' knowledge, critical experimental data are non-existent. It is not commonly realized how different are the requirements of fertility on, for instance, an additive as contrasted to a multiplicative hypothesis. Thus, if one assumes a population with sickle cell trait frequencies as before but with equal survival of Hb^A_β/Hb^A_β and Hb^A_β/Hb^S_β individuals, q, the frequency of the Hb^S_β gene in the population prior to the elimination of Hb^S_β/Hb^S_β individuals, may be determined from the relationship, $.65 = (1-q)^2/(1-q^2)$, from which q^2, the frequency of affected individuals at birth, is 0.04499. We are required now to estimate the (*relative*) fertility differential which must exist to offset this loss of Hb^S_β genes each generation. The equation necessary to determine the fertility differential, on the assumption that fertility differentials are multiplicative, is derived in Table 4; at a value of 35 per cent for the sickle cell trait, f, the excess

TABLE 4—*Computation of the Fertility Differential Necessary to Maintain an* Hb^S_β *Gene Frequency of 0.175 on the Assumption 1) that All* Hb^S_β *Homozygotes Fail to Reproduce and 2) that the Fertility Potentials of Spouses Interact in a* Multiplicative *Fashion*

Mating type	Proportion	Relative fertility	Type of Offspring			Total
			$Hb^A_\beta Hb^A_\beta$	$Hb^S_\beta Hb^A_\beta$	$Hb^S_\beta Hb^S_\beta$	
$Hb^A_\beta Hb^A_\beta \times Hb^A_\beta Hb^A_\beta$	$.65^2$	1	$.65^2$	0	0	$.65^2$
$Hb^A_\beta Hb^A_\beta \times Hb^S_\beta Hb^A_\beta$	$2 \times .65 \times .35$	$1+f$	$(.65 \times .35)(1+f)$	$(.65 \times .35)(1+f)$	0	$(2 \times .65 \times .35)(1+f)$
$Hb^S_\beta Hb^A_\beta \times Hb^S_\beta Hb^A_\beta$	$.35^2$	$(1+f)^2$	$\frac{.35^2(1+f)^2}{4}$	$\frac{.35^2(1+f)^2}{2}$	$\frac{.35^2(1+f)^2}{4}$	$.35^2(1+f)^2$

Calculated proportion of $Hb^S_\beta Hb^S_\beta$ at birth = .045.

Then

$$\frac{\frac{.35^2(1+f)^2}{4}}{.65^2 + (2 \times .65 \times .35)(1+f) + .35^2(1+f)^2} = .045$$

$$f = .37$$

TABLE 5—*Computation of the Fertility Differential Necessary to Maintain an* Hb^{S}_{β} *Gene Frequency of 0.175 on the Assumption 1) that All* Hb^{S}_{β} *Homozygotes Fail to Reproduce and 2) that the Fertility Potentials of Spouses Interact in an* Additive *Fashion*

Mating type	Proportion	Relative fertility	Type of Offspring			Total
			$Hb^{A}_{\beta}Hb^{A}_{\beta}$	$Hb^{S}_{\beta}Hb^{A}_{\beta}$	$Hb^{S}_{\beta}Hb^{S}_{\beta}$	
$Hb^{A}_{\beta}Hb^{A}_{\beta} \times Hb^{A}_{\beta}Hb^{A}_{\beta}$	$.65^2$	2	$.65^2 \times 2$	0	0	$.65^2 \times 2$
$Hb^{A}_{\beta}Hb^{A}_{\beta} \times Hb^{S}_{\beta}Hb^{A}_{\beta}$	$2 \times .65 \times .35$	$2+f$	$(.65 \times .35)(2+f)$	$(.65 \times .35)(2+f)$	0	$2(.65 \times .35)(2+f)$
$Hb^{S}_{\beta}Hb^{A}_{\beta} \times Hb^{S}_{\beta}Hb^{A}_{\beta}$	$.35^2$	$2+2f$	$.35^2(2+2f)/4$	$.35^2(2+2f)/2$	$.35^2(2+2f)/4$	$.35^2(2+2f)$

$$\frac{.35^2(2+2f)/4}{.65^2 \times 2 + 2(.65 \times .35)(2+f) + .35^2(2+2f)} = .045$$

(See Table 4.)

$f = .965$

fertility of the trait bearer, solves for .37, i.e., the Hb_β^A/Hb_β^S individual must be 37 per cent more fertile than normal if this is the entire mechanism whereby the loss of Hb_β^S/Hb_β^S zygotes is offset. If, on the other hand, fertility be defined as additive, and dealing again in relative fertilities, then the equation necessary to solve for f is derived in Table 5; the actual value obtained is 0.97. The difference between expectation on the two hypotheses requires little comment; this is not the place to pursue the interesting problems raised by this demonstration.*

Studies on the evidence for differential survival ideally involve proper, age-stratified population samples of genetically homogeneous groups in areas where sickling rates are relatively high and mortality prior to the age of reproduction is at least 30 per cent, i.e. where the population may be assumed to be under the selective pressures responsible for high gene frequencies. Such samples are not readily assembled in Africa, and it is doubtful if any study thus far performed would meet the requirements of the "census tract" approach. However, the literature contains some seven samples, summarized in Table 6, in which the ideal is approximated (field studies or an approximation thereto, trait frequencies >20 per cent) and in which the Hb_β^C gene, whose presence would complicate the calculations, may be assumed to be absent or to occur in negligible frequencies. A number of other excellent studies have been excluded somewhat arbitrarily because of reliance on hospital series. Even the samples included are not free of criticism. Thus, only one of these studies involves trait frequencies in excess of 30 per cent. It is not clear whether the lower level populations should be considered as in the process of achieving equilibrium frequencies, or whether they have stabilized at lower values. In the two very extensive series of Allard[11] and Delbrouck,[82] malaria pressures may have been blunted in the areas in which the studies were undertaken, and selected populations seem involved. All of the studies agree in showing a higher frequency of the sickle cell trait in adults than in infants. In all except the three studies so indicated, the figures for infants would include surviving cases of sickle cell anemia. Accordingly, the simple ratio of sickling adults to sickling children therefore cannot be taken as the measure of fitness for the sickle cell trait, but only as an (under)approximation. The unweighted average of the seven series is 1.15. Allowance for the inclusion of cases of sickle cell anemia in the figures for children exhibiting the sickling phenomenon would perhaps alter the estimate to 1.16 or 1.17. It may be tentatively concluded that differential survival is certainly the major but probably not the entire factor in the sickle cell polymorphism,

* We are indebted to Dr. T. E. Reed for helpful discussions of this subject.

TABLE 6—*A Summary of Studies in Africa on Age Trends with Respect to the Frequency of the Sickle Cell Trait*

Investigator	Locality	Tribe	Age group	No. tested	No. and % sickling	% trait adults (20-40) / % trait infants and young children
van den Berghe and Janssen, 1950	Belgian Congo and Ruanda-Urundi	Bantu de race Boluba du Kasai (Kanda-Kanda)	infants	145	24 (16.6)	1.11
			adolescents	374	89 (23.8)	
			adults	501	92 (18.4)	
				1020	205 (20.1)	
		Pygmées Ete Ituri (Epulu et Gombari)	infants	119	27 (22.7)	
			adolescents	46	14 (30.4)	1.17
			adults	291	77 (26.5)	
				456	118 (25.9)	
		Bantu de race Mamvu (Epulu)	infants	38	7 (18.4)	
			adolescents	4	1 (25.0)	1.24
			adults	175	40 (22.9)	
				217	48 (22.1)	
Allison, 1956*	Musoma, Tanganyika	Simbiti Zanaki Jita Kwaya	<1 >15	287 654	89 (31.0) 249 (38.1)	1.23
				941	338 (35.9)	

TABLE 6—*Continued*

Investigator	Locality	Tribe	Age group	No. tested	No. and % sickling	% trait adults (20-40) % trait infants and young children
Allard, 1955	Befale, Belgian Congo	Unstated	0-2	953	213 (22.3)	
			15-20	451	93 (20.6)	
			20-30	576	128 (22.2)	1.04
			30-40	646	155 (24.0)	
			40-50	436	116 (26.6)	
			>50	304	60 (19.7)	
				3366	765 (22.7)	
Delbrouck, 1958*	Coquilhatville, Belgian Congo	Mongo	0-3	1275	290 (22.8)	
			3-10	1967	461 (23.4)	
			10-20	1890	452 (23.9)	1.04
			20-30	1820	431 (23.7)	
			30-40	1579	372 (23.6)	
			40-50	1487	395 (26.6)	
			>50	1270	328 (25.8)	
				11288	2729 (24.2)	
Vandepitte, 1959*	†Luluabourg, Belgian Congo	Unstated	<6 mos.	1180	286 (24.2)	1.23
			adults	660	196 (29.7)	
				1840	482 (26.2)	

* Excludes sickle cell anemia.

† The author apparently feels this supersedes his 1954 series on mothers attending a maternity service.

accounting for perhaps two-thirds to three-fourths of the advantage of the heterozygote. This conclusion assumes that the selective pressures in the groups studied are as severe as in tribes which have reached the postulated equilibrium values of 35 per cent of the sickle cell trait among adults. In other words, it is assumed that the groups studied are not in equilibrium but moving towards higher frequencies of the Hb^S gene. If they have actually stabilized at these lower frequencies, then the differential mortality in favor of the sickle cell trait is proportionally more important in maintaining the equilibrium, and, in fact, would appear to be sufficient.

With respect to differential fertility, significant data on this point have been slow to accumulate. The four series which meet the same general restrictions that were placed on the survival data are summarized in Table 7. The data have been collected in a variety of ways, rendering the series difficult to compare. Data are for children ever born. Where possible, the comparison has been limited to trait x normal versus normal x normal marriages. The unweighted average of the four series is 1.13. This estimate is greatly influenced by the results of Allard's[11] study, which appear at marked variance with those of the other three investigators, especially since this same investigator encountered relatively little evidence for differential survival. Be that as it may, a conservative statement on the face of the evidence would be that the observed fertility differential is in the neighborhood of one-seventh to one-fourth the value necessary to maintain equilibrium, depending on the hypothesis adopted regarding the interaction of individual fertilities. All we can really say is that in the total understanding of the dynamics of the Hb^S_β gene towards which we are striving, differential fertility may be a factor which cannot be ignored. It may well be that the relative importance of viability and fertility differentials varies from locality to locality. It cannot be too strongly emphasized how approximate are both the biological data and its mathematical treatment in the derivation of these estimates. Again it should be noted that an equilibrium value of 35 per cent sickle cell trait is assumed in this calculation. Should the value be lower in these particular populations, then the potential role of fertility is greater —but in this case, there is an embarrassment of riches, since the combined evidence for differential mortality and fertility would then be in excess of what is required to maintain gene frequencies of, say, 0.12, the average of the series summarized in Table 6.*

* Note added in proof: Two important and well designed unpublished studies on fertility in relation to sickling in malarious regions have now come to our attention. Roberts and Boyo[320a] (in press) in studies on the Yoruba of southern Nigeria found

Additional evidence for the protection conferred by the sickle cell trait is derived from a study of infant mortality in trait x normal as contrasted to normal x normal marriages. Hiernaux[152] and Allison[18] both report a lower mortality among the children of the former type of mating. Edington,[95] dealing with admissions to a maternity service rather than a field series, found that 181 non-sickling females had given birth to an average of 2.9 children, of whom 32 per cent had died, while 36 sickle cell trait women had an average of 3.3 children, of whom 26 per cent had died. While the bias in these figures obviously precludes their use in a derivation of *f*, the simple fact is that the ratio of the two (incompleted) fertilities is 1.14. Of real interest, and a point to which we will return later, is the fact that the birth weight of infants born to mothers with the sickle cell trait was 3010 grams, as contrasted to 2880 grams for the infants of non-sickling mothers.

The Manner in Which Selection Favors the Sickle Cell Trait in Africa

As a generalization, the action of selection on a genetically determined trait will, even in the simplest case, be found to be complex. The case of the Hb_{β}^{S} gene may be unusual in the extent to which one selective mechanism, differential susceptibility to *P. falciparum* malaria, overshadows all others. Although several investigators had previously mentioned the possibility of such a differential susceptibility,[31, 32, 45, 220, 221, 230] it was Allison[14-16] who first clearly formulated the hypothesis and introduced systematic attempts to prove it. The evidence adduced by him was of three types:

a. Different rates of parasitemia in sicklers and non-sicklers, and lower parasite densities in sicklers once infection is established,

b. Differences between sicklers and non-sicklers in susceptibility to experimental malaria, and

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sickle cell trait (or hemoglobin C trait) x normal marriages resulted in slightly (insignificantly) more livebirths per fertile year of marriage than normal x normal marriages, regardless of whether the mother or father was the heterozygote.

Firschein[113a] (in press) finds that among the Black Caribs of British Honduras, with 23.3 per cent sickle cell trait, the fertility ratio of sickle cell trait mothers to normal (whether based on mean number of children ever born or mean number surviving) is approximately 1.45—sufficient to maintain the observed gene frequencies even in the absence of any differential mortality due to malaria!

Firschein suggests that the mechanism for this differential may be an increased number of abortions among the non-sickling mothers. The addition of these two studies to those already available leaves little doubt that both differential mortality and fertility are involved in the maintenance of the sickle cell polymorphism.

TABLE 7—*A Summary of Studies on Differential Fertility with Respect to the Sickle Cell Trait*

Investigator	Locality	Tribe	Type of marriage	No. marriages	Mean No. children	Rel. fertility trait	Comments
Hiernaux, 1952	Ruanda-Urundi	Bamosso	normal × normal	120	per fa. :3.61* per mo.:3.14	♂ ♂ :.997† ♀ ♀ :1.045	Includes incomplete families
			♂ trait × ♀ normal	46	per fa. :3.42 per mo.:3.13		
			♂ normal × ♀ trait	39	per fa. :3.36 per mo.:3.28		
Allard, 1955	Befale, Belgian Congo	Unstated	♂ normal age 40-50 yrs. × ———	351	1.19	1.336	Includes unspecified proportion of celibates
			♂ trait age 40-50 yrs. × ———	124	1.59		
Allison, 1956	Musoma, Tanganyika		normal × normal	74	3.88	1.049	Includes incomplete families
			trait (both sexes) × normal	84	4.07		
Delbrouck, 1958	Coquilhatville, Belgian Congo	Mongo	♀ normal > 30 yrs. age × ———	1727	1.73	1.092	
			♀ trait > 30 yrs. age × ———	626	1.89		

* Difference between sexes due to polygamy.

† Calculated on basis of mothers.

c. The correlation between the distribution of severe (hyperendemic) malaria and the highest frequencies of the Hb_{β}^{S} gene in Africa.

Evidence bearing on the malaria hypothesis may be of two types, for purposes of convenience termed "direct" and "indirect." The latter type of evidence is circumstantial in nature, tending to implicate malaria as a potentially more fatal disease in non-sicklers, whereas the former, admittedly more difficult to obtain, would consist of data on the relationship between actual death from malaria and the possession of the sickle cell trait. Allison's data were of the indirect type, as indeed were the data in most of the flood of publications on this point which quickly followed the appearance of Allison's three papers.[22, 39, 40, 74, 83, 84, 94, 115, 116, 119, 122, 153, 237, 256, 266, 308-310] An extended review of these papers would form an excellent documentary on the difficulties of establishing a correlation between a genetic trait and a disease susceptibility under field conditions, but such a review, while valuable, would carry us beyond the scope of this paper. The weight of the indirect evidence, and in particular the data on the younger age groups, favors the hypothesis that infections with falciparum malaria are less frequent and less severe in persons with the sickle cell trait, although there are several unexplained inconsistencies. However, it remained for Raper,[310] Vandepitte and Delaisse,[388] and the Lambotte-Legrands[223] to supply direct evidence, to the effect that among children dying of malaria, especially of the cerebral type, there was a relative deficiency of the sickle cell trait. There is now no doubt that malaria plays a role in maintaining the sickle cell polymorphism. However, because of the very high rate of malaria infection in hyperendemic regions and the fact that so many African children die of multiple causes, attempts to specify the precise extent to which malaria is responsible for the differential mortality for which we have seen evidence seem unlikely to yield clear-cut results.

The physiological basis for the differing course of malaria in the two genotypes remains unclear. On the one hand, Allison[14] and Mackey and Vivarelli[256] suggested that hemoglobin S may not be as favorable a medium for the development of the malaria parasite as is hemoglobin A. On the other hand, Miller, Neel, and Livingstone[262] raise the possibility that since in *P. falciparum* infections erythrocytes containing all developmental stages of the parasite except for the very early forms and gametocytes tend to adhere to the walls of the blood vessels, all such parasitized cells with the exception of those lodged in the lungs would no longer be able to renew their oxygen supply as would be the case with a circulating erythrocyte. In the sickle cell trait, the loss of oxygen from the adherent parasitized red cell to both the tissues and the para-

site might raise the proportion of reduced hemoglobin to the level where sickling occurred. If these sickled cells were destroyed at an increased rate, this could interfere with parasite multiplication in an essentially mechanical manner. Viewed in the light of present concepts of the mechanisms of disease resistance, this would appear to involve a most unusual lack of immunological principle—depending on the coincidence of a particular type of life cycle and a protein unique among comparable proteins in its solubility characteristics. It is noteworthy that there is no evidence suggesting that the possession of hemoglobin S confers protection against other species of malaria.

With respect to the basis for the differences in fertility between sicklers and non-sicklers, no mechanism is known at present. It is conceivable that the greater malaria morbidity in childhood permanently impairs the reproductive capacity of the non-sickler. A supplementary explanation draws on the fact that in hyperendemic regions, the balance between the malaria parasite and its host remains precarious throughout life—there is no lasting immunity. Thus, Miller[261] conducted daily blood examinations for a period of a year on 20 young African males living in a hyperendemic region for *P. falciparum*, and presumably with a very strong acquired immunity for falciparum malaria. Parasites were found in 20 per cent of all examinations; the members of the group averaged 1.5 mild clinical attacks during the year of the study. It is therefore conceivable that a greater frequency of subclinical or even clinical attacks of malaria throughout adult life play a role in the relatively impaired fertility of non-sicklers.

The differences in the mortality rates of the children of sicklers and non-sicklers which were commented on above are presumably in large part a reflection of the occurrence of the sickle cell trait in the offspring of mothers with the trait. In addition, Livingstone[244] has drawn attention to the lower birth weight of children born to mothers in whom the placenta can be shown to contain *P. falciparum*. A sub-optimal birth weight may be assumed to lower prospects for survival to some degree. Referring to the data of Edington[95] regarding the higher birth weight of the children of sickle cell trait mothers, Livingstone suggests that the placentae of women with the sickle cell trait are less often parasitized to a significant degree, in consequence of which their infants have a better chance of survival.

Neel,[278] Wilson,[400] and Brass[47] have emphasized that the mortality which must be postulated from malaria, if a differential susceptibility to this disease is the sole factor in the sickle cell polymorphism, would seem excessive to some malariologists,[255, 399] although it must be admitted

that really clear figures do not exist. The recognition of other mechanisms supporting the polymorphism would of course reduce the estimated malaria mortality to what, to some, would be more acceptable figures.

In a search for other selective advantages conferred by the sickle cell gene, a number of observers have correlated admissions to African hospitals by specific disease category with the frequency of the sickle cell trait.[31, 46, 82, 221, 310] Many of these hospital series suffer from a common defect, namely, a sample of mixed tribal origins, with the possibility of different disease patterns and sickling rates in the various tribes. Furthermore, most of the series are predominantly concerned with adults, whereas the genetically most significant segment of the population would be the children. Although no finding emerges as clearly significant at this writing, the following possibilities would seem to warrant further investigation: a decreased susceptibility to leprosy,[21, 82] a decreased susceptibility to hookworm anemia[310] (unconfirmed by the Lambotte-Legrands[224]) but an increased susceptibility to typhoid fever.[221, 310]

Evidences of Pathological Consequences of the Sickle Cell Trait, from Africa and the United States

If in addition to its biological advantages, there are also certain disadvantages to the sickle cell trait, these may be difficult to demonstrate in tropical regions, but more readily detected in areas such as the United States, where large numbers of Negroes are living under non-tropical conditions. Surprisingly, there are only five sizeable series on age-incidence trends in the U.S., all these unfortunately either involving in whole or part hospital in- or out-patients or presenting other complications. The results are summarized in Table 8. The first four series, all based on hospital patients, agree in indicating less sickling among older patients. The results could be interpreted as compatible with less disease (and less cause for hospitalization) in older sicklers, or a higher death rate on the part of sicklers. Even when allowance is made for the probable inclusion of some cases of sickle cell anemia in the younger age group, the magnitude of the differential implied by these figures is really quite striking. Thus, for the combined series, assigning one-third of Pollitzer's[300] "14-29" category to the "20 and below" group, and one-fourth of the "30-" category to "51 and older," the results are: below 20–12.25 per cent; 21-50–11.11 per cent; 51 and above–9.22 per cent. These figures would seem to imply, on one interpretation, a roughly 25 per cent greater susceptibility to death between youth and the advanced years on the

TABLE 8—*The Relation between Age and the Sickle Cell Trait in Some of the Larger Series of Tests for the Trait in American Negroes*

Investigator	Age group	Number tested	Number sickling	Per cent sickling
Diggs, Ahmann, and Bibb, 1933	6-20	1,112	108	9.7
	21-50	1,102	96	8.7
	51-	403	28	7.0
Switzer, 1950	6-20	843	130	15.4
	21-50	1,233	165	13.4
	51-	302	42	13.9
Neel, 1951	12-20	147	15	10.2
	21-50	703	67	9.6
	51-	146	7	4.8
Pollitzer, 1958	14-29	232	42	18.1
	30-	241	26	10.8
Rucknagel, Witkop, and Schmidt, unpublished	0-20	1,476	282	19.1
	21-50	530	101	19.1
	51-	176	46	26.1

part of sicklers. This is an impressive figure, which it is difficult to accept.

The fifth series is based on an intensive field study of a genetic isolate of tri-racial (Negro, Indian, Caucasian) origin found in Southern Maryland. The frequency of sickling in this group is clearly higher than in any other group thus far encountered in a temperate climate.[330] It will be noted that the frequency of the sickle cell trait appears, in contrast with the findings in other studies, to rise with old age in this group. However, this isolate is breaking up, particularly since World War II. Marriages outside the community since World War II (predominantly to Negroes) would decrease the amount of sickling in the 21-50 age group; this in turn would be reflected in the 0-20 age group. Until the actual amount of out-marriage has been established, an opinion on the significance of these findings must be withheld.

With respect to a basis for such a possibly increased death rate, at the moment the chief lead centers about the kidney. The evidence for hyposthenuria, unexplained hematuria, and characteristic renal pathology in individuals with the sickle cell trait was reviewed in Section VI. In addition, Edington[96] has raised the possibility of other pathological findings in the sickle cell trait. On the face of it, these findings would scarcely seem a sufficient basis for such an increased death rate, although if

the renal defect could be a contributory factor in the apparently increased susceptibility of the Negro to severe hypertension, its importance would be magnified.

The need for clarification of this point in any final understanding of the dynamics of the Hb^{S}_{β} gene is obvious. Although on the face of it, most of this increased death rate occurs subsequent to the age of reproduction, and so is of relatively little importance in determining gene frequencies, there is some evidence for an increased death rate below the age of 50. If there is indeed intrinsically a selective disadvantage attached to the Hb^{S}_{β} gene, then the positive factors at work in a tropical environment must be even greater than calculated. Furthermore, the rarity of this gene in Caucasians in temperate climates might well be due to differential selection rather than a lesser frequency of mutation resulting in the Hb^{S}_{β} gene in this group. Although it may seem an anachronistic stand in these days of biochemical excitement, in the opinion of the authors the outstanding gap in our knowledge of the population genetics of the Hb^{S}_{β} gene is this question of age-incidence trends under non-tropical conditions.

Evidence for Selection with Respect to the Hemoglobin C Trait in Africa and Elsewhere

Knowledge concerning the dynamics of the Hb^{C}_{β} gene is meager—far less advanced than for the Hb^{S}_{β}. The maximum heterozygote frequencies thus far encountered are of the order of 20 per cent, but this is not a "pure" figure, since in these same populations there occur Hb^{S}_{β} and also the thalassemia complex (see following section). Thus, at the moment there is no observed equilibrium figure for the Hb^{C}_{β} heterozygote on the assumption of balanced polymorphism comparable to the figure of 35-40 for the Hb^{S}_{β} heterozygote. The only two efforts to derive selective values for the $Hb^{A}_{\beta}/Hb^{C}_{\beta}$ and $Hb^{C}_{\beta}/Hb^{C}_{\beta}$ genotypes have yielded markedly contradictory values, a discussion of which will be deferred until the next section.

With the demonstration of the role of malaria in maintaining Hb^{S}_{β} gene frequencies, the question of course arises whether this same agent might be important to hemoglobin C. The only study to date on this point[97] yielded no evidence that persons with the hemoglobin C trait were in any way protected against malaria; that same study provided suggestive evidence of the protection afforded by the sickle cell trait.

An even more striking failure to implicate hemoglobin C in the same selective system that regulates the frequency of the Hb^{S}_{β} gene emerges

from a recently described "natural" experiment unwittingly performed in the days of the slave trade.[200, 390] In the seventeenth century the Dutch maintained a "factory" at Elmina on the Gold Coast. Most of the slaves transported to their New World possessions of Surinam (Dutch Guiana) and Curaçao were probably derived from there. On Curaçao the slave found himself in a malaria-free environment, whereas in Surinam, malaria was rife. In both Surinam and Curaçao, some Caucasian admixture occurred. In Curacao, blood group gene frequency studies on the inhabitants of the northwestern half of the island, who for various reasons are believed to represent the descendants of the original slaves to a greater degree than those of the other end of the island, suggested about 13-14 per cent Caucasian ancestry. No comparable studies have been carried out in Surinam, where the Negroes studied were to a large extent the descendants of slaves who escaped to the "bush" from the early plantations; the amount of white admixture would presumably have been less. Three groups of Negroes were studied in Surinam. The findings are summarized in Table 9. The test for heterogeneity in the samples falls just short of the 5 per cent level of significance ($\chi^2 = 12.27$, D.f. = 6); all samples agree in AS individuals being three times as frequent as AC individuals. Similar findings are recorded by Liachowitz et al.[243] In Curaçao, on the other hand, type AC is more frequent than AS.

TABLE 9—*The Frequency of the Various Hemoglobin Types in Curaçao and Surinam, after Jonxis, 1959*

Locality	A	AS	AC	S	SC	C	AF	Σ
Curaçao, District III	296	18	28	1	3	0	3	349
	(84.8)	(5.2)	(8.0)	(.3)	(.9)		(.9)	(100.1)
Surinam, Kabel	401	87	27	1	3	0	0	519
	(77.3)	(16.8)	(5.2)	(.2)	(.6)			(100.1)
Surinam, Moengo	126	35	8	2	1	0	0	172
	(73.3)	(20.3)	(4.7)	(1.2)	(.6)			(100.1)
Surinam,	234	31	9	1	0	0	0	275
Stoelman's Island	(85.1)	(11.3)	(3.3)	(.4)				(100.1)

The difference between the two localities in the frequency of both the Hb_β^S and Hb_β^C genes is highly significant. Caucasian admixture cannot account for the results. There is of course no way to reconstruct the original gene frequencies, since even if the exact origins of the slaves were known, the Hb_β^S and Hb_β^C frequencies of 200-300 years ago are unknown. Jonxis[200] interprets the findings as evidence for a selective value of the Hb_β^S gene under "bush" conditions, presumably in consequence of

malaria pressure. Equally interesting to us, and not commented on by the author, is the fact that if one accepts this conclusion, one is almost forced to the position that hemoglobin C is subject to different selection pressures. One alternative to this is the assumption that in a near equilibrium situation the Hb^{C}_{β} allele is being "displaced" in Surinam by the more efficient Hb^{S}_{β} gene, but this then conflicts with evidence summarized by Allison[20] that in the Gold Coast area today higher frequencies of these two genes coexist.

IX. What Are the Potential Implications for Anthropology and "Geographical Pathology" of the Distribution of the Genes Controlling Hemoglobin Synthesis?

From the standpoint of their anthropological implications, the genes responsible for the abnormal hemoglobins can for purposes of convenience be divided into two categories. On the one hand, we recognize certain genes, such as those responsible for G or J, thus far encountered in a very few families of diverse origins. Thus, hemoglobin J, originally described in an American Negro family,[380] has now in addition been encountered in an Indonesian family,[160] Gujerati and Lohana Indian families,[311, 333] a French-Canadian,[252] and a southern Italian.[353] On the other hand, there are certain other genes, of which hemoglobins S, C, and E are excellent examples, which exhibit restricted distributions, but within these distributions, attain very significant frequencies. To be sure, these genes are encountered well outside of their "official distributions," but in the main no more frequently than can reasonably be explained as a result of human migration or rare recurrent mutation. Finally, now, there is an intermediate between these two extremes, well illustrated by hemoglobin D, which is found in about 1-2 per cent of Sikh and Gujerati Indians,[42, 190] but in addition has been reported in American and British Caucasian families,[178, 367] in Italian families,[250, 353] a Turkish family,[8] a Persian family,[169] in Indonesia (Sulawesi),[105] in 17 out of 3918 "Algerians,"[302] and in 4 of 1000 American Negroes.[63] Thus far only two cases have been reported in African (Congolese) Negroes;[385, 387] we have seen an additional case in a Liberian native (Robinson, Zuelzer, Neel, unpublished observation). This hemoglobin would be confused with S in the usual electrophoretic survey.

The anthropological implications of these findings must be tempered by all the usual strictures to be attached to any use of gene frequencies for anthropological ends—namely, by allowance insofar as possible for the roles of mutation, of selection pressures which may differ from popu-

lation to population, and of population structure in altering gene frequencies. In addition, there is the fact that the same hemoglobin phenotype as determined by surveys employing the techniques of electrophoresis, may be the resultant of several different genotypes. The outstanding illustration of this is hemoglobin D, where the three first specimens to be subjected to "fingerprinting" were found to differ biochemically.[34] The distribution of figures given above for hemoglobin D are thus practically useless for the purposes of anthropology and population genetics without further clarification—a clarification which will be extremely laborious.

The Hemoglobinopathies of West Africa

Although an impressive amount of data on the occurrence and distribution of the various hemoglobin abnormalities has been accumulated in recent years, well summarized in the C.I.O.M.S. Conference on Abnormal Hemoglobins,[201] there are still vast and theoretically important areas virtually untouched and, furthermore, even for those areas most intensively investigated there remain important deficiencies in our data. It is clear that the addition of the abnormal hemoglobins to the genetic systems available to the anthropologist-geneticist is a major contribution; it is equally clear that the full impact of this contribution—as, indeed, of the genetic approach in general—cannot yet be evaluated. A full and critical review of what is already available, fragmentary though it be, would result in a truly bulky compilation. Rather than do this, we propose to examine in very considerable detail one single area, an area for which, as the result of a number of recent studies, our information is more complete than for any other part of the world today, and an area which presents in striking fashion many problems of interpretation. This is West Africa.

Hemoglobins S, C, G, K, and N, and the thalassemia phenotype, have all been reported from West Africa. Of these, hemoglobin S, by virtue of numerous surveys of the frequency of the sickle cell trait, has been the most intensively examined. However, the fact that hemoglobin C results from the presence of a gene allelic to that responsible for S means that conclusions regarding genetic equilibrium and tribal relationships which disregard hemoglobin C may be quite misleading. Accordingly, in this discussion we will confine our attention to surveys using electrophoretic techniques.

The basic data available for West Africa are summarized in Table 10 and Figures 4-7. Table 10 lists the findings in all tribes, or groupings

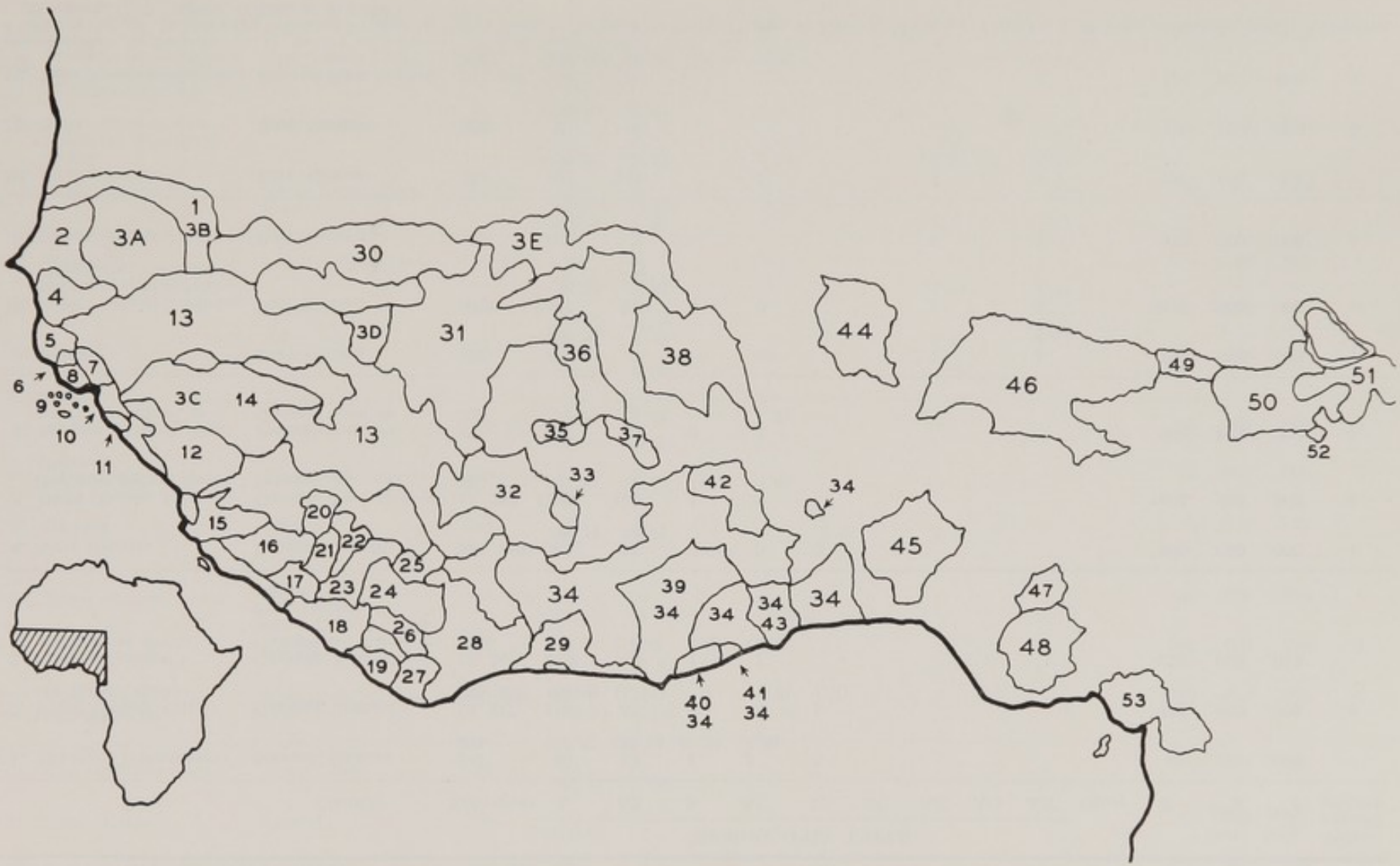

FIG. 4—The distribution in West Africa of those tribes for whom electrophoretic hemoglobin studies have been performed on 30 or more individuals. The same numbers are used in Table 10.

TABLE 10—*A Summary of the Results of the Electrophoretic Surveys of Hemoglobin Carried Out on the Tribes of West Africa to Date.*

Tribe	Country	References	HEMOGLOBIN TYPES										Σ	freq. Hb^S_β	freq. Hb^C_β	Paleo-negroid peoples
			A	AS	S	AC	C	SC	AK	AG	AN	Other				
1. Tukolor (Toucouleur)	Senegal, Gambia	247 286	60 (72.3)	19 (22.9)	1 (1.2)	3 (3.6)	0						83	.120	.018	
2. Wolof (Jolloff, Oulof) (Includes Lebu)	Gambia, Senegal	18, 247, 286, 382	410 (86.9)	57 (12.1)	0	4 (.8)	1 (.2)						472	.060	.005	+
3. "Fulani Groups"[1] (Fula, Peul, Foula)	Gambia, Senegal, Cameroons, Dahomey, Niger, Togo, Fr. and Port. Guinea, Fr. Sudan	18, 247, 286	223 (80.2)	47 (16.9)	1 (.4)	7 (2.5)							278	.086	.013	
4. Serer (Serere)	Senegal, Gambia	247, 286	293 (96.1)	12 (3.9)	0	0	0						305	.020	.000	+
5. Diola (Dyola, Jola) [Includes Felup, Bayot (Baiote)]	Gambia, Port. Guinea, Senegal	18, 286, 382	1290 (91.8)	105 (7.5)	1 (.1)	9 (.6)							1405	.038	.003	+
6. Banyun (Bainuk, Banhum) (Includes Cassangas)	Port. Guinea, Fr. Guinea, Gambia	18 382	237 (79.8)	58 (19.5)	0	2 (.7)							297	.098	.003	+
7. Balante	Port. Guinea	382	767 (98.9)	7 (.9)		0			1 (.1)		1 (.1)		776	.005	.000	+
8. Pepel (Papeis) [Includes Mankanya, Mandyako (Manjacos) (Macanhas)]	Port. Guinea	382	2497 (98.5)	32 (1.3)	0	0			3 (.1)		4 (.1)		2536	.006	.000	+
9. Bijogo (Bijagos)	Port. Guinea	382	627 (99.3)	3 (.5)					1 (.1)		1 (.1)		632	.002	.000	+
10. Bifada(Befadas)	Port. Guinea	382	692 (86.9)	102 (12.8)		1 (.1)			1 (.1)		1 (.1)		797	.064	.001	
11 Nalu	Port. Giunea	382	94 (94.0)	6 (6.0)									100	.030	.000	+
12. Susu (Soussou, Sossos)	Fr. and Port. Guinea	247, 286, 382	105 (89.0)	10 (8.5)		1 (.8)					2 (1.7)		118	.042	.004	+

[1] "Fulani Group" includes the following 9 tribes: a) Fouta Toro, b) Tukulor, c) Fouta Djalon, d) Kita, e) Masina, f) Liptako, g) Sokoto, h) Bauchi, and i) Adamawa. (Murdock, G. P., *Africa*, (1959), p. 413-414.)

13. Malinke (Mandingo, Mandingas) Includes Toronka (Tilbonca) and Mikifore (Miefora)	Fr. Sudan, Fr. and Port. Guinea, Senegal, Gambia	18, 286, 382	1631 (87.4)	208 (11.2)	1 (.1)	22 (1.2)	0	1 (.1)		1863	.056	.006	
14. Fouta Djalon (Fulas) (Includes Pajaninca, Laibe)	Port. Guinea	382	1550 (83.5)	292 (15.7)		6 (.3)		3 (.2)	5 (.3)	1856	.079	.002	
15. Temne (Timne)	Sierra Leone, Port. Guinea	18, 382	39 (69.6)	15 (26.8)	0	2 (3.6)				56	.134	.018	+
16. Mende	Liberia, Sierra Leone	18, 286	102 (68.9)	41 (27.7)		5 (3.4)				148	.138	.017	+
17. Gola	Liberia	286	29 (87.9)	3 (9.1)					1 (3.0)	33	.046	.000	+
18. Bassa	Liberia	286	161 (89.9)	16 (8.9)	1 (.6)	0		1 (.6)		179	.048	.000	
19. Kru	Liberia	286, 247	75 (98.7)	1 (1.3)						76	.007	.000	+
20. Kissi (Gissi)	Fr. Guinea, Liberia	286	55 (84.6)	10 (15.4)						65	.007	.000	+
21. Gbande	Liberia	286	39 (90.7)	3 (7.0)	0	0	1 (2.3)			43	.035	.012	
22. Toma (Buzzie, Loma)	Fr. Guinea	247, 286	92 (86.0)	12 (11.2)	0	1 (.9)			2 (1.9)	107	.056	.005	+
23. Kpelle	Liberia	247, 286	158 (86.4)	18 (9.9)	1 (.5)	3 (1.6)		1 (.5)	2 (1.1)	183	.052	.008	+
24. Ngere (Guere) (Includes Mano)	Liberia, Fr. Guinea	247, 286	113 (95.0)	4 (3.4)		1 (.8)			1 (.8)	119	.017	.004	+
25. Dan (Includes Gio)	Ivory Coast	286	43 (95.6)	1 (2.2)					1 (2.2)	45	.011	.000	+
26. Kran (Krahn)	Liberia	286	40 (100.0)							40	.000	.000	+
27. Grebo (Includes Webbo)	Liberia	247, 286	282 (99.3)	2 (.7)						284	.004	.000	+

TABLE 10—*Continued*

Tribe	Country	References	HEMOGLOBIN TYPES A	AS	S	AC	C	SC	AK	AG	AN	Other	Σ	freq. Hb^S_β	freq. Hb^C_β	Paleo-negroid peoples
28. "Kru Group of South Ivory Coast"[2]		247	63 (95.5)	1 (1.5)		2 (3.0)							66	.008	.015	+
29. "Lagoon Group"[3]	Ivory Coast	98, 247	44 (93.6)	2 (4.3)		1 (2.1)							47	.022	.011	+
30. Soninke (Seraculeh, Saracole)	Gambia, Senegal	18, 247, 286, 382	319 (84.0)	56 (14.7)		5 (1.3)							380	.074	.007	
31. Bambara	Fr. Sudan	247, 286	40 (76.9)	9 (17.3)		3 (5.8)							52	.087	.029	
32. Senufo	Ivory Coast, Upper Volta	247, 334	512 (68.8)	67 (9.0)	3 (.4)	123 (16.5)	17 (2.3)	12 (1.6)	10 (1.4)				744	.055	.102	+
33. Diula	Ivory Coast, Upper Volta	247, 334	460 (69.9)	35 (5.3)	3 (.5)	153 (23.3)	4 (.6)	2 (.3)	1 (.1)				658	.030	.121	
34. "Twi Group"[4]	Ivory Coast	18, 98	76 (68.5)	23 (20.7)		11 (9.9)		1 (.9)					111	.108	.054	+ (in part)
35. Tusyan (Toussian) Includes Vigye (Vike)	Upper Volta	247, 334	551 (73.7)	54 (7.2)		117 (15.7)	10 (1.3)	6 (.8)	7 (.9)	3 (.4)			748	.040	.089	
36. Bobo (Includes Fing, Nieniegue)	Upper Volta	247, 334	1090 (79.0)	48 (3.5)	8 (.6)	203 (14.7)	20 (1.4)	2 (.1)	9 (.7)				1380	.021	.082	
37. Dagari (Dagarti)	Upper Volta	102	72 (74.2)	9 (9.3)		14 (14.4)		2 (2.1)					97	.057	.082	
38. Mossi (Moshie)	Upper Volta	98, 102, 247	143 (74.5)	11 (5.7)		35 (18.2)	3 (1.6)						192	.029	.099	
39. Ashanti	Ghana	18, 98	72 (68.6)	22 (21.0)	1 (.9)	10 (9.5)							105	.110	.048	

[2] "Kru Group of South Ivory Coast" includes 4 principal tribes: 1) Bakwe [includes Ubi (Oubi)], 2) Wobe, 3) Bete, and 4) Dida (includes Godye). (Murdock, G. P., *Africa*, (1959), p. 260.)

[3] "Lagoon Group" of 8 principal tribes (Murdock, G. P., *Africa*, (1959), p. 254), 6 of which are covered by our data: 1) Ebrie (includes Gwa), 2) Avikam, 3) Ari (Abidji), 4) Ajukru, 5) Abe, and 6) Assini (includes Nzimah and Abure). In addition we have added a 7th, the Attie.

[4] "Twi Group" (as given in Murdock, G. P., *Africa*, (1959), p. 252-254) is composed of the 31 tribes: a) Baule, b) Ari, c) Ajukru, d) Avikam, e) Alagya, f) Anyi, g) Abe, h) Attie, i) Ebrie, j) Mekyibo, k) Assini, l) Brong, m) Ashanti, n) Fanti, o) Ga, p) Akyem, q) Adangme, r) Guang, s) Krachi, t) Atyuti, u) Adele, v) Tribu w) Kebu, x) Buem, y) Akoposo, z) Ewe, aa) Avatime, bb) Basila, cc) Fon, dd) Popo, and ee) Gun.

40. Fanti (Fante)	Ghana	18, 98, 102	133 (65.2)	47 (23.0)		22 (10.8)	1 (.5)	1 (.5)				204	.118	.059
41. Ga (Includes Akripon)	Ghana	18, 98, 102	209 (73.9)	49 (17.3)		22 (7.8)		2 (.7)	1 (.3)			283	.090	.042
42. Dagomba	Ghana	97, 102	774 (69.7)	92 (8.3)		209 (18.8)	22 (2.0)	14 (1.2)		1[5]	2	1114	.048	.110
43. Ewe	Ghana, Togo	18, 98, 102	154 (66.4)	53 (22.9)	1 (.4)	23 (9.9)	1 (.4)					232	.116	.052
44. Zerma (Zjerma, Zabrama)	Fr. Sudan	18, 247	47 (66.2)	14 (19.7)		9 (12.7)		1 (1.4)				71	.106	.070
45. Yoruba	Nigeria	18, 122, 247, 395	931 (68.7)	326 (24.1)	3 (.2)	84 (6.2)		10 (.7)			Unclassified: 1 (.1)	1355	.125	.035
46. Hausa	Nigeria	18, 247	72 (75.8)	19 (20.0)		4 (4.2)						95	.100	.021
47. Igala	Nigeria	395	127 (81.9)	28 (18.1)						[6]		155	.090	.000
48. Ibo (Includes Ouanbi)	Nigeria	18, 247, 232	227 (73.5)	76 (24.6)		5 (1.6)			1 (.3)			309	.123	.008
49. Bede (Bedduwai)	Nigeria	320	62 (67.4)	30 (32.6)								92	.163	.000
50. Kanuri (Kanouri)	Nigeria	320	79 (68.1)	37 (31.9)								116	.160	.000
51. Shuwa	Nigeria, L. Chad	320	83 (79.0)	22 (21.0)								105	.105	.000
52. Wakura	Cameroons	320	75 (72.1)	23 (22.1)		6 (5.8)						104	.111	.029
53. "Southern Cameroons Group"[7]		226	170 (75.6)	52 (23.1)	2 (.9)	1 (.4)						225	.120	.002

[5] Reported by Ager and Lehmann (1958) subsequent to the original report by Edington and Laing (1957).

[6] There is no mention of hemoglobin N in the original survey, but Ager and Lehmann later credit a specimen of this type to the Igala.

[7] "Southern Cameroons" Group. We have assumed that Languillon's "Bantu of Southern Cameroons" corresponds to Murdock's "Northern Cluster of Northwestern Bantu" which includes 9 major tribes: Bubi, Duala, Koko, Kossi, Kpe, Kundu, Ngumba, Puku, and Seke, 4 of which (the Koko, Kossi, Kpe, and Kundu) are located within the area of West Africa included in this map. (Murdock, G. P., *Africa* (1959), p. 274-275.)

of contiguous and related tribes, for whom 30 or more electrophoretic hemoglobin determinations have been performed, the number 30 having been arbitrarily selected as the minimal figure necessary to provide insight into the tribal composition. Tribal names are as given in Murdock's recent compilation.[274] Figure 4 indicates the location of these tribes. Of the 301 tribes listed by Murdock for West Africa, 48 are treated separately in Table 10, while 55 more are included in five groupings (entries 3, 28, 29, 34, and 53). Figures 5 and 6 are graphic summaries of the data on hemoglobins S and C. The fragmentary nature of our present data is obvious. This leads to unfortunate blank areas in the map which convey the erroneous impression of absences of the genes under discussion. However, we have felt this to be preferable to the even more unfortunate expedient sometimes adopted in situations of this type, of extrapolating and "smoothing out" distributions in a manner which conveys the impression of far more knowledge than actually exists.

The findings may be briefly characterized as follows: In the savannah country of West Africa, extending from the Lake Chad region in the east to the Atlantic in the west, and lying between the Sahara Desert on the north and, in the main, a tropical rain forest belt on the south, the Hb^S gene attains a frequency in the neighborhood of 0.09. South of this band, the frequency of the Hb^S gene is quite variable. In general, however, from Ghana east the frequency of the Hb^S gene averages somewhat higher than in the interior, of the order of 0.12. Relatively high frequencies of this gene are also encountered in the Temne and Mende of Sierra Leone and also in the Banyun of the Gambia and adjacent areas. In view of the argument to be developed, it should be pointed out that these "highs," except in Sierra Leone, are the general regions in which the great rivers of interior West Africa—the Senegal, the Gambia, the Volta, and the Niger—break through to the Atlantic ocean. The rivers of Africa have of course been major channels for trade and conquest.

By contrast, the Hb^C_β gene shows a striking restriction in its distribution which has been commented on by many investigators.[18, 102, 286] Although there are still critical deficiencies in our knowledge, the Hb^C_β gene shows a clearly defined high, of the order of 0.10, in a region very roughly contained in the great bend of the Niger river. In all directions from this high, gene frequency falls off rapidly.

Finally, now, it should be pointed out that there are two areas in which both the Hb^S_β and Hb^C_β genes have negligible frequencies—namely, as shown in Figure 4 and Table 10, in the region Portuguese Guinea (the Balanta, Pepel, and Bijogo) and in the region of southeastern Liberia and the southern Ivory Coast (the Kru, Ngere, Kran, Dan, Grebo, and

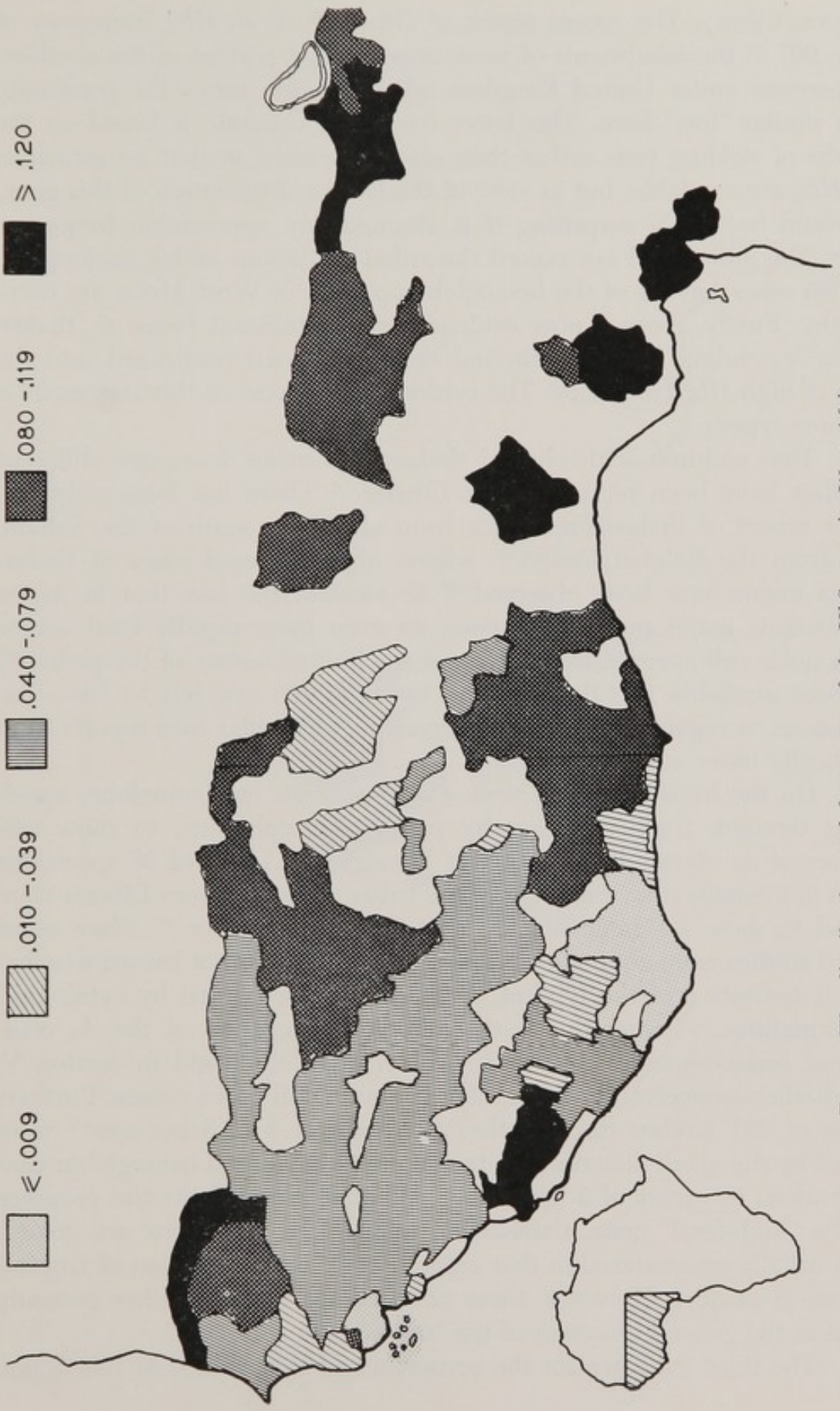

FIG. 5—The distribution and frequency of the Hb^{S}_{β} gene in West Africa.

Lagoon tribes). The recent report of Gibson[126] of an Hb^S_β frequency of only .007 in the inhabitants of some areas of that portion of the southern Cameroons under United Kingdom administration raises the possibility of a similar "low" here. This latter frequency estimate is based on the results of sickling tests rather than electrophoresis, so that no estimates for Hb^C_β are available, but in view of the known distribution of this gene, it would be highly surprising if it attained any appreciable frequency here. The author did not record the tribal affiliations of his subjects.

Two other aspects of the hemoglobinopathies of West Africa are noteworthy. Firstly, there is now evidence for a significant focus of "thalassemia" extending from Liberia and the Ivory Coast northward into the area of high Hb^C_β frequency. The evidence in support of this statement is of three types:

1. Two children with clinical thalassemia major from two different families have been reported from Liberia.[291] There has been only one other report of thalassemia major from all Africa south of the Sahara, this from the Belgian Congo,[387] where, also, scattered cases of thalassemia minor have been observed.[368] In view of the fact that in Africa thalassemia major probably pursues an even more rapidly fatal course than sickle cell anemia, and in view of the limited nature of the pediatric services available in Liberia, these two children are felt to have implications as regards frequency far greater than similar case reports from medically more advanced regions.

2. On the basis of starch block electrophoresis on hemoglobin specimens thought, from screening by paper electrophoresis, to show evidences of an elevated proportion of hemoglobin A_2, 11 of 98 specimens from individuals from a number of the tribes of southeastern Liberia were found to show a significant elevation of the A_2 fraction.[291] Since other blood studies were not made on these individuals, it is not known whether this is indicative of the type of thalassemia accompanied by cytological abnormalities, whether this is the hereditary elevation of the A_2 component unaccompanied by cytological changes discussed in section V, or whether—conceivably—the finding is due to still other causes. Furthermore, of 281 Grebos from southeastern Liberia 8 (2.8 per cent) were found by the alkali denaturation technique to have fetal hemoglobin concentrations in excess of 5 per cent.[247] Whether this reflects the presence of the "high-fetal" gene, thalassemia minor (the elevations are greater than usually encountered in that entity), or is a manifestation of tropical disease is unclear. However, these two findings taken together certainly point in the general direction of the "thalassemia complex."

3. The third evidence for the occurrence of thalassemia in this region

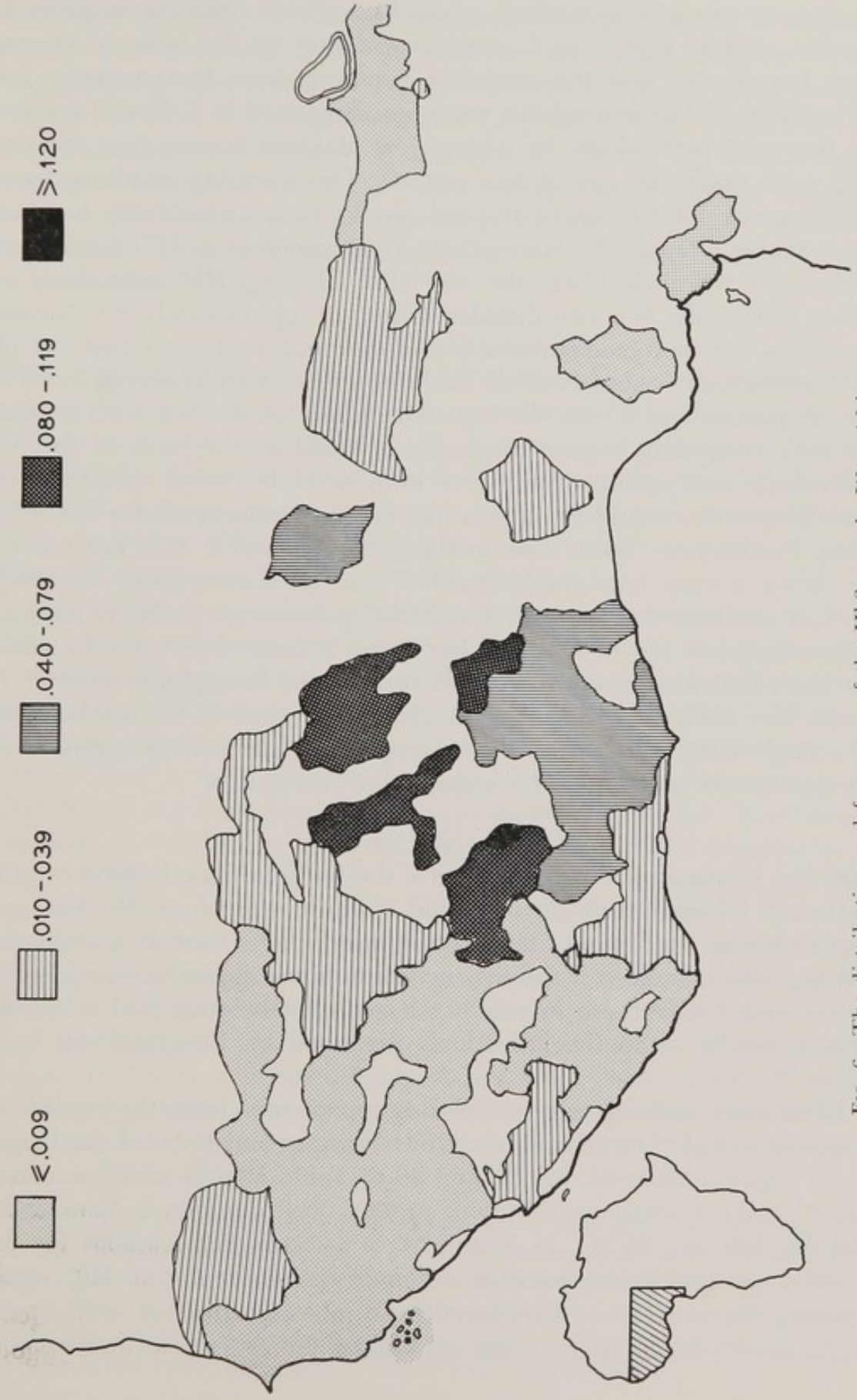

FIG. 6—The distribution and frequency of the Hb^C_β gene in West Africa.

involves an excess of individuals whose hemoglobin upon electrophoresis appears entirely type S or C, over expectation on the basis of known gene frequencies and the mortality resulting from homozygosity for Hb^S_β or Hb^C_β.[286] The hemoglobin surveys summarized in Table 10 involve for the most part adults. In Africa, few children homozygous for the Hb^S_β gene reach the age of five years.[222] The mortality resulting from homozygosity for Hb^C_β under tropical conditions is undoubtedly less but the available estimates[18, 97] are conflicting (discussion on p. 237). Somewhat arbitrarily, we shall place the mortality of Hb^C_β / Hb^C_β individuals in Africa during the first two decades of life at approximately 0.5. Nevertheless, in our combined material for Liberia and the Ivory Coast, out of 1281 individuals (tribes 17-29 of Table 10), there were observed 74 with the AS pattern and 2 with the S pattern. Expectation, even with normal survival, computing a gene frequency of 0.029 on the basis of the AS individuals and ignoring the error introduced by tribal variations in gene frequency, would be approximately one homozygote for the Hb^S_β gene. Furthermore, there were in this same material 8 AC's but one C, the latter a very improbable event if due to homozygosity for Hb^C_β. Further evidence that these all-S and all-C patterns are probably not due to homozygosity for the Hb^S_β and Hb^C_β genes is derived from the fact that the three individuals in question had near normal hemoglobin levels.[286] It seems very likely that these individuals are the result of the combination of a single Hb^S_β or Hb^C_β gene with a gene of the thalassemia complex. If so, a *minimum* estimate of the frequency of this gene is

$$\frac{2+1}{(74+8+2+1)} = \frac{3}{85} = .036.$$

This is a minimum estimate because if the Hb^S_β-thalassemia gene combination in Liberia results in increased mortality—which on the basis of experience in the United States seems quite likely—then in a study involving almost exclusively adults such as ours in Liberia we would tend to encounter only those in whom the gene combination had relatively benign results, as in the individuals described by Edington and Lehmann.[100, 101]

Even more striking evidence of this type comes from the studies of Sansarricq et al.[334] on the Senufo, Diula, Tusyan, and Bobo of the Upper Volta region (tribes 32, 33, 35, and 36 of Table 10), to which our own data[247] make a small contribution. Among 3530 individuals there were 204 AS, 596 AC, 22 SC, 14 S, and 51 C patterns. Expectations for the S and C patterns if they result from homozygosity for Hb^S_β or Hb^C_β, again ignoring the small error introduced by combining tribes of unlike gene frequencies (which in any event only biases the estimate of homozygote

frequency upwards), and assuming normal survival, would be approximately 3 or 4 S's and 27-30 C's. Here is very strong evidence for the presence of a thalassemia-type gene. To what extent the all-C patterns truly result from homozygosity for the Hb^C_β and to what extent from the Hb^C_β-thalassemia combination is unknown. If survival for Hb^C_β homozygotes is as calculated by Allison,[18] then perhaps the ratio of the two types is about 1:3. For purposes of calculation, we will adopt that ratio. Then a *minimum* estimate of the frequency of the thalassemia complex genes in these tribes becomes

$$\frac{14 + \tfrac{3}{4} \times 51}{(204 + 596 + 14 + \tfrac{3}{4} \times 51)} = .061.$$

Thus a variety of observations and calculations point to a combined frequency of genes of the thalassemia complex in the Volta-Ivory Coast-Liberia region of something like .05. For calculating the gene frequencies of Table 10, we have adopted the convention of regarding the all-S and all-C patterns as the result of a thalassemia gene in combination with an Hb^S_β or Hb^C_β gene. While this is not strictly accurate, it seems the most convenient way to meet this situation.*

The second outstanding characteristic of the hemoglobins of West Africa is the occasional occurrence of hemoglobins K and N in representatives of many of the tribes studied. The locations in which hemoglobins K and N have been encountered are shown in Figure 7. Although there are sometimes technical problems in distinguishing between the various "fast" hemoglobins, on the basis of present knowledge it appears that N has not been encountered outside of West Africa. K—although reported in Italy[353]—has been encountered in frequencies comparable to those of West Africa only in North Africa. This hemoglobin was first encountered among the Kabyles in the region of Tigzirt by Cabannes and Buhr,[51] where it was found in 13 of 114 individuals tested. Subsequent publications from this same group on the results of an extensive survey of Algerians record 13 AK individuals among 3918 tested.[302] It appears that these are the same 13 AK individuals as in the original report. If this is so, then in North Africa hemoglobin K would appear to have a restricted distribution indeed. It is noteworthy that in India hemoglobin L appears to present a picture analogous to that presented by K and N in West Africa.[371]

* Note added in proof: Since this manuscript was submitted, an evaluation of all the data from Liberia and the western Ivory Coast has led to the conclusion that in those regions the frequency of genes of the thalassemia complex is approximately .02-.03 (Neel, Robinson, Zuelzer, Livingstone and Sutton, Am. J. Human Genet., in press). The available evidence thus suggests a greater frequency of the thalassemia complex in the Upper Volta than in Liberia and the Ivory Coast.

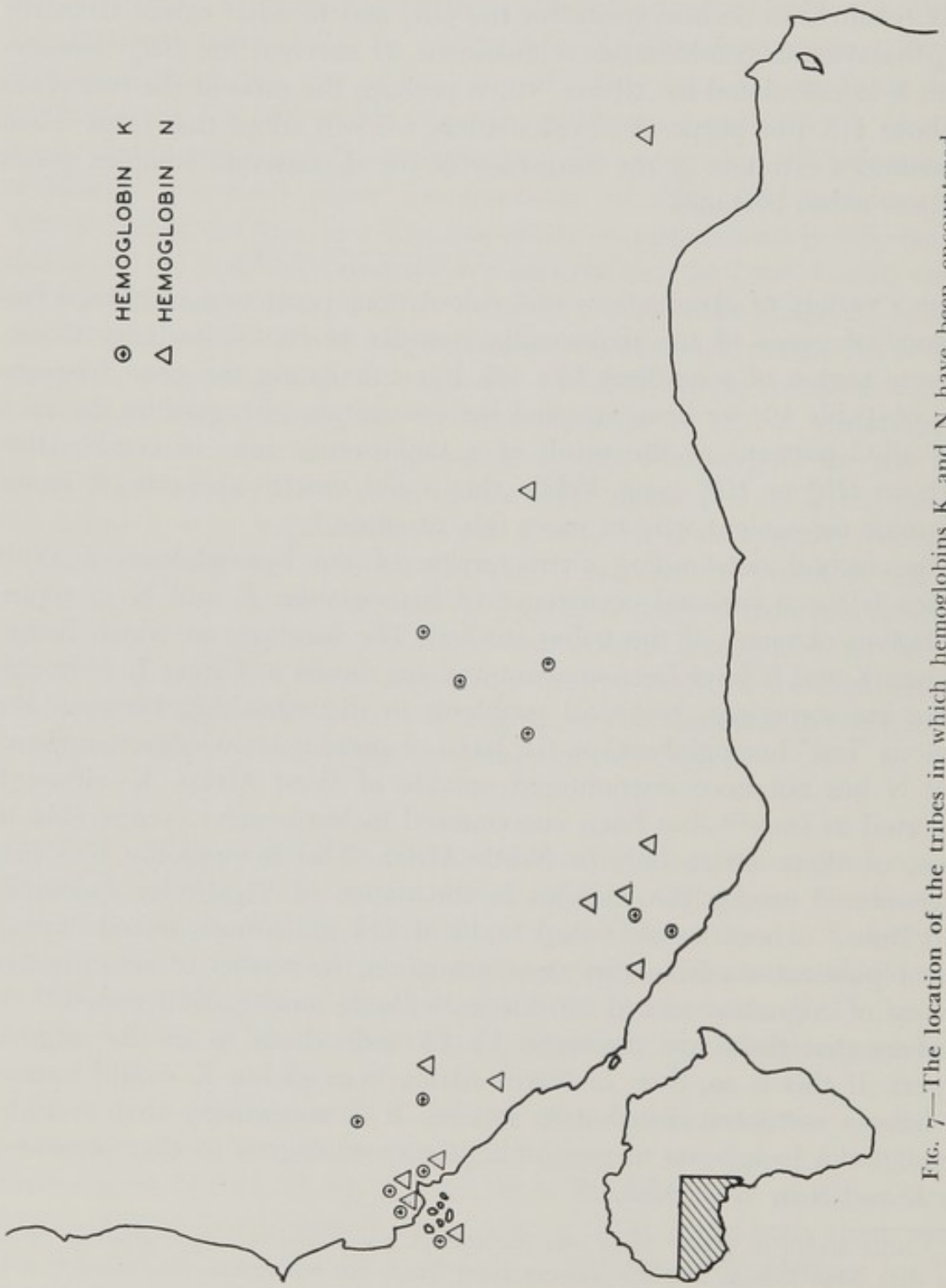

FIG. 7—**The location of the tribes in which hemoglobins K and N have been encountered.**

The Anthropology of West Africa

For the attempt which we will now make to give anthropological and genetic perspective to these striking findings, certain background data concerning the anthropology of West Africa become necessary. Although the principal developments of the past 1000 years are reasonably well recorded, beyond this there is relatively little information. It seems likely that West Africa has been inhabited by man from very early times onward, but thus far there have been no systematic efforts to uncover evidences of the past in West Africa comparable to those for East and South Africa. From our standpoint, the most important geographical feature is the subdivision of the area into a series of climatic "bands," these being, from the Sahara Desert southwards, dry grassland, savannah, park savannah, and tropical rain forest. As the Sahara Desert has expanded during the past 5000 or so years, these areas have probably been slowly shifting southwards. The traditional routes of commerce and conquest in West Africa have been east-west, through the savannah country. Exchange between the interior north and the southern coastal areas has been primarily along the great rivers of West Africa, and certainly not over mountain ranges such as occur in the interior of Liberia.

West Africa contains a variety of cultures and physical types. The languages of West Africa for the most part fall into the Niger-Congo grouping. However, within this grouping there is great linguistic diversity, suggesting that West Africa has been inhabited for a relatively long time by the speakers of Niger-Congo. In Figure 8 we have indicated the distribution of the principal subdivisions of the Niger-Congo linguistic grouping, following Greenberg.[132] Thus far there has been no published attempt to derive a glotto-chronology for these languages.

Repeatedly, students of West African anthropology have been impressed by the occurrence throughout West Africa of tribes whose general cultural level was far below that of some of their neighbors. These have been referred to as paleonegroid, indigenous, or relict tribes. Culture has many dimensions—the problem of an objective rating-scale is obvious. Perhaps for this reason, no one has as yet, to our knowledge, attempted a systematic classification of these "indigenous" tribes. From the writings of Reeve,[313] Baumann and Westermann,[27] Richard-Molard,[319] Jones,[196] and Murdock,[274] we have drawn up the list of such tribes contained in Table 11, also presented graphically in Figure 9. This is not a complete list. Thus, certain of the anomalies brought out on the map, such as the "islands" of apparently advanced culture surrounded by a more retarded people, may indicate merely that no investigator has yet

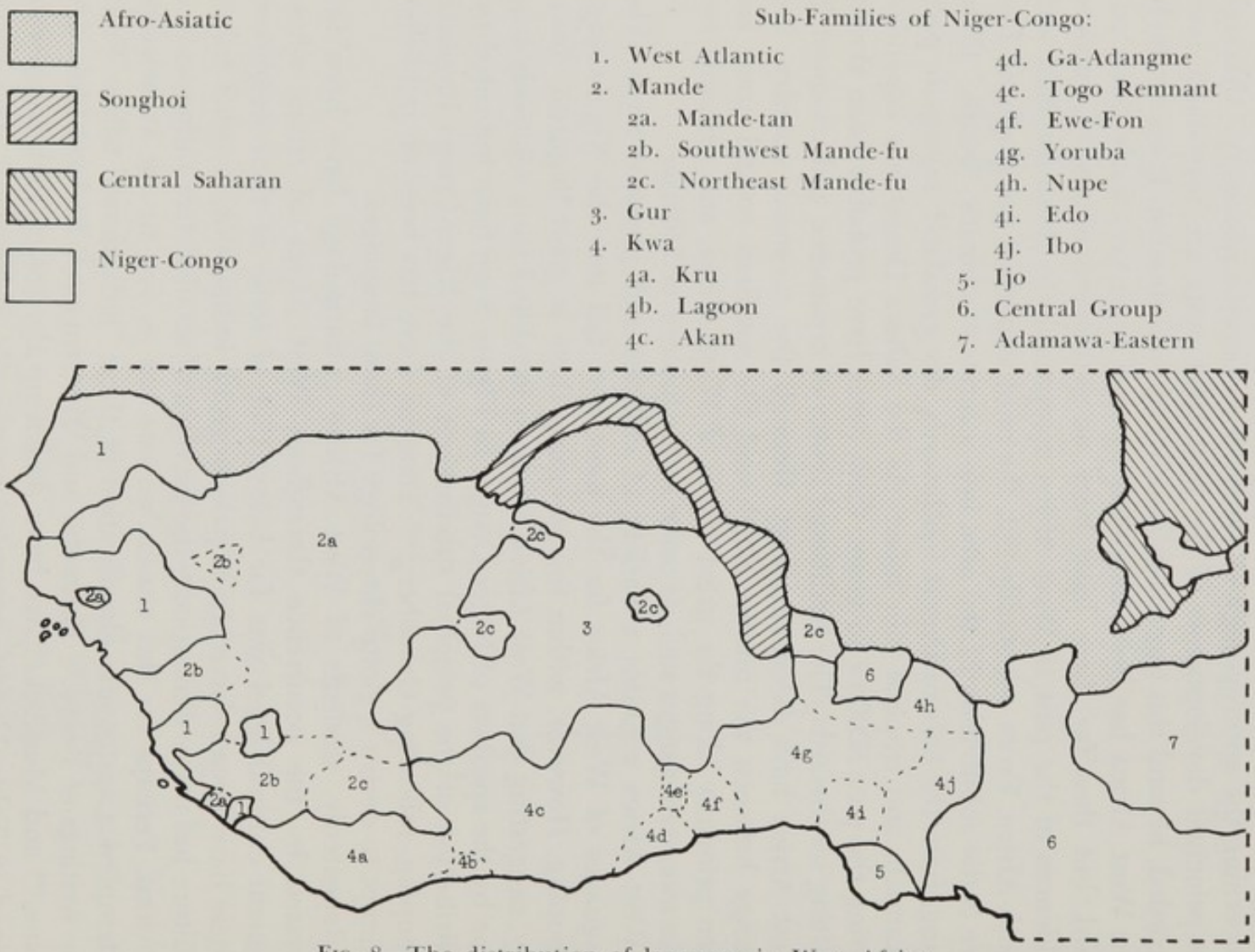

FIG. 8—The distribution of language in West Africa.

characterized these people to the point where a decision regarding cultural status is possible. It will be noted that this classification cuts across the linguistic classification, i.e. we find both "advanced" and "primitive" cultures recorded in five of the seven principal subdivisions of the Niger-Congo languages; for the remaining two it may be merely a matter of lack of information. It will be further noted that to a very considerable extent these "paleonegroid remnants" are to be found in the tropical rain forest belt or transitional regions. To what extent they have been forced into these areas by other culturally superior and more war-like tribes, and to what extent they represent long-time inhabitants of these areas, is not clear, and undoubtedly varies from tribe to tribe.

TABLE 11—*A Partial List of the "Paleonegritic" Tribes of West Africa (after Baumann, Jones, Richard-Molard and Reeve)*

West Atlantic speakers	Gagu	Buem
Wolof	Guro	Akposo
Serer	Gan	Ewe
Diola	Bozo	Avatime
Banyun	Diula	Basila
Balante		Egba
Pepel	*Kwa speakers*	Popo
Bijogo	Kran	Ekiti
Tenda	Sapo	
Nalu	Kru	*Gur speakers*
Baga	Bete	Senufo
Bulom	Bakwe	Minianka
Temne	Dida	Wara
Limba	Avikam	Dogon
Kissi	Ajukru	Bobo
Sherbro	Alagya	Dagari
Gola	Ebrie	Birifor
Fulani	Assini	Lobi
	Mekyibo	Grunshi
Mande speakers	Ari	Gurensi
Dialonke	Abe	Konkomba
Susu	Attie	Kabre
Koranko	Baule	Naudeba
Mende	Krachi	Somba
Toma	Atyuti	
Kpelle	Adele	*Central speakers*
Dan	Tribu	Tiv
Ngere	Kebu	Fia

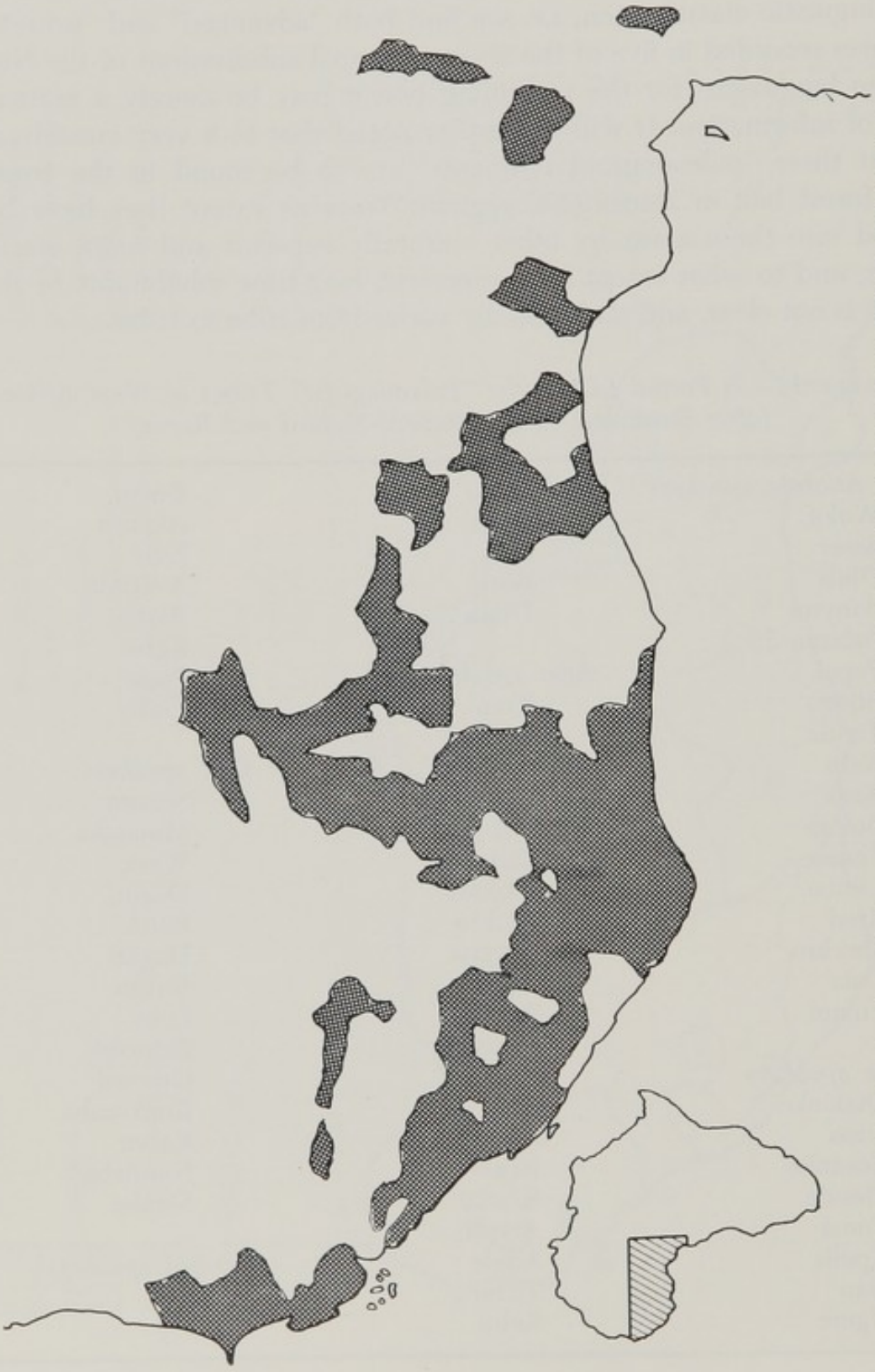

Fig. 9—The distribution of the so-called relict, "paleonegroid," or indigenous tribes of West Africa.

The Interpretation of the Distribution of Hemoglobin Abnormalities in West Africa

We return now to the anthropological and genetic significance of the hemoglobinopathy genes in West Africa. Any interpretive attempt is immediately confronted with the question of the origin or origins of the genes under consideration. A keystone of modern genetics is the concept that mutation is repetitive. It seems highly probable that the genes responsible for the various hemoglobinopathies have each arisen repeatedly in the past, although, from the quantitative standpoint, the responsible mutations may be relatively rare. This notwithstanding, the present distribution of both hemoglobin C and S in West Africa seem explicable on the basis of one or a relatively few principal foci of origin for either the gene or a critical selective factor, and we shall pursue that hypothesis in what follows, although recognizing that it may be an oversimplification.

Livingstone[246] has marshalled impressive evidence for the Sudan region of the eastern two-thirds of West Africa being the principal area from which the Hb_{β}^{S} gene was disseminated throughout Africa. For the detailed arguments, to which we subscribe, the reader is referred to the original paper. This viewpoint explains the present pattern of distribution in part on the basis of large-scale population movements, some of which are subject to documentation,[107, 108, 274] but to an even greater extent to the "flow" of a gene of high selective value, i.e. its introduction into an area by a relatively few migrants, with a subsequent relative increase in gene frequency due to positive selection. An additional possible factor in the distribution will be discussed below. Some years ago, one of us,[279] in considering the over-all picture of the Hb_{β}^{S} gene in West Africa, was led to speculate that "the mutations responsible for these hemoglobin types arise very infrequently but under special circumstances then become widely disseminated. Either the mutation is very rare or the 'special circumstances' are, geologically speaking, of recent origin—otherwise, one would expect the genes to be much more widely disseminated." In point of fact, the two alternatives suggested are not mutually exclusive, but could be combined. There is evidence for a major center of plant domestication in areas now occupied by the Mande speakers of the Niger-Congo linguistic grouping (cf. Fig. 8).[274] In addition, as Livingstone[246] has emphasized, there is evidence for the extensive introduction, about the beginning of the Christian era, of domesticated plants and agricultural techniques, and iron working, into West Africa from Asia Minor via Egypt. Without wrought iron tools, clearing land for

agricultural purposes could only proceed on a very small scale. With the introduction of iron working and the appropriate plants, agriculture first became possible on a really significant scale in West Africa. It is Livingstone's thesis that the resultant ecological changes as well as the increase in population numbers created just those conditions under which malaria becomes a very major cause of morbidity and mortality. If then it is, as seems probable, a relative resistance to malaria that is a major factor in the frequencies attained by the sickle cell trait in Africa, the conditions under which this resistance becomes really important are no more than a few thousand years old in northeastern West Africa, while in the tropical forests of southwestern West Africa, these conditions have only come into being in the past millenium. The highest frequencies of the sickle cell gene in West Africa are today encountered in parts of Nigeria, the Gold Coast, Sierra Leone, and the Gambia—these are postulated to be areas where the Hb^{S}_{β} gene was present at a relatively early date and, with the introduction of agriculture and the more intense malaria pressures than in the savannah country, achieved relatively high frequencies. Conversely, the very low frequencies of this gene in some of the "paleonegroid" tribes of Portuguese Guinea, Liberia, and the Ivory Coast may be attributed to the fact that the gene has only very recently been introduced into these areas.[239, 245, 280]

The situation with regard to the Hb^{C}_{β} gene is very different. The reader will have noted the remarkable correspondence between the area of relatively high Hb^{C}_{β} frequency and the Gur sub-family of the Niger-Congo languages. These are the Voltaic peoples of Murdock[274]—a collection of many culturally diverse, usually small tribes, whose agricultural practices are rather advanced, quite likely as a result of borrowing from the Mande to the west, but who otherwise, as a whole, exhibit a rather primitive culture. They appear to have been compressed into their present distribution by the more powerful tribes to the north, west, and east. The degree to which the Hb^{C}_{β} gene is restricted to this group suggests—to the extent that language defines a genetic community—that this gene did not attain an appreciable frequency until the linguistic differentiation of Africa was well advanced. On these grounds, then, the gene, at least in its present frequency, would appear to be of relatively recent origin. Unfortunately from the standpoint of establishing the genetic isoclines which could be of great help in understanding the history of this gene, there are, as Figure 6 shows, very critical gaps in our knowledge of its distribution.

The question immediately arises, as to whether the Hb^{C}_{β} gene attained appreciable frequencies before, at approximately the same time, or subse-

quent to the Hb^{S}_{β} gene. A related question is whether appreciable frequencies of the Hb^{S}_{β} gene first appeared in the same people as the Hb^{C}_{β} gene or further to the east. In our opinion, these questions cannot be answered at this time, and, indeed, may never receive definitive answers. In the face of constant selective pressures, the "older" gene should have attained equilibrium frequencies over wider areas. However, inasmuch as the only two studies of the selective value of $Hb^{S}_{\beta}/Hb^{A}_{\beta}$ versus $Hb^{C}_{\beta}/Hb^{A}_{\beta}$ performed in areas where their distribution overlaps to a significant extent have yielded conflicting evidence regarding the relative values of the two genotypes, we are scarcely in a position to reach decisions concerning equilibrium. Furthermore, although, as already brought out, a differential susceptibility to malaria is probably the major factor in maintaining the Hb^{S}_{β} polymorphism, at the moment we have no really good clues as to what is responsible for the Hb^{C}_{β} polymorphism. We are thus scarcely in a position to conclude which gene has been subject to favorable selection pressures for the longer period. The indirect evidence bearing on this point is confusing. Thus, the fact that the Hb^{S}_{β} gene appears to have penetrated into areas not yet reached by Hb^{C}_{β} (e.g. Liberia and the Ivory Coast) could be construed as an argument for the greater antiquity of the Hb^{S}_{β} gene—but might also to some unspecifiable extent reflect a greater selective advantage of Hb^{S}_{β}. Likewise, the fact that the Hb^{C}_{β} gene has a significantly higher frequency in Algerians as a group than the Hb^{S}_{β} gene[50] could indicate that the former has been diffusing into North Africa over a longer period of time than the latter, or, conversely, that the selective disadvantage which may exist for the $Hb^{S}_{\beta}/Hb^{A}_{\beta}$ genotype outside of a tropical and subtropical ecology may have resulted in a more significant elimination of Hb^{S}_{β} than of Hb^{C}_{β} genes subsequent to their diffusion into North Africa. If the ecological developments favoring the attainment of considerable frequencies are roughly comparable for the two genes, then each might have been present in one or several populations at very low frequencies until the advent of malaria and other "new" selective agents, and then have increased in frequency at the same time.

What may we conclude from the fact that the Hb^{S}_{β} and Hb^{C}_{β} genes are absent in such diverse groups as the West Atlantic speakers of Portuguese Guinea, the Kwa speakers of Liberia and the Ivory Coast, and, probably, some of the tribes (still inadequately identified) of the southern Cameroons? If West Africa had been peopled by a small band of immigrants who subsequently increased greatly in numbers and became linguistically subdivided, we would be correct in concluding that the appearance of the two genes under discussion, at least in significant numbers, postdated the onset of linguistic diversification. In this case, the glotto-

chronology which will in time undoubtedly be forthcoming for the languages of West Africa would be of great help in dating the appearance of these two genes. However, in view of the probable great antiquity of man in West Africa, it is conceivable that these two genes even predate the onset of linguistic diversification, although not of course in their present frequencies. In any event, it does seem highly probable that major components—and perhaps all—of the early inhabitants of West Africa lacked hemoglobins S and C. The simple fact that hemoglobin C is predominantly encountered in "paleonegroid" groups and S in culturally more advanced groups is not sufficient to date the relative antiquities of high frequencies of these two genes.

The fragmentary information available concerning the thalassemia complex raises the possibility that with further data here, too, we will encounter a rather strikingly restricted distribution, but one not correlated with a linguistic subdivision to the same extent as hemoglobin C. Thus, the direct and indirect evidences on the basis of which we have postulated relatively high frequencies of the thalassemia gene(s) in the Kwa speakers of Liberia and the Ivory Coast have not been encountered to nearly the same degree in the Kwa speakers of Ghana and Nigeria. As already noted, there is evidence of a considerable amount of the thalassemia complex among the Voltaic Gur-speakers. On any type of simple diffusion-plus-selection hypothesis, the impulse would be to interpret this pattern as suggesting the greater antiquity of the thalassemia complex than the Hb^{C}_{β} gene, since in this instance both are found in the paleonegroid groups. In this connection, it is noteworthy that thalassemia has a rather high frequency in North Africa, although exact figures are not available.[302] However, conclusions here are stringently limited by the probable heterogeneity of the thalassemia complex. Thus, at this stage of our knowledge it is entirely conceivable that the thalassemia of the Gur speakers tends to differ in kind from that of the Kwa speakers—and both from the thalassemia of North Africa. With current techniques, an attack on this question is possible. For instance, there is already evidence that the thalassemia of the American Negro is less often accompanied by an elevation of the A_2 hemoglobin component than the thalassemia of the American Caucasian.[73]

In rounding out this discussion of the distribution of the abnormal hemoglobins in West Africa, reference must be made to the "trace" hemoglobins, N and K. Table 10 and Figure 7 show that these have been encountered almost exclusively in the western half of West Africa, where their distribution corresponds to a noteworthy degree to that of the paleonegroid elements. It is tempting to speculate that these are hemo-

globins of great antiquity, which, unlike S, C, and the thalassemia complex, did not confer a marked selective advantage with changing ecology, and so remained at low gene frequencies. *Indeed, it seems possible that modest frequencies of the* Hb^S_β *and* Hb^C_β *genes throughout West Africa predated the appearance of certain critical selective factors, the distribution of these two genes prior to the advent of the selective pressures which result in their present frequencies being roughly comparable to what we see today with respect to hemoglobins K and N. In this case, the present-day patterns of distribution for* Hb^S_β *and* Hb^C_β *reflect both the result of selection of a low-frequency allele by an advancing wave of changing selective pressures as well as the pattern created by the migration of individuals from an area of high gene frequency to one of low.* The alternative concept, of "the right mutation at the right time," demands, of course, a low mutation rate, although, in view of the considerations of Section VI, perhaps not unusually low.

The Evaluation of Genetic Equilibrium

To what extent are the West African populations in genetic equilibrium for the $Hb^S_\beta - Hb^C_\beta - Hb^A_\beta$ system? Allison[18] has made a preliminary calculation of the fitness of the various genotypes in this system on the basis of electrophoretic studies of hemoglobins of 1042 adult Africans from Ghana, especially southern Ghana. Utilizing an equation derived by Penrose, Smith, and Sprott[298] in their treatment of the problem of stability in systems of multiple alleles, *and assuming as a point of departure genetic equilibrium in the southern Gold Coast* (see below), Allison[20] has concluded that with the exception of Gambia, "the other West African populations tested (Nigeria, Ghana, Sierra Leone) have lived long enough in relatively uniform environments to show frequencies of the abnormal hemoglobin genes close to those predicted for a semi-stable equilibrium from the estimated fitness of the several genotypes." Quite aside from the question of the validity of assuming genetic equilibrium at this locus in southern Ghana, the subsequent demonstration of the low Hb^S_β, low Hb^C_β areas in Portuguese Guinea, Liberia, and the Ivory Coast suggest that large areas of West Africa are a far cry from genetic equilibrium. Thus, Penrose, Smith, and Sprott[298] have shown that a *semi-stable* equilibrium can be attained in a three-allele system of the type under discussion if $k_1a_2 + k_2a_3 = k_3$, where a_2 and a_3 are the frequencies of two of the three alleles, and k_1, k_2, and k_3 are constants. Graphically this is a straight line with the intercepts at either the x or y axis defining gene frequencies when the Hb^S_β or Hb^C_β allele is missing

from the system. Under a variety of different relative viabilities for homozygote and heterozygote than assumed in the situation above, one can also attain a *stable* equilibrium in a three-allele system. These latter relations do not lend themselves to any simple formulation such as the above. In Figure 10 we have plotted the values for the Hb^{S}_{β} and Hb^{C}_{β} genes for the tribes summarized in Table 10. Assuming only that some tribes have reached values satisfying the conditions for a semi-stable equilibrium defined above, an approximate graphic solution for this equation with respect to this system is given by the straight line best fitting the greatest value of x (within the limits of sampling error) observed for each value of y. Such a line has been drawn freehand in Figure 10. The subspace adjacent to this line contains a variety of gene frequencies which meet the condition for a stable equilibrium if slightly different assumptions are made as regards the value of the genotypes concerned; but in general, assuming constancy of selective pressures, entries below a line drawn from .08 on the ordinate to .08 on the abscissa —which include about half the tribes thus far studied—are certainly not in equilibrium, even with due allowance for sampling error. The possibility must be considered that in the low S and C areas there occurs thalas-

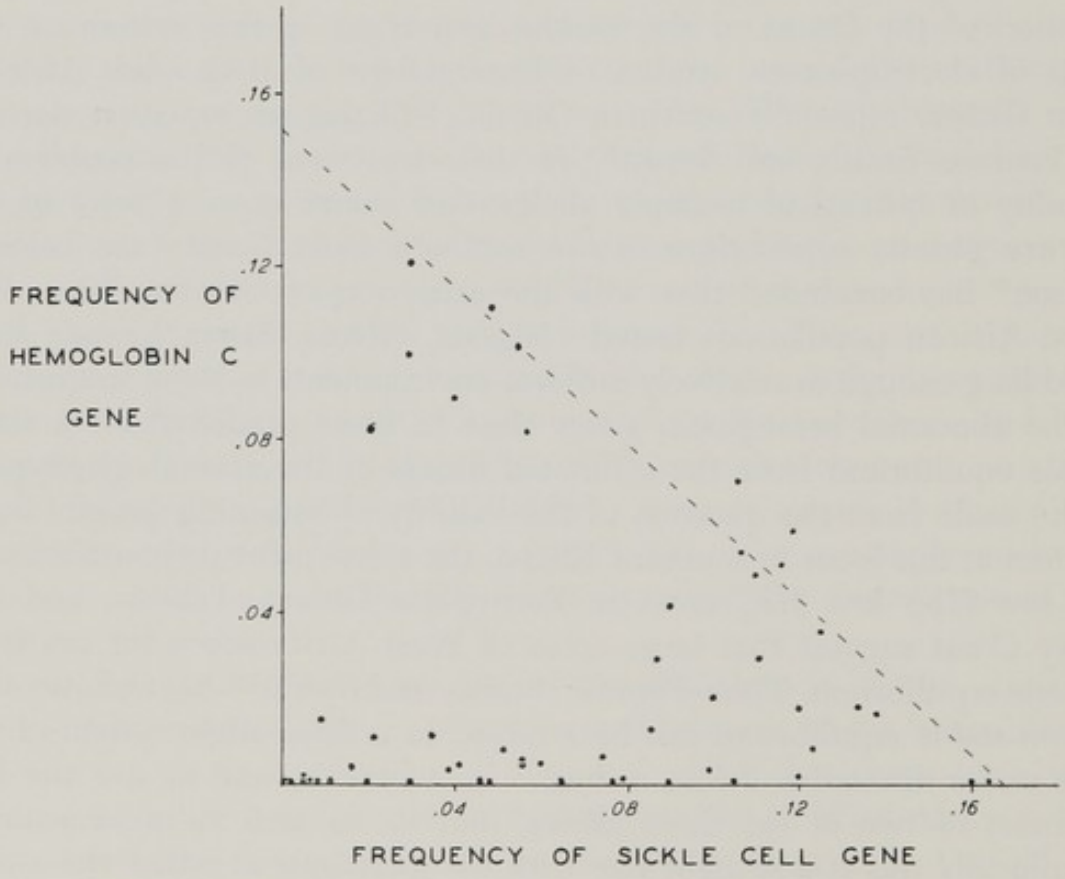

FIG. 10—Frequency of hemoglobin C gene and sickle cell gene.

semia due to an allele at the Hb_β locus at a sufficient frequency to maintain a stable four-allele equilibrium. While this possibility cannot be completely discarded for the Upper Volta, it seems doubtful it would apply to southeastern Liberia and the Ivory Coast or to Portuguese Guinea.

Additional reasons for reservations concerning population equilibrium emerge from the work of Edington and Laing.[97] On the basis of studies utilizing primarily adult inhabitants of southern Ghana, with Hb_β^S and Hb_β^C gene frequencies of 0.100 and 0.059 respectively, *and assuming equilibrium*, Allison[18] estimated the fitness of Hb_β^A/Hb_β^A, Hb_β^S/Hb_β^A, and Hb_β^C/Hb_β^A individuals at 0.976, 1.138, and 1.103 respectively, recognizing from his data the possibility that "the estimates may not be quite correct." Edington and Laing,[97] on the other hand, have provided age-stratified data for 1040 inhabitants of northern Ghana, with Hb_β^S and Hb_β^C frequencies of 0.050 and 0.108 respectively. When the data are subdivided by age into subjects <1 year of age and subjects >1 year of age, the genotype frequencies are not significantly different, but such differences as are observed are in the direction expected if there is positive selection for Hb_β^S and Hb_β^C heterozygotes. If we take the data at face value, and equate the figures for "<1 year of age" to zygote frequencies, and the figures for ">1 year of age" to adult frequencies—an obvious oversimplification—then the approximate fitness values for Hb_β^A/Hb_β^A, Hb_β^S/Hb_β^A, and Hb_β^C/Hb_β^A can be calculated to be 0.948, 1.042, and 1.129 respectively. In the latter calculation, no assumption regarding equilibrium is involved. In the derivation of both fitness values, no allowance has been made for differential fertility. The tenuous nature of calculations utilizing nonsignificant differences is obvious, as is the acute need for more data. Intuitively, one would suspect that Edington and Laing's value for the Hb_β^S/Hb_β^A genotype is too low. However, the fact is that these are the only two bodies of data available for comparing in one and the same study the fitness of Hb_β^S and Hb_β^C heterozygotes, and they yield rather different results for the Hb_β^S heterozygote. It seems most unlikely that the different selective values estimated in the two studies are entirely an example of the dependence of selection coefficients on allelic frequencies, although this may be a factor.

An even more striking discrepancy between the two studies, although one with a very large sampling error, is that whereas in Allison's study the fitness of Hb_β^C/Hb_β^C is estimated at 0.55, in Edington and Laing's study it can be estimated at 2.25! This estimation involves the assumption that all individuals with the all-C pattern are Hb_β^C/Hb_β^C in genotype. We have already seen that this assumption is on shaky grounds in areas where the thalassemia complex also occurs. However, the data

of Edington and Laing fail to show the accompanying relative increase in the all-S patterns we would expect if this were the entire explanation. Taken at face value, these data preclude the conditions demonstrated by Penrose, Smith, and Sprott[298] to be necessary for a stable equilibrium in a three-allele system.

The clash of data presents us with an interesting dilemma. We can, on the one hand, invoke sampling error, in which case we have at present little grounds for reaching any conclusions concerning the relative selective values of the genes concerned and current population trends. We can, as one of several other alternatives, accept the change of the apparent selective values of Hb_β^S within a distance of 300-400 miles as valid, in which case, malaria pressures being as similar as they are for the two regions, we must admit to the gene participating in several selective systems—a possibility already raised on other grounds in the section on selection.

In a two-allele, balanced polymorphic system at equilibrium, where the fitnesses of the three genotypes concerned are $1-s$, 1, and $1-t$, the frequencies of the two alleles are

$$\frac{t}{s+t} \text{ and } \frac{s}{s+t}$$

(references and demonstration in Li.)[242] The data of Allison[18] and Edington and Laing[97] yield fitness values for the Hb_β^A/Hb_β^A, Hb_β^A/Hb_β^C, and Hb_β^C/Hb_β^C genotypes, on the assumption that fitness is determined entirely by differential survival. We will rather arbitrarily set the value of the Hb_β^C/Hb_β^C genotype at 0.5 for both sets of data. Allison's data then yield estimates of s and t of .115 and .546, from which q, the equilibrium value for Hb_β^C is calculated to be .174. Edington and Laing's data yield values for q of .222. Both estimates are tempered by the possible occurrence of a thalassemia allele in the system. However, taking the estimates at face value, they are both in excess of the value of 0.15 yielded by the graphic solution of the equilibrium equation which was presented in Figure 10. This discrepancy may find an explanation in some one or combination of the following: 1. the available data may provide estimates of fitness that are too high, 2. equilibrium frequencies for Hb_β^C may not yet have been reached in any but a very few tribes, and/or 3. the presence of thalassemia in tribes with high Hb_β^C frequencies may be depressing the levels attained by the latter gene. Again it should be remarked that the possible role of differential fertility has been ignored.

Incidentally, if the fitness values of Allison[18] for the Hb_β^A/Hb_β^A and Hb_β^A/Hb_β^S genotypes given above are accepted, and the value for Hb_β^S/Hb_β^S set at 0, as is now known to be correct, this same approach

suggests that equilibrium would be achieved at a gene frequency of approximately .123, corresponding to a frequency of the sickle cell trait, after the death of homozygotes and the action of selection, of 25 per cent rather than the 40 per cent observed in several tribes in East Africa and assumed in a number of calculations to represent the equilibrium value. In view of the occurrence of hyperendemic malaria in extensive regions of West Africa where the sickle cell gene is also present, it is difficult to understand the apparent differences in equilibrium gene frequency between East and West Africa. The possibility arises that the gene has actually been present (and subjected to selection pressures) longer in parts of East than West Africa.

The foregoing treatment involves numerous assumptions and approximations, necessitated in large part by the fragmentary data available despite the efforts of many persons. Its critical tone is not meant to detract from the signal contributions which have been made in this field, but rather to emphasize how much remains to be learned concerning the polymorphism frequently cited as the best understood for man or any other animal.

Other Genetic Clines in West Africa

As is apparent from Figure 5, the cline exhibited by the Hb^{s}_{β} gene from northwest to southeast Liberia is striking, the gene frequency falling from about 0.08 in the northwest to essentially zero in the southeast.[245] This situation presents an unusual opportunity to explore the extent to which a cline in one genetic system may occur independently of similar findings in other systems. Livingstone et al.[248] and Sutton et al.[374] have reported on observations in Liberia and the Ivory Coast on 12 other genetic systems, namely, the ABO, MN, Rh, Kell, Duffy, Diego, Henshaw, U, P, Kidd, and Jay systems controlling erythrocyte antigens, and the genetically controlled serum haptoglobins. As regards the erythrocyte antigens, only with respect to two of the "minor" groups, Henshaw and U, was there any evidence for a cline, which in any event cannot at this stage be said to parallel the Hb^{s}_{β} cline closely. Livingstone et al.[248] point out that if we accept the general thesis

> . . . that the gene frequencies of many blood group systems are maintained by a balanced polymorphism, the similarity of blood group frequencies throughout Africa implies that the selective forces maintaining these balanced polymorphisms are relatively constant throughout this region. Whatever these forces are, it is noteworthy that the great changes which resulted from the adoption of an agricultural economy by hunting populations and which are responsible for the

spread of falciparum malaria and the sickle cell gene have not affected them. In addition to the primitive "relict" populations in Portuguese Guinea and Liberia, the Pygmies and Bushmen have frequencies of the blood group genes which are similar to those of surrounding Bantus. Since the transition to an agricultural economy results in changes in the diseases prevalent among the population, the absence of marked differences in the frequencies of the blood group genes between agricultural and hunting populations in Africa seems to imply that these diseases are not involved in the selective forces maintaining the blood group gene frequencies. However, since it seems most likely that a great many diseases are involved in these selective forces, an alternative explanation is that the equilibrium frequencies are the same in both a primitive hunting population and in an agricultural population but are maintained by quite different kinds of selection.

The situation with respect to the inherited serum haptoglobins appears to be different. The three principal types of serum haptoglobin patterns are determined by a pair of genes, Hp_1 and Hp_2. For the data from Liberia and the Ivory Coast, the weighted linear regression of Hp_1 frequency on sickle cell trait frequency is significant at the 5 per cent level. The chief known function of the serum haptoglobins is that of complexing with free hemoglobin and reducing renal loss of hemoglobin. This is a mechanism for the conservation of iron, an essential mineral for which, especially in populations with high levels of intestinal parasitism, the metabolic balance is precarious. Malaria results in an increased rate of erythrocyte destruction. In individuals with the Hp_1 gene, the hemoglobin-binding capabilities of the haptoglobins are greater than in individuals with the Hp_2 gene,[289] so that Hp_1Hp_1 individuals would be less subject to iron loss following hemolysis than Hp_2Hp_2 persons. The available data[228] do not permit a decision as to just how important this iron-conserving mechanism might be where malaria was hyperendemic. We can only conclude that here may be a plausible physiological basis for the parallel clines presented by the Hb^S_β and Hp_1 genes—both confer an advantage in the presence of malaria.

Concluding Remarks

Although the general principles have been established that Hb^S_β and Hb^C_β are members of a polymorphic system, and that falciparum malaria is an important factor in maintaining that balance, the reader will have seen from this and the preceding sections that many of the details concerning this polymorphism remain exceedingly obscure. We have no satisfactory estimate of the mutation rates of the genes con-

cerned. We do not know what handicap, if any, these two genes confer in a non-tropical environment, i.e. the baseline against which positive selection works. We are unsure as to how much of the polymorphism is due to differential mortality and how much to differential fertility, and if the latter to any significant extent, the responsible mechanisms. We are actually not sure whether it is the Hb^{S}_{β} or the Hb^{C}_{β} gene which confers the greater selective advantage—or whether the relative advantage may vary sharply from area to area. Finally, many important details regarding the distributions of these genes remain unknown, not only in West Africa but in other parts of the world, such as India, where the Hb^{S}_{β} gene is rather widespread throughout the so-called aboriginal tribes but is also present in the "schedule castes."[41, 49, 62, 91, 115, 233, 234, 350, 351, 372]

Clearly, the elucidation of many of these questions entails immense expenditures of effort. From the scientific standpoint, is the problem that important? In the authors' opinions, yes. The demonstration of a role of malaria in maintaining the Hb^{S}_{β} polymorphism is a fundamental advance in knowledge, but it would be unfortunate if the appeal of this advance discouraged full exploration of other possibilities. On the basis of our present information, here is the most favorable opportunity in human genetics today to gain detailed insight into the precise workings of a polymorphic system. In view of the current great interest in such genetic systems, this opportunity should certainly not be missed. This is true even though we can say with considerable assurance that this particular situation, with the loss at equilibrium of approximately 4 per cent of individuals prior to reproduction, cannot be typical of very many of the genetic polymorphisms of man or other animals. Thus, it is unlikely that many such systems could still exist undetected, while, on the other hand, the total known loss from fertilization until adulthood in Western societies is not great enough to make allowance for many systems entailing a loss of 4 per cent of the population. An unusual aspect of the Hb^{S}_{β}–Hb^{C}_{β} polymorphism is that here we may be witnessing an early stage in the attempts of our species to evolve a genetic immunity to a parasite which is either relatively new or whose importance has recently increased, with the normal prospect that this would in time be superseded by a less wasteful polymorphism or genetic fixation of one or several alleles conferring a relative immunity. On the other hand, these latter genetic systems, simply because they are less wasteful, will be much more difficult to come to grips with in the requisite detail; it is these extreme systems which provide the insight and experience from the vantage point of which the less spectacular systems can be attacked.

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CHAPTER 7

Morbidity of Children from Consanguineous Marriages

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CUSTOMS AND FOLKLORE RELATING TO CONSANGUINEOUS MARRIAGES ARE found even among the most primitive people.[44, 82] Many cultures are characterized by intricate preferences for, or injunctions against, some kinds of consanguineous marriage. Incest taboos are quite general, though the definition of incest varies widely and often is biologically confused. Thus some societies ban marriages of paternal cousins, but accept or encourage marriages of maternal cousins, while other societies adhere equally firmly to the opposite rule. Some American states permit marriage of double first cousins, but outlaw uncle-niece and half-sib marriages; yet the genetic relationship is the same in all three cases. Roman Catholic canonical law prohibits marriages of godparents and godchildren, which normally have no genetic relationship. Even if the prevalence of customs regulating consanguineous marriages has a basis in primitive observations of inbreeding effects, as Tylor[73] and others unconvincingly claim, the overlay of social conventions is clearly the dominant factor in defining the degrees of relationship between which marriages are encouraged or disallowed.

Observations and speculations about the biological effects of consanguineous marriages were made long before the development of genetics, and probably before the dawn of recorded history. Darwin[11] was one of the first to carry out systematic experiments on the effects of self-

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fertilization in normally outbred plants, and Mendel[33] drew attention to the increase in homozygosity that accompanies inbreeding. After the rediscovery of Mendel's work in 1900, Bateson[1] and Garrod[17] were quick to recognize that cases of rare recessive diseases are often the issue of consanguineous marriages, and that the frequent occurrence of parental consanguinity is evidence for a recessive component in diseases whose inheritance is uncertain. With the development of human population genetics in recent years, consanguineous marriages have assumed importance not only for their immediate medical effects, but also as a delicate instrument for the analysis of genetic phenomena in man.

Types and Frequencies of Consanguineous Marriages

The terminology of consanguinity is complicated. In English, the children of the same parents are called sibs, their children are first cousins, and their grandchildren are second cousins. Children with one common parent are half-sibs, and their children are half-first cousins. The child of one's first cousin is called a first cousin once removed, and the grandchild of one's first cousin is a first cousin twice removed. Roman and canonical terms, which are used in many countries, are compared with their English equivalents in Table 1, and the common types of consanguineous marriage are diagrammed in Figure 1.

A compilation of anthropological observations on consanguineous marriages in primitive societies was made by Murdock,[44] who indicated the "preferred" marriage system with no evidence on what kinds and frequencies of consanguineous marriages actually occur. Freire-Maia[15] gives

Table 1—*A Comparison of Terms for Consanguinity*

English	Roman	Canonical	F
Sibs	2	I	1/4
Uncle-niece	3	II/I	1/8
First cousins	4 (cousins of 1st degree)	II	1/16
First cousins once removed (1-1/2 cousins)	5 (cousins of 2nd degree)	III/II	1/32
Second cousins	6 (cousins of 3rd degree)	III	1/64
First cousins twice removed	6 (cousins of 3rd degree)	IV/II	1/64

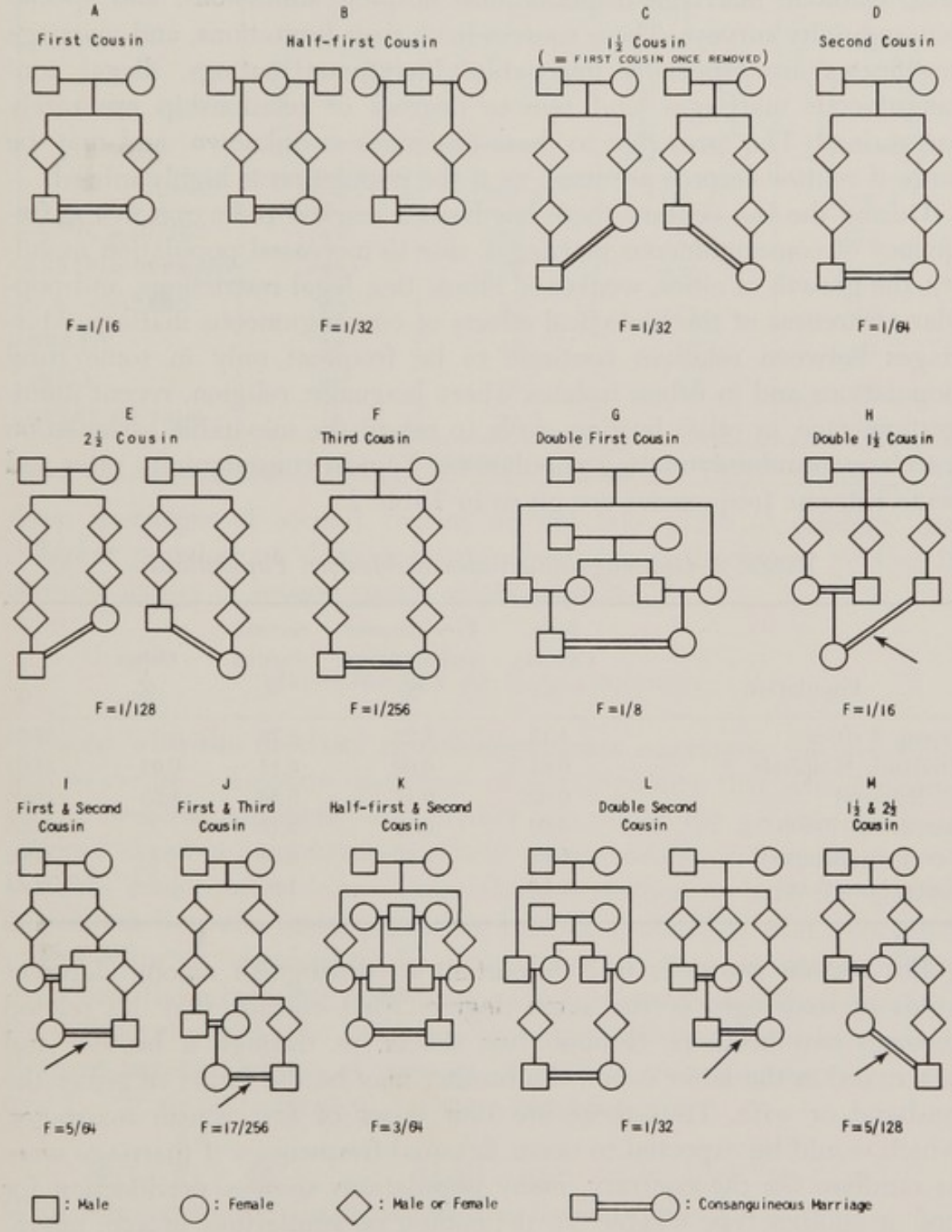


FIG. 1—Consanguineous matings.

a valuable summary of the frequencies of different types of consanguineous marriages in civilized populations, extracted from civil marriage records, Catholic marriage dispensations, hospital admissions, and special consanguinity surveys. These sources have their limitations, and marriage certificates are especially unreliable. Incestuous matings, illegal consanguineous marriages, and remote degrees of relationship are rarely ascertained. The error due to these omissions is unknown, and may be large if routine records are used, or if the population is highly inbred.

During the last century there has been a marked reduction in the frequency of consanguineous marriages, due to increased population mobility, the growth of cities, weakened ethnic ties, legal restrictions, and popular awareness of the biological effects of consanguineous matings. Marriages between relatives continue to be frequent only in some rural populations and in urban isolates where language, religion, recent immigration, race, or other barriers serve to retard the inevitable assimilation into large, randomly mating populations. Typical consanguinity rates and some extreme frequencies are given in Table 2.

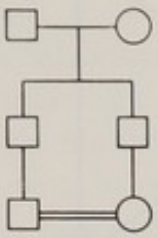
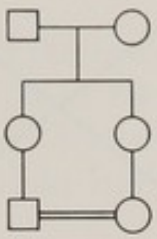
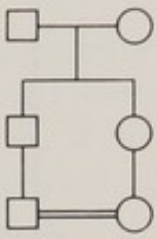
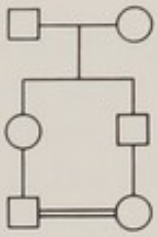
TABLE 2—*Consanguinity Rates in Modern Populations*

Population	First Cousins %	First cousins once removed %	Second cousins %	Other %	α
Japan, 3 cities	4.13	1.22	1.70	?	.0035
England, Hospitals	0.61	0.00	0.11	0.04	.0004
U. S., rural	0.00	0.44	0.88	8.85	.0006
Brazil, São Paulo	0.66	0.12	0.16	?	.0005
Germany, Munster	0.08	0.09	0.84	?	.0002
India, Marathas	10.00	——	1.9	?	.0064

For certain purposes it is important to distinguish among different kinds of marriages of the same degree. First cousins may be related through two brothers, through two sisters, or through a brother and sister, and in the latter event, the brother may be the father of either the husband or wife. Thus there are four types of first cousin marriages, which would be expected to occur in equal frequencies if marriage were at random. On the contrary, many populations show a predilection for one or another type (Table 3), depending on similarities in age, migration patterns of brothers and sisters, and the customs and economic considerations which regulate marriage preferences.[34]

Nonrandomness also characterizes marriages of first cousins once removed, in which the wife is almost always of the younger generation.

TABLE 3—*Types of first cousin marriage*

	 TYPE 1	 TYPE 2	 TYPE 3	 TYPE 4
JAPAN, 3 CITIES	149	229	127	184
AUSTRIA-GERMANY	430	656	472	591
U.S., CHICAGO	23	39	22	20
BRAZIL	55	46	53	57
INDIA	80	75	87	48
GREAT BRITAIN	12	32	17	14

□: MALE ○: FEMALE □—○: CONSANGUINEOUS MARRIAGE

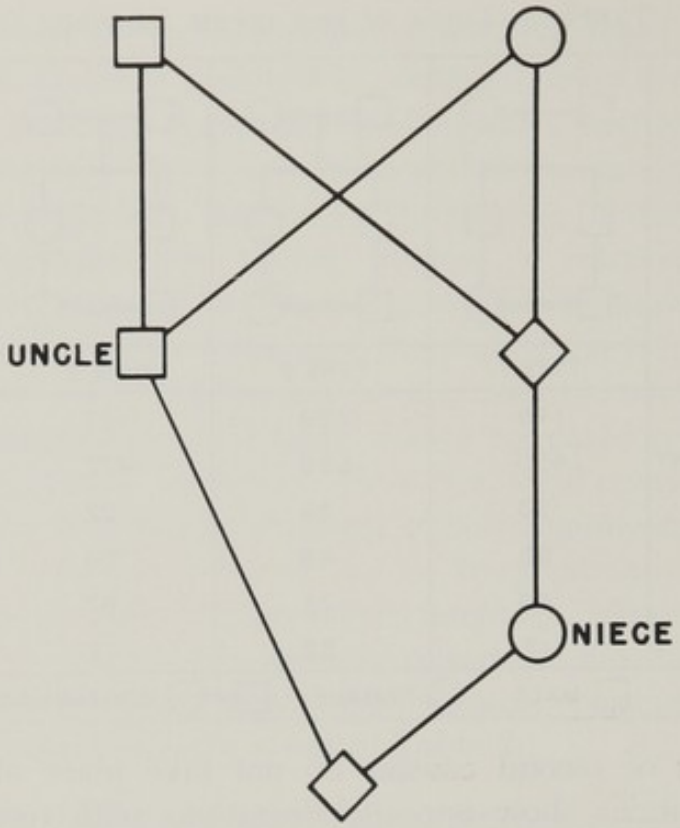
Even marriages of second cousins do not take place at random, and different populations show opposite deviations with respect to the 16 possible modes of second cousin relationship.[34]

MEASUREMENT OF CONSANGUINITY

Faced with the diversity of consanguineous marriages, the geneticist had to devise a measure of degree of consanguinity that would permit him to describe succinctly, and predict accurately, the genetic effects of different types of consanguinity. These requirements are best satisfied by Wright's coefficient of inbreeding, which is defined as both the genetic correlation between uniting gametes and the probability that two genes in the offspring are identical by descent from a common ancestor.[79] By definition, the inbreeding coefficient (which is conventionally designated F) is proportional to the reduction in heterozygosity due to inbreeding. If there are two alleles at a locus, A and a, with frequencies p and $q = 1 - p$, respectively, the genotype frequencies are

AA	Aa	aa
$p^2(1-F) + pF$	$2pq(1-F)$	$q^2(1-F) + qF$

With inbreeding the frequency of the heterozygote Aa goes from 2pq under random mating ($F = 0$) to zero under complete inbreeding ($F = 1$), and the frequency of the homozygote aa goes from q^2 under random mating to q under complete inbreeding. All of the genetic consequences of consanguineous marriages follow from these facts.



$$F = \left(\frac{1}{2}\right)^4 + \left(\frac{1}{2}\right)^4 = \frac{1}{8}$$

FIG. 2—Uncle-niece marriage.

Calculation of F can be illustrated by an uncle-niece marriage (Fig. 2). The probability that the uncle transmits to his child the gene which he received from his mother is ½, and the probability that the same gene is transmitted to the uncle's sib, and then to the niece, and finally to their child, is $(½)^3$. Thus the probability that their child is homozygous by descent from the uncle's mother is $(½)^4$. This is also the chance of identity by descent from the uncle's father, and since the two events are mutually exclusive, the total probability of identity by descent for the child of an uncle-niece marriage is the sum of the component probabilities, or

$$F = (½)^4 + (½)^4 = ⅛.$$

Notice that in the diagram there are five connecting paths from each common ancestor to the child of the consanguineous marriage and back to the common ancestor. The general formula is

$$F = \Sigma\,(½)^{n-1}$$

where n is the number of paths and the summation is taken over all common ancestors. Therefore F is 1/16 for marriages of first cousins, 1/4

for parent-offspring and brother-sister incest, 1/64 for second cousins and for first cousins twice removed, etc. Other values of F are diagrammed in Figure 1.

A complication is introduced if the common ancestor was the child of a consanguineous marriage, for then his inbred descendant might be homozygous through identity by descent even if the ancestor transmitted a maternal gene to one child and a paternal allele to the other. The general formula becomes

$$F = \Sigma\,(1/2)^{n-1}\,(1 + F_A),$$

where F_A is the inbreeding coefficient of the common ancestor.

A second complexity arises if the relationship is so distant that selection, mutation, or unrecognized illegitimacy is likely in the line of descent. These factors reduce the importance of relationship beyond second cousins, which in any case is often difficult to ascertain.

Sex linkage presents a final complication. Consanguineous marriage does not affect the genotype frequencies in sons, which have only a single X chromosome. The distribution in daughters is the same as for autosomal genes, except that lines of descent through normal males should be ignored in computing the inbreeding coefficient.

As an over-all measure of inbreeding in the population it is convenient to take the average inbreeding coefficient, designated α. For example, if one per cent of the population comes from matings between first cousins and another two per cent are from second cousins, but all others are from unrelated parents, the average inbreeding coefficient would be $\alpha = .01(1/16) + .02(1/64) = .0009375$. In general, if c_i is the frequency of persons with inbreeding coefficient F_i, then $\alpha = \Sigma\, c_i F_i$. When consanguineous unions differ from unrelated ones in fertility or legitimacy, the values of α calculated from individuals, marriages, and matings will not be the same, but the differences are usually negligible. Table 2 gives estimates of α for a few populations.

Consanguinity and Rare Recessive Diseases

The early workers in human genetics noted that some rare diseases, such as albinism, phenylketonuria, and amaurotic idiocy, showed a marked excess of consanguineous parentage (Table 4). The parents themselves were nearly always normal, and the frequency of the disease in the susceptible families, after allowance for the way in which they were ascertained, was close to ¼, while relatives outside the sibship were seldom affected. All of these characteristics are symptomatic of rare recessive diseases, of which several hundred have now been identified in man.

TABLE 4—*Consanguineous Parentage and Recessive Diseases*

Trait	Population	Incidence of first cousin marriages among parents (k)	Gene frequency (q)
Albinism	Caucasian	.18 — .24	.0021 — .0030
	Japanese	.37 — .59	.0029 — .0075
Infantile amaurotic idiocy	Caucasian	.27 — .53	.0006 — .0018
	Japanese	.55 — .85	.0007 — .0034
Ichthyosis congenita	Caucasian	.30 — .40	.0010 — .0015
	Japanese	.67 — .93	.0003 — .0022
Congenital total color-blindness	Caucasian	.11 — .21	.0025 — .0055
	Japanese	.39 — .51	.0041 — .0069
Xeroderma pigmentosum	Caucasian	.20 — .26	.0019 — .0026
	Japanese	.37 — .43	.0057 — .0075

Many of these fall into the category of inborn errors of metabolism, with an identifiable defect in an enzyme or other protein. Thus albinos lack dopa oxidase, patients with galactosemia lack p-gal transferase, and phenylketonurics cannot convert phenylalanine to tyrosine.

It is easy to understand why consanguineous parentage is such an important factor in these diseases. A carrier of a rare gene is unlikely to marry another carrier if mating is at random, but this is more likely to happen if he marries a relative. To be exact, the chance that a gene with frequency, q, becomes homozygous by random mating is q (1−F), while the chance of homozygosity due to inbreeding is simply F. If q is large relative to F, the increase of the condition with inbreeding will not be appreciable, but if q is small, the effect of consanguinity will be marked. The proportion of cases in the population due to consanguinity is $\alpha/[q(1-\alpha)+\alpha]$ or approximately $\alpha/(q+\alpha)$, as shown in Figure 3.

Dahlberg[10] made these considerations the basis of an estimate of the recessive gene frequency, q, for various diseases. He assumed that mating is at random except for a proportion c of first cousin marriages. The trait frequency is then $q^2(1-c/16)+qc/16$ in the general population, of which the contribution of cases from cousin marriages is $c[15q^2/16+/16]$. The ratio of these two probabilities is

$$k = c(15q+1)/(c+16q-qc),$$

which is the proportion of cases of the disease whose parents are first cousins. Solving for q, we obtain

$$q = \frac{c(1-k)}{16k - 15c - ck}.$$

Some of the gene frequency estimates that have been made in this way are given in Table 4.

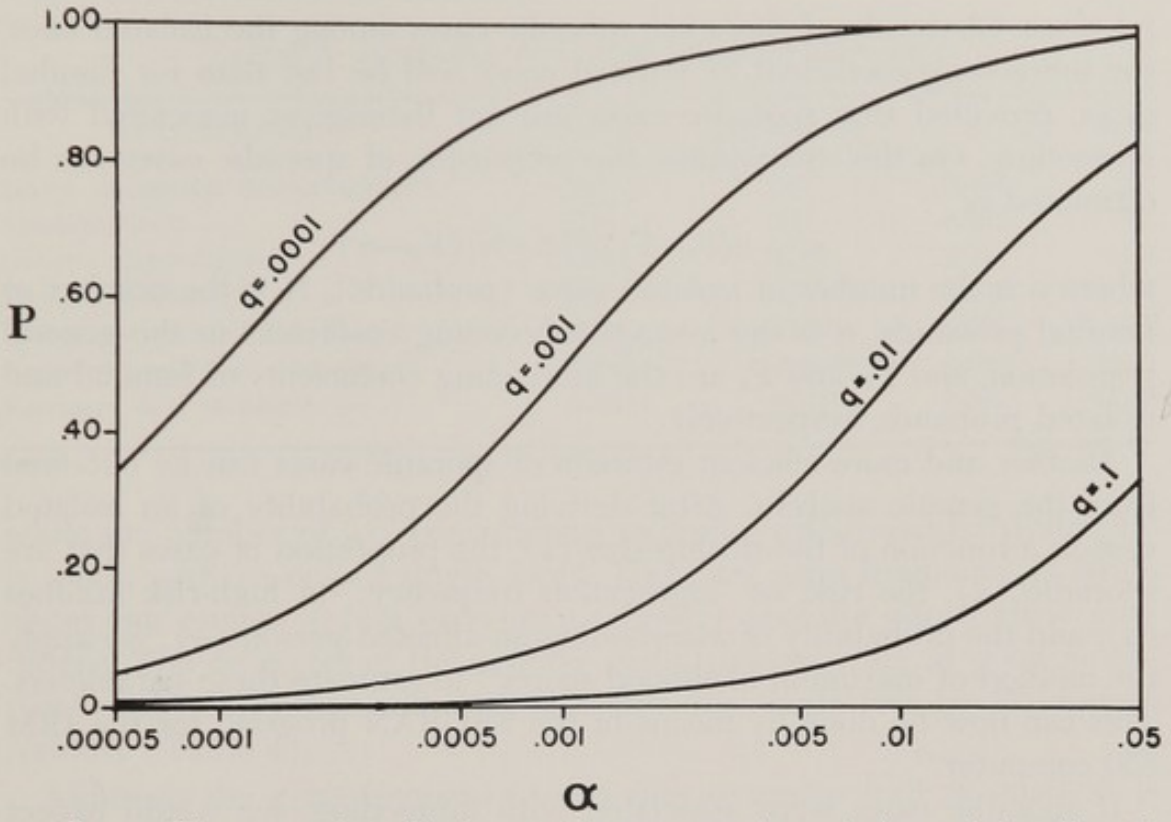


FIG. 3—The proportion (P) of cases of a rare recessive trait due to inbreeding ($\hat{z}$) at different gene frequencies (q).

The limitations of Dahlberg's formula are that it assumes without proof that all cases are due to a single locus and that it ignores information from consanguineous marriages other than first cousins. Sporadic cases, multiple loci, and diverse types of consanguinity are complications which invalidate this approach. Kimura[26] has removed the restriction to first cousins, but retains the assumptions of only a single locus and no sporadic cases. Krooth[27] assumes that consanguineous marriages are restricted to first cousins and that no cases are sporadic, but admits more than one locus.

Recently a more general theory has been elaborated and applied to data on limb-girdle muscular dystrophy, deaf mutism, and low-grade mental defect.[6, 40, 42] Familial cases from normal parents can be shown by genetic analysis to be recessive, but a large proportion of the isolated cases are sporadic. (It is important to distinguish in principle between two kinds of isolated cases: chance isolated cases, which are of the same etiology as familial cases; and sporadic cases, which are of different etiology.) Sporadic cases are due to other genetic mechanisms and to maternal and environmental factors. The proportion x of all cases that are sporadic can be estimated in two independent ways.

The first method uses the inbreeding coefficients of isolated and famil-

ial cases. Obviously, if there are sporadic cases among the isolated ones, the inbreeding coefficient of isolated cases will be less than for familial cases, provided that sporadic cases are not themselves associated with inbreeding. On this assumption, the proportion of sporadic cases can be estimated as

$$x = n(F_F - F_I) / (n+N)(F_F - \alpha)$$

where n is the number of isolated cases (probands), N is the number of familial probands, α is the average inbreeding coefficient in the general population, and F_F and F_I are the inbreeding coefficients of familial and isolated probands, respectively.

Another and more efficient estimate of sporadic cases can be obtained from the genetic analysis. After deriving the probability of an isolated case as a function of the sibship size (s), the proportion of cases that are sporadic (x), the risk, or "segregation frequency," in high-risk families (p), and the probability of ascertaining an affected person (π), we apply the method of maximum likelihood scores[39] to estimate these parameters. This can now be done by means of the SEGRAN program for the IBM 650 computer.[42]

If sporadic cases were associated with inbreeding, we would expect the estimate of x from the inbreeding coefficients to be lower than the estimate from the genetic analysis. Since this is not the case for any of the three diseases studied so far, there is no reason to suppose that sporadic cases are related to inbreeding or even to homozygosity.

Having estimated x and knowing the incidence of the disease in the population, we can estimate the frequency of recessive cases, which is $I = \Sigma[q^2(1-\alpha) + q\alpha] \doteq \Sigma q^2 + \alpha\Sigma q$, where the summation is over all loci at which recessive mutants occur that can produce the disease. Among familial cases (or among all cases if none of them is sporadic) the average inbreeding coefficient will be

$$\begin{aligned}\overline{F} &= \Sigma c_i F_i(q^2+F_iq)/\Sigma c_i\,(q^2+F_iq) \\ &= [\alpha\Sigma q^2 + (\sigma^2+\alpha^2)\Sigma q]/(\Sigma q^2+\alpha\Sigma q)\end{aligned}$$

where c_i is the frequency of F_i in the general population and σ^2 is the variance of F_i. Solving these two equasions for Σq and Σq^2, we obtain

$$\begin{aligned}\Sigma q &= I(F-\alpha)/\sigma^2 \\ \Sigma q^2 &= I - \alpha\Sigma q.\end{aligned}$$

The number of loci contributing to the disease is at least $n = (\Sigma q)^2/\Sigma q^2$ and the average gene frequency per locus is about $\Sigma q/n = \Sigma q^2/\Sigma q$. More refined estimates by maximum likelihood and formulae for the variances of the estimates have been published, together with a method for estimating mutation rates from these data.[42] As shown in Table 5, the number of loci that can produce deaf mutism or low-grade mental defect is large,

TABLE 5—*Morbidity in Consanguineous Marriages*

Trait and reference	Unrelated	First cousins	B	B/A
Major congenital malformation[60]	.010	.017	0.093	9.1
Abnormality[62]	.098	.161	1.164	11.3
Genetic abnormality[3]	.079	.188	2.020	24.6
Mental deficiency[3]	.013	.055	0.694	54.0
Low-grade mental deficiency[12]	.0036	.0138	0.167	46.1
Recessive muscular dystrophy[40]	.000034	.000509	0.008	225.9
Recessive deaf mutism[6]	.000181	.005176	0.080	442.0

while the number of loci contributing to limb-girdle muscular dystrophy is small, perhaps only a single locus. The total gene frequency is large, about one gamete in four carrying a recessive gene for one of these conditions. The estimates of the average gene frequency are in good agreement with each other and with estimates for single loci by Dahlberg's formula (Table 4).

Although the gene frequency for a rare recessive trait is small, it is much larger than the incidence of the disease. The frequency of heterozygous carriers is $2q(1-q)$, or about twice the gene frequency, and, therefore, is typically between one per thousand and one per hundred loci. Furthermore, the number of loci at which rare recessive genes occur is very large, probably of the order of thousands. About one person in two carries a gene which, if homozygous, would cause limb-girdle muscular dystrophy, deaf mutism, or low-grade mental defect, and most of us carry at least one recessive gene which, if homozygous, would cause severe impairment or death.[36] The fact that carriers for many nominally recessive genes can be detected by suitable tests suggests that an appreciable part of the genetic variability in a population is due to the heterozygous carriers for rare recessive diseases.[22] The clinical implications of this evidence have scarcely begun to be explored.

Some organisms have a region which is common to the X and Y chromosomes. Genes in this homologous region are said to be partially, or incompletely, sex-linked. A rare recessive gene of this kind shows a typical inbreeding effect and gives 3:1 ratios in intercrosses (crosses between heterozygotes). However, when the gene in the father is on the X chromosome, there will be an excess of affected daughters and a complementary deficiency of affected sons, while if the gene is on the Y chromosome, the converse will hold.

Haldane[19] made a search for partial sex-linkage in man, and found evidence that several recessive diseases might be inherited in this way.

Other investigators added to the list, until it began to seem unlikely that such a large proportion of tested loci should crowd onto a hypothetical region of a single chromosome. Meanwhile, Sachs[57] attacked the cytological evidence that the X and Y chromosomes pair during meiosis and thus called into question the physical basis of partial sex-linkage. If there is no crossing over between the X and Y, homozygotes for a partially sex-linked recessive gene should be always of the same sex within a sibship, and there should be no association with inbreeding in sibships with affected boys.

Although the cytological evidence is contradictory,[13] a purely genetic test for recessive partial sex-linkage was developed.[37] Ex hypothesi, in consanguineous matings there should be a positive association between the sex which prevails among affected individuals in the sibship and the sex of the parent through whom the father is related to the mother. Since no such association has been found,[37] it is highly probable that what had been interpreted as recessive partial sex-linkage is really due to sex-biased viability, penetrance, ascertainment, or some other association which varies among families with respect to sex. Partial sex-linkage has not been observed in other mammals.

Morbidity Risks

For any specific recessive disease or group of diseases, the risk associated with an inbreeding level F is $(1-F)\Sigma q^2 + F\Sigma q$. Some representative risks are shown in Table 5. Once a clearly recessive case has occurred in a sibship, the parents are proven to be carriers and the risk of recurrence is one in four for each additional sib.

If the recessive cases cannot be clearly recognized or if penetrance is incomplete, an empirical risk must be used. Since homozygosity increases linearly with the inbreeding coefficient, the mean number of abnormalities of a specified type per zygote is $A + BF$, where A is the mean number of defects of either genetic or environmental origin under random mating and B is the rate of increase with inbreeding. If $A + BF$ is small, it is approximately equal to the morbid risk. However, if it is not small, a Poisson correction for multiple independent defects should be made, so that the risk is[36]

$$P = 1 - e^{-(A+BF)}$$

Estimates for limb-girdle muscular dystrophy, deaf mutism and low-grade mental defect (exclusive of known trauma, infection, anoxia, hydrocephalus, or mongolism) are given in Table 5.

Several studies on broader categories of morbidity have been pub-

lished, based on comparisons of children of first cousins, unrelated parents, and sometimes other degrees of relationship. Schull[60] has reported on major congenital malformations in the material collected by the Atomic Bomb Casualty Commission.[48] Consanguinity was recorded through a nearly complete registration of pregnant women in Hiroshima, Nagasaki, and Kure during 1948-1954, followed by home visits shortly after birth. The inbreeding effect was consistent among cities. Followup studies of this group are now being made on defects that developed after birth, growth characteristics, and intelligence.

Another study[69] was based on Catholic marriage dispensations issued during 1919-1925 in three French departments. The consanguineous families were visited by the authors, who took notes on conspicuous abnormalities. The same information was obtained by town clerks for a control sample of unrelated parents married during the same period and selected without regard to medical history. The dangers in this sampling scheme are obvious, but the data satisfy tests of internal consistency.

The only recent study in this country[62] considered consanguineous marriages (nearly all between first cousins) in the Chicago region, ascertained through Catholic marriage dispensations issued during 1936-1956. These were compared with a control sample of the families of married sibs, preference being given to a sister of the wife.

Böök[3] examined all marriages between first cousins in three north Swedish parishes. For each cousin family another family living in the nearest house or farm was selected as a control. He chose for analysis abnormalities commonly considered to have a major genetic component, not necessarily recessive. Population data on mental deficiency (corresponding approximately to an I.Q. less than 70) were compared with consanguineous marriages between normal or gifted parents.

As a final sample, we may consider data collected a century ago through correspondence with physicians and recently reclassified.[2, 68] This material probably gives an upper estimate of the effect of inbreeding, not only because of unintentional bias in selection of families, but also because the population lived in a more rigorous environment and abnormality was broadly defined to include infectious diseases. Bias in selection of families can be minimized, although probably not eliminated, by considering only degrees of consanguinity, all of which were ascertained by the same procedures, and omitting the unrelated parents who were ascertained differently. When this is done, the inbreeding effect is linear.

The estimates of A and B from these data (Table 5) vary consider-

ably, partly because morbidity is not defined in the same way in different studies. However, they agree in showing a considerable increase of morbidity with inbreeding, to a lesser degree for congenital malformations than for defects with later onset.

Mortality Risks

In addition to the sources for morbidity risks, we have a study by Arner[36] of deaths before age 20 in the children of consanguineous marriages recorded in early American genealogies. Non-consanguineous marriages of the parents' siblings served as controls. Estimates of inbreeding effects on mortality are given in Table 6, using the formula

$$\text{mortality} = 1 - e^{-(A+BF)}$$

Table 6—*Mortality in Consanguineous Marriages*

Trait and reference	Unrelated	First cousins	B	B/A
Stillbirths and neonatal deaths[69]	.044	.111	1.124	24.4
Infant and juvenile deaths[69]	.089	.156	1.431	15.1
Early deaths, Hiroshima[60]	.031	.050	0.371	11.5
Early deaths, Kure[60]	.035	.041	0.044	1.2
Juvenile deaths[2]	.160	.229	1.734	10.8
Postnatal deaths[62]	.024	.081	0.934	38.9
Miscarriages[62]	.129	.145	0.261	2.0

In general there is good agreement about the effects of inbreeding on perinatal and juvenile deaths, which increase at much the same rate. The principal exception is the Nagasaki and Kure material, which differs not only from Hiroshima, but from all the other studies. This is the more remarkable because for congenital malformations the inbreeding effects were consistent. Since death rates are more susceptible to socioeconomic factors than are malformation rates, it seems likely that there is some environmental disturbance in the Nagasaki and Kure material. Consanguineous marriages differed significantly from non-consanguineous ones in maternal age, parity, and frequency of induced abortions. The population is heterogeneous, the consanguineous marriages being mostly rural in origin. It is difficult in such complex material to obtain controls as adequate as neighbors or siblings provide in more homogeneous communities. With this exception, mortality appears to be approximately doubled by first cousin parentage.

Quantitative Traits

Data on metrical traits and inbreeding were collected by Morton[38] from the records of the Atomic Bomb Casualty Commission in Japan. Insofar as inbreeding effects on the mean depend only on homozygosity, without selection or epistasis, they are expected to be linear. Thus

$$m_F = m_O - (m_O - m_1)F$$

where m is the mean, $m_O - m_1$ is estimated as a regression coefficient, and the subscripts O, 1 and F denote no inbreeding, complete inbreeding, and intermediate inbreeding, respectively. The data, analyzed within city, parity, and other groups, are consistent with this expectation. The effects of inbreeding on live birth weight, weight at 8-10 months, height at 8-10 months, and chest girth at 8-10 months are small but significant, while the effects on live birth gestation time and head girth at 8-10 months are non-significant. The estimated effects are shown in Table 7. It seems unlikely that such small changes in size could have appreciable effects on morbidity.

Table 7—*Quantitative Traits in Consanguineous Marriages*

Trait	Unrelated	First Cousins	B	Standard error of B
Weight of live births (Gm.)	3074	3046	− 540	120
Length of gestation (wks.)	40.13	40.13	− 0.1	0.7
Weight at 8-10 mos. (Gm.)	7818	7722	−1200	480
Height at 8-10 mos. (mm.)	689.6	687.3	− 28	13
Head girth at 8-10 mos. (mm.)	442.8	442.8	− 10	7
Chest girth at 8-10 mos. (mm.)	427.7	426.8	− 29	10

The genetic variance due to additive genes increases linearly with inbreeding.[79] This suggests a method for estimating the fraction of the total variance that is genetic,[21] neglecting the effects of rare recessive genes. Unfortunately, enormous samples are required to get accurate estimates. Table 8 shows that there is no significant effect of inbreeding on the variances, although the increase observed for measurements at 8-10 months is consistent with evidence of higher heritability for these traits than for birth weight.[35]

Genetic Isolates and Outcrossing

Mating of close relatives is not the only kind of inbreeding. If migration is limited, members of the same community will tend to be dis-

TABLE 8—*Variances of Quantitative Traits in Consanguineous Marriages*

Trait	$\sigma^2 + BF$	Standard error of B
Weight of live births (dkg.)	1750 − 177 F	736
Length of gestation (wks.)	5.0 − 1.0F	2.3
Weight at 8-10 mos. (dkg.)	7500 + 9035 F	6337
Height at 8-10 mos. (mm.)	538 + 504 F	445
Head girth at 8-10 mos. (mm.)	158 + 153 F	132
Chest girth at 8-10 mos. (mm.)	351 + 464 F	297

tantly related, even if they themselves recognize no kinship, or if their culture prohibits marriage of relatives. It is convenient to distinguish between recognized consanguinity, which is usually known to the marriage partners and can be ascertained from them, and more obscure and distant relationship, which is due to restriction of the mating group by limited migration, or "isolation by distance."[79] The importance of this factor is poorly understood, and except for the work of Malécot,[30, 31] The mathematical theory has been worked out only for some special cases.

In most countries, isolated communities can still be found where geographic or social barriers, custom, and small population size enforce consanguineous marriages among many, or even a majority, of the inhabitants. It is debatable whether these isolates are typical of primitive communities or whether, on the contrary, population movement and occasional long-range dispersal make village endogamy always the exception rather than the rule. In aboriginal populations there is often a surprisingly high frequency of intertribal marriages.[28]

Wright[79] considered two migration patterns: the island model, in which a fraction of the population is replaced each generation by long-range migrants drawn at random from other populations; and the continuous model, in which short-range migrants tend to be drawn from neighboring populations. Clearly both long-range and short-range dispersal is important in human populations, and the distinction between the two is somewhat arbitrary. In the continuous model, the effect of migration depends on the variance of the distance between birthplace of parent and offspring, and the size of the local population, or "neighborhood," is defined in terms of this variance. Wright[78] calculates that, if mating is at random within neighborhoods of 200 married couples, the long-term inbreeding coefficient will be about .02. He suggests that this is likely to be an upper limit to the inbreeding of primitive popu-

lations. This value would be decreased by mutants, long-range migrants, selection, and avoidance of consanguineous marriages. It would be increased by variations in population size or fertility and by preference for consanguineous marriage.

Neel[46] takes as the probable long-term inbreeding coefficient of primitive human populations the maximum value presently attained in isolated communities, or about .006. Other workers have accepted this as a reasonable estimate, given the small amount of information we possess.[36]

Since contemporary societies have much smaller inbreeding levels than this, and the frequencies of consanguineous marriages have declined in recent years, it is evident that modern populations cannot be in genetic equilibrium. Relaxation of inbreeding has reduced the frequency of homozygotes, and therefore the intensity of selection against rare recessive genes. These gene frequencies should slowly increase, as the result of mutation, until a new balance between mutation and selection is reached. It follows that recessive diseases are less common under present inbreeding levels than they were in the past, and than they will become in the future.

It is not clear how important the genetic effects of isolation by distance are in modern populations. When local populations, or "isolates," merge with one another, the frequency of heterozygotes increases. Does "isolate-breaking" explain the increase in body size in modern populations, or the reduced frequency of institutionalized defectives in some urban areas?[10]

Hultkrantz[24] found that the height of Swedish men examined for universal military conscription increased 9 cm. in 100 years, with no effect of a severe famine in the 1860's. Broman, Dahlberg, and Lichtenstein[5] reported that height of boys increased 15 cm. during a 50 year period, while adult height increased 5 cm. These differences are rather greater than those between social classes measured at the same time. In Sweden the frequency of institutionalized mental defectives is greater in rural districts than in the cities, despite the expectation that rural defectives are more often cared for at home. Dahlberg suggested that both increased size and decreased incidence of defect were due to isolate-breaking, and other investigators have accepted this hypothesis.[65]

Some defects are clearly due to rare recessive genes, and any reduction in the frequency of consanguineous marriages will reduce the incidence of these diseases. If the average inbreeding coefficient were lowered from .006 to .0006, this would probably reduce the frequency of recessive diseases by more than 50 per cent. Thus it seems likely that isolate-breaking will have important consequences for conditions that

are to a considerable extent determined by rare recessive genes. Whether this is the true explanation of rural-urban differences in the incidence of Swedish defectives, or whether differences in medical practice, institutionalization, migration, or other factors are more important, could only be decided by a much more penetrating study than has been carried out so far.

We have more information about the effects of isolate-breaking on body size. Morton[38] found that in Japan the effect of an inbreeding coefficient of .02 was to reduce birth weight by 11 Gm., weight at 8-10 months by 24 Gm., and height, head girth, and chest girth by fractions of a millimeter. However, during the period 1949-1954, the material of the Atomic Bomb Casualty Commission showed an increase of 7 Gm. per year in birth weight among children of unrelated parents. A comparison of babies born at the Red Cross Hospital in Tokyo in 1926-27 and again in 1937-38 showed an increase in birth weight of 74 Gm.[70] Osaka babies examined in 1924-25 at the age of 8-10 months had increased more than 200 Gm. in weight and several millimeters in height, head girth, and chest girth, compared with Japanese standards published in 1902.[25] Japanese babies born in Hawaii during 1952-53 average more than 100 Gm. greater in birth weight than the babies studied by the Atomic Bomb Casualty Commission in Japan.[7] There have been comparable increases in birth weight in modern European populations. Thus, at least for measurements at birth and during infancy, the increase is far too great to be attributed to isolate-breaking. More plausible possibilities include better nutrition of mother and fetus, reduced hazards from infection, better prenatal and postnatal care, reduced obstetrical risks for large babies, and increased maternal age and size. The persistent difference in size between social classes in many countries suggests the importance of socioeconomic factors. To the extent that the secular trend is genetic, it probably owes more to increased survival of large parents than to isolate-breaking.

This conclusion of course cannot be extrapolated to temporal changes in adult measurements. Dahlberg[10] has suggested that inbreeding may have a greater effect on adult than juvenile characteristics. There is no evidence that this is the case. In fact, data on other mammals show no great increase of inbreeding effects with age, except perhaps for weight, which at birth is considerably influenced by maternal factors.[35] On the contrary, the inbreeding effect seems to be less for adult measurements than for rate of growth, as if the inbred animals attained nearly the same final size but approached it more slowly.[4] It may be doubted whether any appreciable fraction of the increase in size observed dur-

ing recent generations in Japanese and Western populations can be attributed to increase in heterozygosity through isolate-breaking. However, the evidence on this question is clearly insufficient, and Hulse[23] has presented data which suggest an increase in size with isolate-breaking in Switzerland. The interpretation of his results is complicated by temporal trends and differences between endogamous and exogamous matings in religion, family size, and possibly other factors.

In the absence of a satisfactory genetic theory for the effects of isolation by distance, Dahlberg[9] attempted to describe isolate effects from the frequency of consanguineous marriage alone. He argued that if each marriage had exactly c children which survive to maturity, then the average person in the population would have c(c—1) cousins of the opposite sex. Thus if marriage of first cousins took place entirely at random in a population of size n, the frequency of cousin marriage would be 2c(c—1)/n. By equating the observed frequency to this and the average family size to c, he solved for n, the "isolate size," and obtained values of a few hundred in European populations. He also calculated isolate size from the expected frequency of uncle-niece and aunt-nephew marriage, and obtained estimates of the order of thousands in European populations. He commented: "We know that the upper limit is decidedly too high, because, as far as the formula is concerned, it is conditioned by a frequency for marriages between parents' sibs and sibs' children that is clearly too low. There is also reason to suppose that the figure for marriages between cousins is too high, and thus has given too low a figure for the isolates. Evidently nothing can definitely be said in this respect, even though the calculations may give grounds for a supposition that the isolates have to be denoted by three, rather than by four figures."

These estimates of isolate size have two main disadvantages. First, they rest on the obviously incorrect assumptions that sibship size is constant and that close consanguineous marriages take place at random. Frota-Pessoa[16] removed the first restriction, but only by introducing the equally incorrect assumption that there is no familial correlation in fertility. The second assumption has been criticized at length by Morton,[31] who showed that not only are all types of close consanguineous marriage nonrandom, but estimates of isolate size from different degrees of relationship (uncle-niece through second cousins) are highly inconsistent.

The other disadvantage of Dahlberg's estimate of isolate size is more disturbing than mere lack of plausibility. There is no quantitative theory to relate isolate size with heterozygosity. Obviously, the smaller the isolates, the less heterozygosity should be, but this does not tell us what magnitude of effects to expect with isolate-breaking. The readiness with

which some human geneticists have accepted a theory which is neither plausible nor useful is testimony to the difficulty of work in this field.

At least in principle, it should be possible to approach isolate-breaking from a strictly empirical point of view, if we had data on the distance between birthplaces of mother and father (d), recognizable consanguinity (F), and a trait in the progeny (Y). Separating recognizable consanguineous marriages which do not take place at random, the relation among these variables might reasonably be taken as

$$Y = A + BF + Ce^{-kd}$$

where A, B, C, and k are constants to be estimated.[30, 31] For apparently unrelated couples from the same small community, d equals zero, and their children are expected to differ from outbred controls by an amount C. Then the effective inbreeding coefficient of apparently unrelated individuals from the same community is C/B, which depends not only on the past migration pattern, but also on selection and mutation pressures. This very important topic in human population genetics should be studied while sedentary populations still exist, to provide a basis for understanding the effects of isolate-breaking.

At the opposite extreme from small isolates is the outcrossing of ethnic groups, or races. The increase of heterozygosity that accompanies such crosses can in theory be described in the same way as for crosses between isolates. However, not only is heterozygosity increased, but novel genotypes may be created as the result of combining gametes from populations that have been exposed to different selection pressures. There is unfortunately very little information about the biological effects of outcrossing in man, apart from casual observations that fertility and viability are not conspicuously increased or impaired, and that metrical traits in the offspring are usually intermediate between the parental groups, with a slight increase in variance in the F_2 and later generations.[54,72] Studies on the genetic effects of outcrossing on morbidity are now being conducted in Hawaii, but the results have not yet been published.[41]

Genetic Counseling

The term "genetic counseling" is commonly applied to two different processes: the estimate of a genetic or empirical risk figure appropriate to a given family situation; and the combination of social work, psychology, and medicine incident to discussing this risk and other relevant considerations with the patient. Only the first of these is an aspect of genetics, per se. Both aspects of counseling have received more encouragement than is altogether wise at their present state of development.[20]

The data that were presented above on morbidity and mortality risks apply to consanguineous marriages before the appearance of an abnormal child. Once such a child is born, the parents are likely to be more interested in the specific risk of recurrence of the same malformation, rather than in a more general risk. In some cases, as for limb-girdle muscular dystrophy, deaf mutism, and low-grade mental defect, the cases born to related parents are almost entirely recessive, after exclusion of the obvious exogenous cases. Therefore the risk for subsequent children is approximately one in four.

It should not be assumed that all abnormalities from related parents are recessive. There is no clear evidence that the recurrence risk for cleft palate is higher when the parents are related.[14] The risks must be determined on the evidence for each disease. The smaller the recessive component, the less relevant consanguineous parentage is likely to be.

The probability that a recessive homozygote will have an affected child is $q(1-2F) + 2F$, or nearly $q + 2F$, where q is the gene frequency. Since q is ordinarily about .002, this risk is quite small, except in consanguineous marriage. If the homozygote marries a first cousin, the risk will be .127, or about 64 times as great as in a marriage to an unrelated person. The risk for children of normal sibs of an affected homozygote is nearly $(q+2F)/3$, which is increased by consanguinity to the same relative extent. From the standpoint of genetic risks, consanguineous marriage is especially to be avoided for a known carrier of a recessive gene.

The situation is more complicated if sporadic and recessive cases cannot be distinguished. The effects of consanguinity will then be less. Particular cases are best handled by consultation with a medical geneticist.

The immediate genetic risk has no close relation to the long-term effect on the population. If the population size is decreasing, if nominally recessive genes are advantageous in heterozygotes, if the diseases they cause can be completely ameliorated in the future, the long-term effect of consanguineous marriage at the present time is harmful. If the population size is increasing, if nominally recessive genes are disadvantageous in heterozygotes, and if the diseases they cause cannot be completely ameliorated in the future, the long-term effect of consanguineous marriage may be beneficial, by eliminating two deleterious alleles at once. The nature and time of action of both heterozygous and homozygous effects determine which type of elimination is preferable. For example, if heterozygotes were eliminated through decreased fertility and homozygotes through premature mortality, the former might well be considered innocuous, and any factor tending to promote homozygosity might be considered unfavorable. Since we do not have nearly enough infor-

mation to decide these questions for even a single gene, it is apparent that genetic counseling should and must be based on its immediate effects, and not on unpredictable long-range ones.

The Genetic Load

Studies on consanguineous marriage indicate that most individuals in a population are heterozygous for one or more recessive genes which, if made homozygous, would cause severe impairment or death.[36] According to one hypothesis, these genes are maintained in the population because recurrent mutations balance the elimination by selection. If there is any effect in heterozygotes, it is unfavorable. Such genes are said to constitute the mutation load, since the damage they cause is proportional to the mutation rate. On this model, the situation may be represented as follows for a single locus:

Genotype	*GG*	*GG'*	*G'G'*
Frequency	$q^2(1-F)+qF$	$2q(1-q)(1-F)$	$(1-q)^2(1-F)+(1-q)F$
Relative fitness	$1-s$	$1-hs$	1

Here q is the frequency of the mutant gene *G*, s is the probability of death, sterility, or defect due to homozygosis for *G*, and h is a measure of dominance, being 0 if *G* is completely recessive and 1 if G causes the same impairment in a homozygote as in a heterozygote.

The *expressed genetic load* is the extent to which the population fitness (or whatever component is being measured) is reduced in comparison to what it would be if all individuals were of the optimum genotype *G'G'*. This is $s[q^2(1-F)+qF] + hs[2q(1-q)(1-F)]$ per locus. The total genetic load is the extent to which the population fitness (or the component that is being measured) would be reduced if an average gamete were made completely homozygous. This is qs per locus. By summing the expressed loads over all loci and collecting the terms which do or do not involve F, we see that the expressed load is $A + BF$ per zygote and that the total load Σqs lies between B and $A + B$ per gamete, where

$$A = \Sigma x + \Sigma q^2 s + 2\Sigma q(1-q)sh$$
$$B = \Sigma qs - \Sigma q^2 s - 2\Sigma q(1-q)sh$$

and x is the load due to any particular environmental cause of death. These loads are measured in terms of the type of death, infertility, or defect under study. Thus we may speak of a load of lethal equivalents expressed as early fetal death, of sterile equivalents, or of detrimental equivalents expressed as congenital malformations, mental defect, deaf mutism, albinism, or in any other specified way. A load of $A + BF$ lethal, sterile, or detrimental equivalents is defined as giving a probability of $e^{-(A+BF)}$ for survival, fertility, or nonaffection, respectively.

According to an alternative hypothesis nominally recessive genes, although harmful in homozygotes, are actually favorable in heterozygotes, and therefore they are maintained at a stable equilibrium in frequencies that depend on selection alone and are independent of the mutation rate. Because the harmful effects are due to segregation from favored heterozygotes, this is called the *segregation load*, which may be represented as follows for alleles G^i,G^j and gene frequencies q_i, q_j:

Genotype	G^iG^i	G^iG^j	G^jG^j
Frequency	$q_i^2(1-F) + q_iF$	$2q_iq_j(1-F)$	$q_j^2(1-F) + q_jF$
Relative fitness	$1 - s_i$	1	$1 - s_j$

Again we find that the expressed genetic load is $A + BF$ lethal, sterile, or detrimental equivalents per zygote, and the total load Σqs lies between B and $A + B$, where

$$A = \Sigma x + \Sigma q^2 s,$$
$$B = \Sigma qs - \Sigma q^2 s,$$

and the summation, as before, is over all genotypes and causes of defect. There are other genetic loads due to transient polymorphism for genes that are being eliminated from the population by selection, to maternal-fetal antigenic incompatibility, to gametic selection, or meiotic drive,[58] and to other causes, but these are believed to be less important sources of genetic variability than the mutation and segregation loads.

Attempts to analyze the genetic load have taken advantage of the fact that the expressed and total loads can be estimated from consanguineous marriages for any specified type of impairment. There is a remarkable theorem, which may be expressed as follows: For a locus with k alleles maintained by selection alone in stable equilibrium in a randomly mating population, with no restrictions on the magnitudes or modes of selection, the product of any gene frequency and its selection coefficient in the homozygote is always less than or equal to the expressed genetic load at that locus, and the total genetic load is less than or equal to k times the expressed load.* Two corollaries of this proposition have been used to argue that the genetic load revealed by consanguineous marriage is largely mutational.[42]

* This theorem can be proved by the following argument, suggested by Prof. J. F. Crow as a generalization of results obtained by the author and by Crow earlier.[8, 42] Let $1 - s_{ij}$ be the fitness of genotype $G^i\,G^j$ relative to the best genotype and let q_i be the frequency of G^i ($i = 1,2, \ldots, k$). The general formula for selection pressure[77] is $\Delta q_i = q_i\ (W_i - \bar{W})/\bar{W}$, where $\bar{W}$ is the mean fitness of the population and W_i is the mean fitness of G^i. In a randomly mating population, $\bar{W} = {}_i\Sigma_j\ q_iq_j\ (1-s_{ij})$ and $W_j = {}_j\Sigma q_j\ (1-s_{ij})$. At equilibrium, $W_i = \bar{W}$, or ${}_j\Sigma q_j s_{ij} = {}_i\Sigma_j q_i q_j s_{ij}$, which by definition is the expressed genetic load. Since $q_i s_{ii}$ is less than or equal to ${}_i\Sigma q_i s_{ij}$ for all k alleles, it follows that

$$\Sigma q_i s_{ii} \leqslant {}_k{}_j\Sigma q_i s_{ij}$$

or $q_i s_{ii} \leqslant \frac{1}{k}$ total genetic load $\leqslant$ expressed genetic load.

The first corollary is that the ratio of the total to the expressed load is less than or equal to the number of mutually heterotic alleles which must be postulated to explain the genetic load as segregational. If we are measuring fitness, B is less than or equal to the total genetic load, and A is greater than or equal to the expressed genetic load, so that B/A is the minimum number of heterotic alleles per locus.[8] As we have seen in Tables 5 and 6, B/A is typically 15 or more even for deaths, malformations, and other morbidity which must contain a large nongenetic component, while for nominally recessive conditions B/A is commonly more than 200. For these defects this is larger than estimates of the total number of contributory loci or complementary alleles. Since local populations in the past were not of greater order than this, it is clear that selection against rare homozygotes could not maintain such a high number of alleles at each locus. Moreover, the average allele frequency would have to be less than B/(B/A), or A, which for rare recessives is of the same magnitude as the mutation pressure, and so small that nearly every affected person would be expected to come from close consanguineous marriages. Clearly these expectations do not fit the facts, and so it seems unlikely that the genetic load is maintained by heterozygote advantage or any other purely selective system.

However, it should be noted that this argument is not rigorous unless we are measuring total fitness. For example, if one allele were eliminated by mortality and another allele by sterility, we could not obtain from mortality data alone a valid estimate of the number of alleles maintained by selection.

The second corollary is more general. It states that if at any locus there is one allele (or set of non-complementary alleles) producing defect in double dose, and any number of alleles which are manifested differently, the lower limit to the expressed segregation load at that locus equals the product of the frequency and selective disadvantage of the extreme allele. Since the mortality and sterility in limb-girdle muscular dystrophy, deaf mutism, and low-grade mental defect is high, the expressed load for these genes would have to be nearly as great as the total gene frequency, or about ¼ (Table 5). This is an incredible intensity of selection when we consider that only about 100 loci are represented in these data, while the total number of loci per gamete is probably at least 10,000.[36] Even if we ignore the load due to mutation and exogenous factors, the expressed segregation load would be equivalent to selective elimination through death or infertility of nearly all zygotes formed. This appears to refute the suggestion by Penrose[53] that recessive

genes for low-grade mental defect and other abnormalities are maintained as a segregation load through heterozygote advantage.

A third argument that rare recessive genes represent a mutation load is based on the fact that mutation rate estimates from this assumption are in good agreement with rates estimated for dominant and sex-linked genes in man and for recessive genes in Drosophila and the mouse.[48]

Finally, the model of impairment proportional to the genetic variance of additive genes cannot fit the consanguinity data, because it requires that B/A be unity.[76]

Recently Neel[49] has suggested that many sporadic malformations in man are "segregants (phenodeviants) resulting from the existence and functioning of complex (multilocal) genetic homeostatic systems." This theory has not so far been tested or even formulated clearly, but any formulation must take account of the fact that the inbreeding coefficient for isolated cases of limb-girdle muscular dystrophy, deaf mutism, and low-grade mental defect agrees quantitatively with the assumption that sporadic cases are not associated with inbreeding. If sporadic cases are not demonstrably related to homozygosis, the hypothesis of phenodeviants must be dismissed as either false or operationally undefined. Undoubtedly most sporadic cases are due to maternal and environmental factors and to a variety of non-recessive genetic mechanisms, including polygenes, heterozygotes with low penetrance, dominant mutations, deletions, and aneuploidy.[42]

Evidence that the total load revealed by inbreeding is a mutation load should not be construed as indicating that the expressed genetic load in a randomly mating population is largely mutational. On the contrary, the general theorem of the segregation load shows that at least 1/k of the total load is expressed, compared with a much smaller fraction (typically about 2 per cent) for the mutation load.[36] Even if, as seems likely, balanced polymorphism is limited to a small fraction of loci which account for a negligible fraction of the total load revealed by inbreeding, these loci collectively and in some cases individually must make an appreciable contribution to the genetic load expressed under random mating.[8]

Experimental Studies

Human genetics derives much of its insight from experiments on lower organisms. They indicate that inbreeding effects are linear, as the theory for morbidity risks and genetic loads predicts, but there is a paucity of good data.[45, 67, 75]

In Drosophila, refined genetic techniques allow whole chromosomes to be made homozygous. When this is done with chromosomes from natural and laboratory populations, a whole Pandora's box of defects is opened. In a typical study, 28.6 per cent of second chromosomes of *Drosophila willistoni* were lethal when homozygous, 12.6 per cent were semilethal, 57.5 per cent were subvital, 31.0 per cent were sterile, and 15.9 per cent produced anatomical defects.[51] If this genetic load is measured as lethal equivalents, a surprisingly high proportion, nearly 50 per cent, is due to chromosomes that are completely lethal in homozygotes. The contribution of detrimental genes is increased under crowded conditions, raising the question of whether the detrimental/lethal ratio as measured is representative either of natural populations or of an equilibrium load under laboratory conditions. Among newly induced mutations the detrimental/lethal ratio is low, but presumably increases as lethals are eliminated by selection.[18]

Although mammals do not yet permit the elegant manipulations of Drosophila genetics, some evidence has been obtained that the inbreeding effect on premature mortality in the European bison is also largely due to full lethals, and not to minor subvitals.[63] If this is true in man, it provides some justification for interpreting the expressed mutation load, especially in radiated populations, in terms of genes with large homozygous effects.

Maternal inbreeding considerably reduces fetal size and increases mortality.[4] There is little evidence about inbreeding effects on early fetal deaths, except for a tantalizing report that inbreeding of the fetus is more important than maternal inbreeding in causing deaths under 35 days gestation, but is relatively less important in late fetal deaths.[32]

Isolate-breaking has been subjected to intensive study in Drosophila. Interpopulational F_1 hybrids commonly exceed the parental groups in viability, longevity, and fecundity, but the F_2 hybrids fall below the F_1 and often below the parental groups.[74, 80, 81] Clearly two processes are involved. On the one hand, outcrossing increases heterozygosity, initially without recombination of the co-adapted parental genotypes. Then, in the F_2, novel genotypes are formed by recombination, which have not been subjected to the sieve of selection which produced the parental populations. There is a suggestion that crosses which do not give appreciable F_1 effects do not break down in the F_2. Double cross corn populations and crosses of inbred lines of guinea pigs show heterosis with little or no subsequent breakdown.[45, 75] Heterosis, like amphidiploid advantage, is probably increased if the environment is alien to the parental populations.[64, 71]

Problems for the Future

Morbidity in consanguineous marriages has been studied only for late fetal, infant, and juvenile deaths and for a few specific defects and metrical traits. Many more studies are needed, especially for early fetal deaths, fertility, intelligence, adult measurements, specific types of morbidity not measured by mortality, and effects of maternal inbreeding. These will not only provide empirical risks, but will answer basic questions about whether sporadic cases are associated with inbreeding, whether the genetic load revealed by inbreeding is mutational or segregational, and how the genetic load is expressed in modern populations. Attention should also be given to primitive populations under intense natural selection, to determine the equilibrium load instead of its temporarily reduced value under relaxed selection. Populations under chronic radiation must be studied both for their own interest and to predict the genetic consequences of increasing levels of radiation. The effects of isolate-breaking and the role of genetic isolates in determining our present genetic load must be attacked now if they are ever to be understood, since population mobility is enlarging isolates all over the world. Reciprocal crosses between populations (diallel crosses), in addition to their interest in connection with isolate-breaking, provide the best means for separating maternal and zygotic causes of morbidity and variation. Finally, analysis of balanced polymorphisms like the blood groups and hemoglobins will indicate whether they contribute appreciably to the genetic loads revealed by inbreeding or expressed in a randomly mating population.

Other organisms must be used to obtain adequate evidence for inbreeding effects on early fetal deaths and on many aspects of the theory of the genetic load. Linearity of the inbreeding effects on the inbreeding coefficient provides the only operational distinction between the classical mutation hypothesis and the synergistic model advocated by Lerner, Wallace, Neel and others.[29, 49, 74]

Clearly the modern studies on morbidity in consanguineous marriages have opened a field, of both genetic and medical interest, the reaches of which have yet to be explored.

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Chapter 8

Chromosomes and Human Disease

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For over half a century cytogeneticists have been studying the chromosomes of plants and invertebrates almost exclusively. By correlating the numerical and morphological abnormalities of chromosomes with the unusual expression of the characteristics they transmit, the cytogeneticists have given proof to the chromosome theory of heredity and have uncovered a vast amount of knowledge about chromosome structure and behavior. The study of human chromosomes played almost no part in this increase of knowledge, and was in fact almost completely neglected as a worthwhile subject for research until as recently as 1956. Progress in human cytogenetics thus stems from this late date, when improved methods of cytological technique applied to appropriate material revealed for the first time that the correct chromosome number in man was 46.[131] Only then did the study of human chromosomes in relation to disease become a realistic proposition.

Attempts to study human chromosomes had of course taken place long before this. It is probable that they were first observed in tumor cells by Arnold in 1879.[3] Hansemann[59] in 1891 and Flemming[39] in 1898 attempted to determine the normal chromosome number in man, but their estimates were crude and did not allow them to do more than suggest that it was in the order of 24. Similar studies by a succession of investigators during the next twenty years reported numbers of 16, 24 or 38 with most in favor of 24.

Quite different results were first reported by Winiwarter[138] in 1912

using more convincing techniques. He found 47 chromosomes in testicular material and 48 in ovarian tissue, suggesting that in man the sex determining mechanism was of the XO/XX type. Nine years later Painter[104] demonstrated the Y chromosome in primary spermatocytes and concluded that 48 was the correct diploid number in both sexes. It is of interest that he stated he could count only 46 chromosomes in some of the clearest mitotic figures. In a later paper[105] he confirmed 48 as the correct number and showed very clear pictures of the XY sex bivalent at the first meiotic metaphase. Mild controversy between those who favored 47 and those who favored 48 persisted for the next ten years, but thereafter the human chromosome constitution of 46 autosomes (or non-sex chromosomes) and two sex chromosomes, with an XY/XX sex determining mechanism became established as the opinion of the majority until 1956. By analogy with Drosophila, the Y chromosome was thought to be inert as far as sex determination was concerned, there being a balance between masculinizing genes carried by the autosomes and feminizing genes carried by the X chromosomes.

As a result of his observations on human spermatogenesis, Painter[105] predicted the existence of individuals with unusual combinations of sex chromosomes, and, in particular, intersexes with an XXY sex chromosome complement. This raised the question of the sex chromosome constitution of hermaphrodites, but no satisfactory attempt was made to follow this up until 1942 when Severinghaus[121] described an XY sex chromosome complex in a male pseudohermaphrodite.

Nuclear Sex

Further information about the sex chromosomes at meiosis was accumulated by Koller[78] and other workers, but apart from this no significant advance was made on human chromosomes or genetic sex diagnosis until 1949, when Barr and Bertram[6] made the important observation of nuclear sex dimorphism in mammals. Like so many others in history, this discovery was made while the investigators were looking for something quite different. Barr and Bertram were engaged in some experiments on nerve fatigue, specifically on the effects of electrical stimulation of the hypoglossal nerve of the cat. While examining the altered neurons histologically they noted a distinctive mass of nuclear chromatin which varied in position from the nucleolus to the nuclear membrane. This "nucleolar satellite" could be seen only in some cats and was conspicuously absent in others. Various explanations of this inconsistency had to be excluded before it was realized that the nucleolar satellite was

present in female cats but not in males. The importance of this finding was immediately recognized by Barr and his colleagues who extended the initial observation to other mammals including man. The nucleolar satellite was renamed the sex chromatin.[129]

FIG. 1—Nucleus of oral mucosal cell in normal female showing sex chromatin body. (Cresyl echt violet × 3,000 approx.)

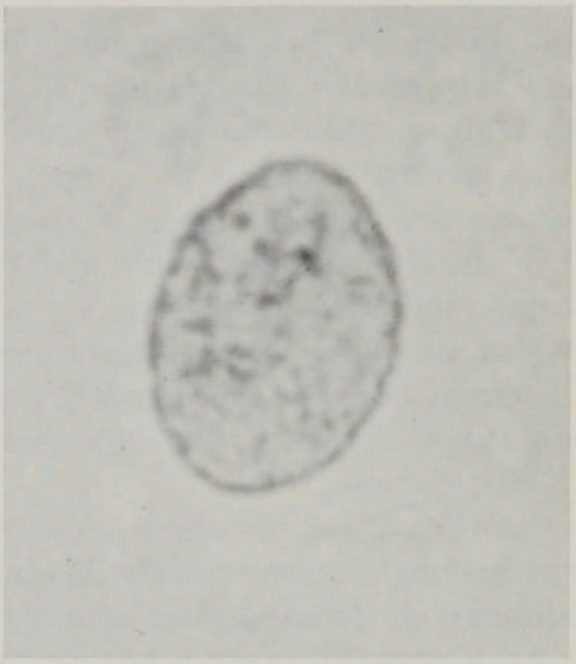

FIG. 2—Nucleus of oral mucosal cell in a normal male. (Cresyl echt violet × 3,000 approx.)

Between 30 and 60 per cent of cell nuclei in all tissues of the human female contain the sex chromatin body, which is a plano-convex mass about one micron in diameter composed largely of deoxyribonucleic acid (DNA) characteristically lying in close contact with the nuclear membrane (Fig. 1).* Although the true sex chromatin is absent in normal males (Fig. 2), a chromocenter indistinguishable from the sex chromatin is seen in less than 1 per cent of nuclei. Given satisfactory preparations, this degree of sex dimorphism makes the distinction between "male" and "female" tissues almost infallible. With regard to the exact nature of the sex chromatin occasionally seen in male preparations it is highly probable that one of the two or more frequently prominent nucleolar chromocenters is mistaken for the sex chromatin when it lies in close contact with the nuclear membrane (Fig. 3).

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* Figures 1, 2, 4, 5, 6, 7, and 10 are reproduced from "Genetic Factors in Disorders of Sexual Differentiation" Chapter 19, *Modern Trends in Endocrinology*, Second Series, edited by Dr. H. Gardiner-Hill, Butterworths, London.

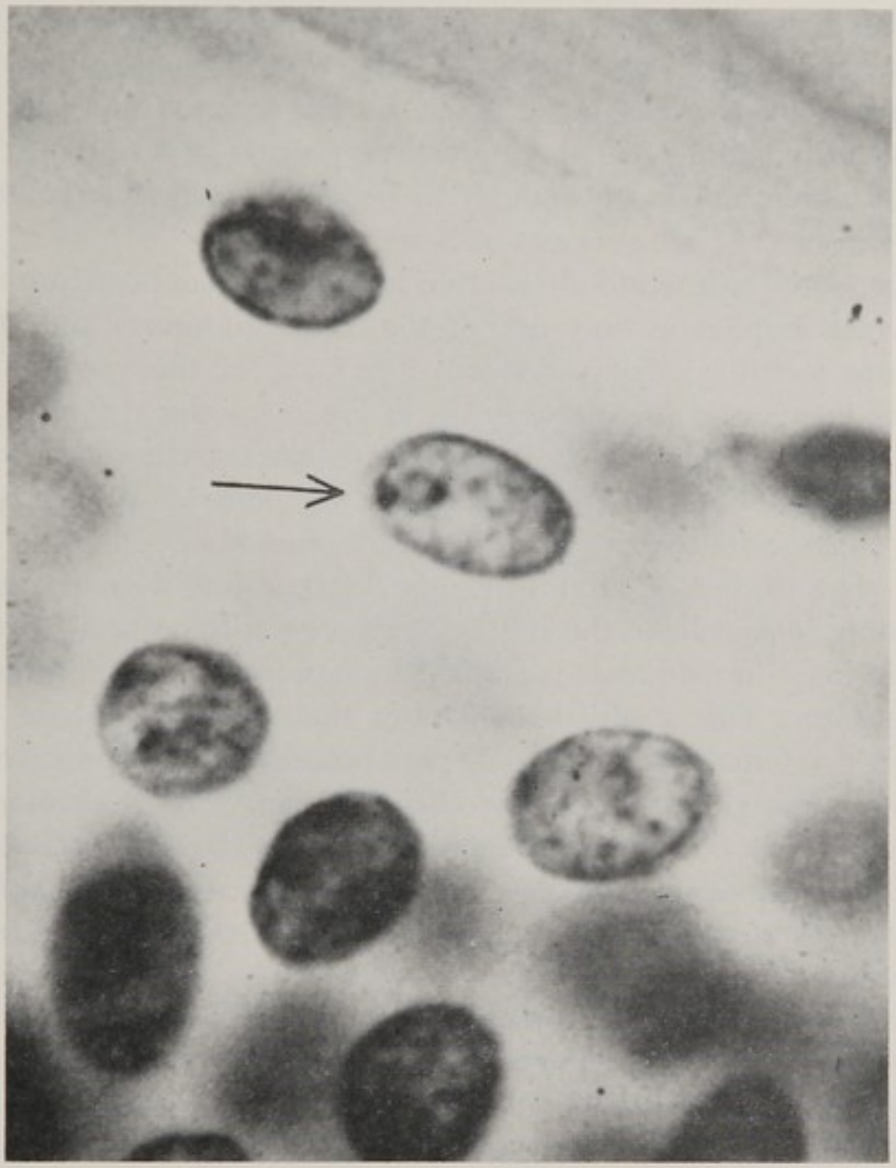

FIG. 3—Nucleus of squamous epithelial cell in a normal male showing one of the two nucleolar chromocenters (on either side of the nucleolus) simulating sex chromatin. (Feulen × 2,000 approx.)

The Origin of Sex Chromatin

The origin of the sex chromatin body remains the subject of much speculation. Its DNA composition relates it chemically to the chromosomes. Its identification in the fetus long before gonadal differentiation and its persistence in tissue culture indicate that it is an inherent characteristic of the cell, uninfluenced, for example, by the sex hormones. An early hypothesis suggested that it represents heteropyknotic portions of the two X chromosomes visible in the resting nucleus due to somatic pairing.[7, 87] This hypothesis was based largely on the circumstantial evi-

dence that the sex chromatin was observed only in individuals with two X chromosomes, and also on the fact that it sometimes appears as if it were formed by two distinct masses. Its absence in the male was interpreted as indicating that the equivalent portion of a single X chromosome was either not large enough to be distinguished from the other nuclear chromocenters, or was not heteropyknotic.

A rather more attractive alternative hypothesis has been proposed recently which implicates only one of the X chromosomes and thus does not require somatic pairing. Evidence in favor of this comes from observations in nuclear sex dimorphism in lower species. In birds, butterflies and moths the female also shows a nuclear sex chromatin mass, but as the female and not the male is the heterogametic sex, this sex chromatin mass is associated with only one sex chromosome.[54, 79, 123]

The recent studies of Ohno and his collaborators[103] on the nuclei of regenerating liver cells in the rat also support the hypothesis that the sex chromatin body in mammals is formed by heteropyknotic portions of one X chromosome. This study demonstrates that at the prophase stage of mitosis in the female one of the X chromosomes shows positive heteropyknosis compared with the other chromosomes. This X chromosome is bent acutely at its center and resembles in shape the sex chromatin body seen in intermitotic nuclei. The male rat does not show differential heteropyknosis of any of the prophase chromosomes, and does not have sex chromatin.

Further evidence is given by the sex chromosome studies in the triple-X[68, 33] and triple X/Y[32] syndromes described below. These conditions are diagnosed by the nuclear sex anomaly of a double complement of sex chromatin in intermitotic nuclei (Fig. 4). These individuals have three X chromosomes and not four as might be expected from the early hypothesis on the origin of the sex chromatin.

The major implication of these studies is that the two X chromosomes in female somatic nuclei have different properties. One can only speculate how such differentiation could occur. It would appear that only two opportunities exist for the differentiation or selection of the X chromosomes, namely during gametogenesis or at the time of fertilization.[35]

Nuclear Sex and Human Intersexuality

While nuclear sex may be determined from any human cellular tissue including peripheral blood,[22] the most convenient method for clinical use is provided by the oral mucosal smear technique.[94] A scraping of the

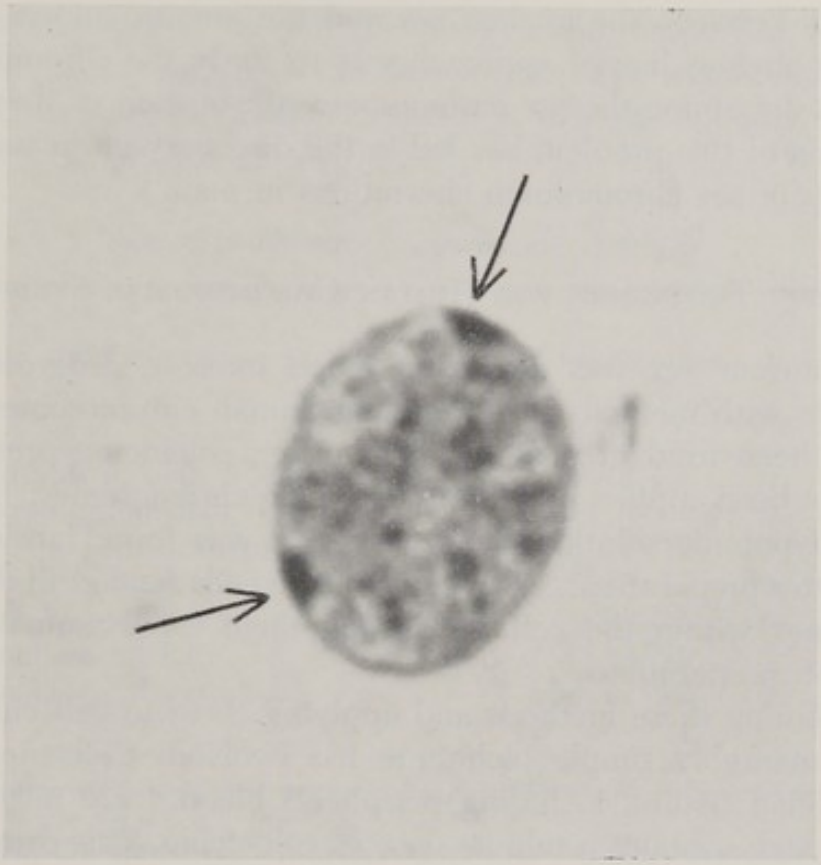

FIG. 4—Nucleus of oral mucosal cell from an individual with the triple X/Y syndrome. Two sex chromatin masses are present. (Cresyl echt violet × 3,000 approx.)

inside of the cheek is smeared onto a microscope slide, which is dropped into an ether-alcohol fixative and stained several hours later by a nuclear dye such as cresyl violet. This simple test has become the single most important diagnostic tool in the management of human intersexuality. In clinical use it is customary to refer to a diagnosis of female-type nuclear sex as chromatin-positive, to a diagnosis of male-type nuclear sex as chromatin negative, and to the other nuclear sex anomalies as being double or triple chromatin-positive.

As soon as nuclear sexing methods became established they were applied to cases of hermaphroditism. Approximately two thirds of true hermaphrodites were found to be chromatin-positive; male pseudohermaphrodites were found to be chromatin-negative, and female pseudohermaphrodites were found to be chromatin-positive.[56] Then in 1954, female patients with gonadal aplasia (Turner's syndrome) were reported to be chromatin-negative,[111, 139] and two years later male patients with a particular variety of primary testicular failure known as Klinefelter's syndrome[76] were discovered to be chromatin-positive.[110, 14] In these two disorders it became particularly important to know what this

discrepancy between the nuclear sex and the somatic appearance could mean. The obvious line of approach was to study the chromosomes and if possible, determine the sex chromosome constitution of these patients. The solution of this problem has led to the discovery of an unexpectedly large group of sex chromosome aberrations in man.

New Techniques for Human Chromosomal Studies

While nuclear sex was attracting much interest, progress was also being made with methods for examining human chromosomes. Many of these had been used in plant cytology (e.g., colchicine pretreatment), but had not been applied previously to human chromosomes. The osmotic effect of hypotonic solutions before fixation was found accidentally to lead to better preparations, as swelling of the cells tended to disperse the chromosomes within the cell, separating them for examination in the final squash preparations.[90, 66, 64]

By combining these methods and applying them to cell culture materials, a remarkably simple technique has evolved. Cells derived from normal human tissues, including peripheral blood,[65] are incubated in a medium which contains a minute dose of colchicine. This drug interferes with the formation of the mitotic spindle apparatus, and so arrests cell division at the metaphase stage, accumulating large numbers of these cells in the culture. The colchicine-treated cells are then exposed to hypotonic salt solution for several minutes before fixation and staining with aceto-orcein; in the final stages, squash or smear preparations are made and this serves to spread the chromosomes in one optical plane for accurate counting and photography under the oil immersion lens.

Chromosomes at the metaphase stage of mitosis appear as rod-shaped structures split longitudinally into two chromatids which lie side by side and are held to one another at a constricted area, the centromere, which is the site of the spindle fiber attachment. The chromosomes vary both in length and centromere position, so that the total length and the ratio of the lengths of the short arm to the long arm are individual characteristics of each chromosome. The measurable differences between several pairs of chromosomes are too small to allow exact identification of every chromosome[141] but this difficulty will no doubt be eventually resolved once techniques become sufficiently refined. In some preparations five pairs of chromosomes, numbers 13, 14, 15, 21 and 22, possess satellites on their short arms.[37] A satellite may be considered as part of the densely coiled and contracted metaphase chromosome which appears to be separated from the rest of the chromosome by an uncoiled region. It is

thought that the stalk of the satellite is uncoiled because it is adjacent to a *nucleolar organiser* region on the chromosome. The formation of nucleolar material, specifically ribonucleic acid, at this site appears to prevent the usual process of coiling.

TABLE 1—*Human Chromosome Analysis*

Group	Size and Centromere Position	Idiogram Number	Number in Each Group	
			Male	*Female*
I	Largest, median & submedian	1– 3	6	6
II	Large, submedian	4– 6	6	6
III	Medium-sized, submedian	X and 7–12	13	14
IV	Medium-sized, sub-terminal	13–15	6	6
V	Small, median & submedian	16–18	6	6
VI	Smallest, median	19&20	4	4
VII	Smallest, sub-terminal	Y and 21, 22	5	4
				46

At present, chromosome analysis depends largely on classifying the chromosome complement into seven groups on the basis of total length and arm ration (Table 1). The sex chromosome constitution is determined by the number of chromosomes in Groups III and VII. In males, there are thirteen chromosomes in Group III and five chromosomes in Group VII, whereas in females the numbers are fourteen and four, respectively. This is readily shown if the 46 chromosomes from a normal male are arranged in a karyotype in order of decreasing length (Fig. 5). There are 22 different autosomes, or non-sex chromosomes, which occur in pairs of equal length, and one pair of chromosomes of unequal length, the X and Y sex chromosomes. The X is usually the seventh largest in size and the Y is frequently the largest in Group VII. Although there is some evidence that the lengths of the sex chromosomes may show slight variation from one individual to another,[38] the female has what appears to be an identical pair of X chromosomes and no Y chromosome, and consequently about 4 per cent more genetic material than the male.

The normal human chromosome constitution, first accurately demonstrated by Tjio and Levan[131] in 1956, has been amply confirmed both in somatic tissue[19, 41, 132, 89] and in meiotic figures from testicular material.[40, 38] Only one conflicting report of diploid numbers other than 46 in normal individuals has been published since.[77] Kodani, while accepting 46 as the basic number, believes that inert supernumerary chromosomes

FIG. 5—Karyotype of a normal male. $2n = 46$ (XY).

are responsible for consistent counts of 47 or 48 in six of fifteen Japanese men in his study using testicular material. There are as yet no confirmatory reports of chromosome numbers other than 46 in normal individuals,[91] with three rather unique exceptions which, however, can hardly be classed as normal. Fraccaro et al.[48] have reported a chromosome number of 47 in the "normal" father of a mongoloid child, and Carter et al.[18] and Penrose et al.[109] have described families in which reciprocal translocation between two chromosomes, apparently without significant loss of genetic material, has resulted in both normal and mongoloid offspring with counts of 45 and 46 respectively. These cases will be considered in greater detail below.

Human Chromosome Aberrations

Before listing the remarkable series of chromosome anomalies which has been discovered to be associated with specific types of human congenital disease, it would seem appropriate to define briefly several terms used in cytogenetics.

There are two main types of human chromosomal abnormalities. The first group comprises individuals who have an abnormal number of chromosomes. Where the number is an exact multiple of the gametic or haploid number (23), the term euploid is used, and multiples over the diploid are collectively termed polyploid. Where there is an irregular number of chromosomes, this is referred to as aneuploidy. For example, an individual may be aneuploid because three instead of two representatives of a particular chromosome are present in his somatic chromosome complement; he is thus *trisomic* for the particular chromosome. A special type of numerical abnormality, chromosomal mosaicism, has been demonstrated in individuals whose cells appear to be derived from two or more stem lines each with different chromosome numbers. Chromosome counts in these cases consistently show a proportion of cells with one chromosome number, while another proportion has a different number.

A second group of human chromosome abnormalities is due to a disturbance in chromosome structure rather than number, although both may co-exist. These abnormalities are identified by detecting an abnormal chromosome in the karyotype or by detecting differences in length between what should be homologous chromosomes. Chromosome breakage occurring during meiosis in the parental gametes may result in the detachment and subsequent loss of a portion of an arm from the remainder of the chromosome. This is referred to as a chromosome deletion. Breakage occurring in two non-homologous chromosomes

may result in the exchange of fragments between the chromosomes. This is called a reciprocal translocation. There is some evidence for the existence of another type of structural abnormality known as an *isochromosome*. This develops during cell division as a result of transverse instead of longitudinal fracture of the centromere. Thus two daughter chromosomes are formed which may be of unequal length but always have a median centromere. The arms of each individual daughter chromosome are thus genetically identical.

It will be apparent that only the gross types of chromosome abnormality are considered here, for these alone will be recognized by the cytological techniques at present available. Small chromosomal deletions, duplications, and inversions are invisible in these preparations. In the vast majority of so-called "genetic" diseases, typified by a specific Mendelian type of inheritance, and presumably due to specific gene mutation, the chromosome defect must be considered to be at the molecular level and far beyond the limits of the microscope. Nonetheless, the conditions described below are also genetic diseases, but because the chromosomal aberrations which produce them are so gross, the associated disabilities usually prevent transmission of the disease. Thus, with a few important exceptions, individuals with the chromosome anomalies described to date are usually unable to reproduce, are sporadic in origin, and typically show multiple severe defects.

Classification of Human Chromosomal Aberrations

The various types of chromosome aberrations provide a useful basis for classification. Table 2 lists the conditions in which a simple numerical abnormality of the autosomes has been found.

Table 2—*Numerical Abnormalities of the Autosomes*

Clinical Condition	Chromosome Constitution	
Mongolism	2n + 1 = 47	*T.21 or T.22
Group IV trisomy syndrome	2n + 1 = 47	T.13, 14 or 15
Group V trisomy syndrome	2n + 1 = 47	T.17 or T.18
Triploidy syndrome	3n = 69	

* Trisomy of chromosome 21

The Autosomal Trisomy Syndromes

In January 1959, the report of an extra autosome in each of several cases of mongolism by Lejeune and his collaborators in Paris,[83] heralded

the beginning of a new field of investigation in medicine and must be considered one of the most significant discoveries in recent years.

Mongolism is a not uncommon congenital disorder associated with severe mental retardation. It is estimated to occur approximately once in every six or seven hundred births and accounts for about 5 per cent of institutional defectives. Characteristic features include short stature, stumpy limbs, small round head, and typical facies with open mouth, protruding fissured tongue, short nose, epicanthic folds, and oblique palpebral fissures. The hands and feet are broad with short fingers and toes. A single transverse palmar crease with a single interphalangeal crease instead of two on the little finger are often present. Other typical abnormalities are speckled iris (Brushfield spots), blepharitis, dry skin, umbilical hernia, webbing of the fingers and toes, extra digits, and congenital heart disease. Both sexes are affected, with a slight preponderance of males, giving a sex ratio at birth of about 1.24:1. Sixty per cent of mongols die before the age of ten years, the major causes of death being broncho-pneumonia and congenital heart disease.[17] Leukemia appears to be more common among mongols than among normal children; one study reports that of 677 children dying of leukemia, 17 were also mongols.[125]

Before the advent of chromosome studies, the evidence in favor of a genetic etiology in mongolism was derived mostly from twin studies which showed complete concordance in monozygotic and complete discordance in dizygotic twins.[1, 92] Occasional families were reported in which mongolism was present in more than one generation and had recurred several times in the same sibship. Mongoloid mothers were also known to have had mongoloid offspring.[84] Further evidence of a genetic effect has come from study of palm prints in mongols and their relatives.[108] It was found that the characteristic palmar pattern of mongols is found in a significantly higher incidence among relatives of mongols, particularly in the familial cases, than among relatives of non-mongols. As the palm patterns are thought to be genetically determined, this was considered evidence of a specific gene effect in mongolism.

The chromosome number of 47 in mongolism has been confirmed by numerous investigators.[67, 11, 33] The extra chromosome is due to trisomy of one of the smallest (Group VII) autosomes (Fig. 6). At first it was thought to be chromosome 21, largely on the basis of satellite identification, for at this time satellites had not been identified on chromosome 22. At the present time it is not possible to distinguish between pairs 21 and 22.[37]

Two other trisomic syndromes are now established. They are similar to

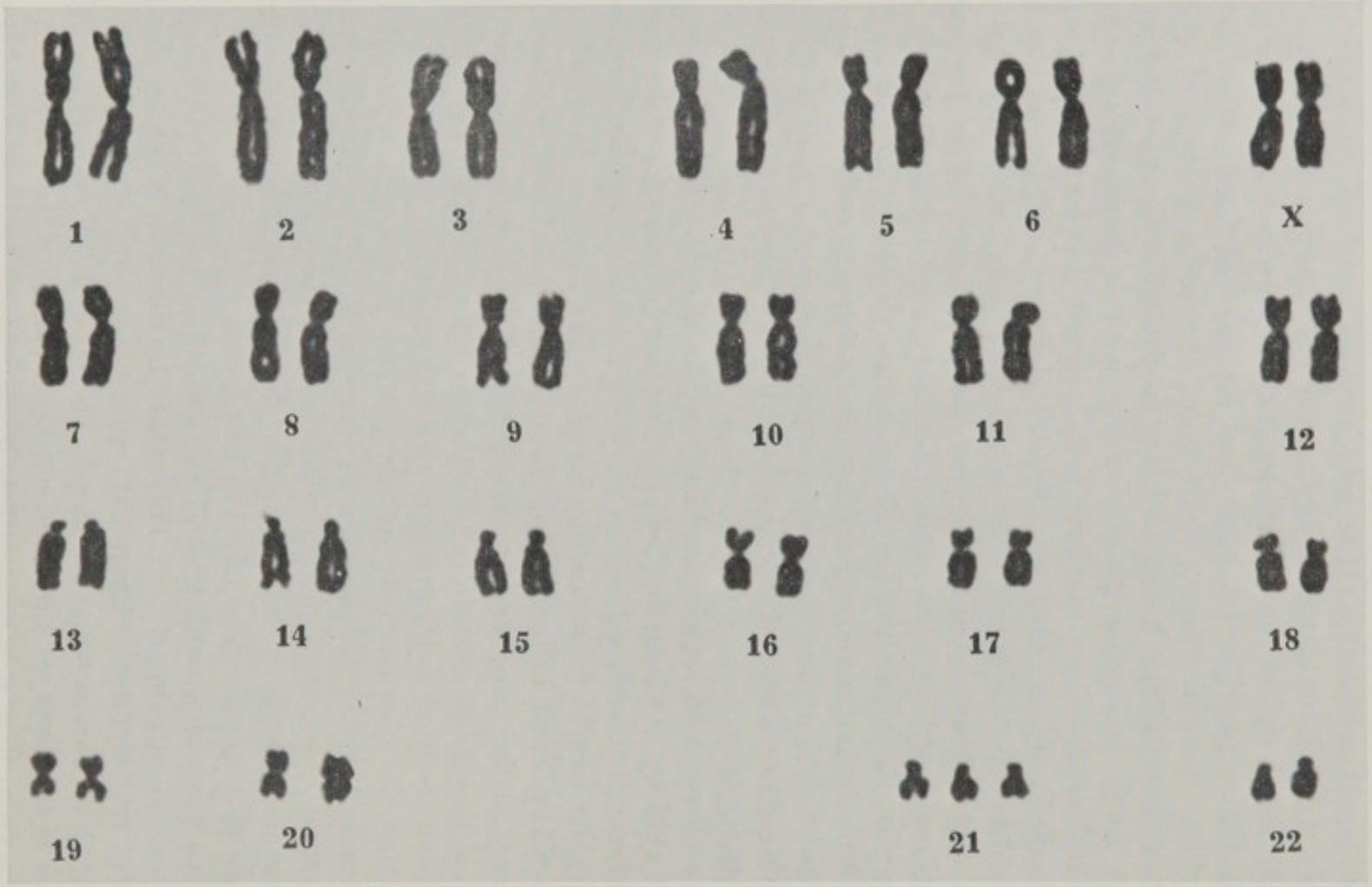

FIG. 6—Karyotype of a female mongol. 2n + 1 = 47 (XX) T.21 (or T.22).

mongolism in that mental defect and multiple congenital anomalies are invariably present in the limited number of cases studied to date. The chromosome responsible for each of these syndromes has not yet been identified more specifically than by chromosome group. One trisomy involves either chromosome 13, 14 or 15 in Group IV,[106] the other, either chromosome 17 or 18 in Group V.[122] A single report of trisomy of chromosome 22 in the Sturge-Weber syndrome has not been confirmed.[62] *

The distinctive features of the Group IV trisomy syndrome appear to be anophthalmia (or microphthalmia), cleft palate, hare lip, polydactyly, trigger thumbs, hemangiomata, and congenital heart disease. (Fig. 7 illustrates an example of this syndrome recently studied in this department.) The cases of the Group V trisomy show anomalies of the ears, spasticity, "rocker-bottom" feet, flexion deformity of the fingers, micrognathia, umbilical hernia and congenital heart disease.

A Possible Triploid Individual

A preliminary announcement of chromosome studies in a retarded male child with a diagnosis of porencephaly reports a chromosome number of 69 in cells from an eleven day skin culture.[12] In over twenty-five cells examined, all chromosomes are present in triplicate and the sex chromosome constitution is XXY. Further confirmation of this important finding is awaited, as it is to date the only report of a viable polyploid mammalian individual. It should be noted that many normal human tissues, such as liver and bone marrow, contain a small proportion of polyploid cells although the diploid number of 46 remains the basic somatic chromosome number.

Abnormal Sex Chromosome Complements

Seven different abnormal sex chromosome complements are known to date (Table 3). The reports on the first two of these followed closely the publication of the chromosome abnormality in mongolism, and were the direct consequence of attempts to solve the nuclear sex anomalies in the Klinefelter and Turner syndromes.

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* In three patients with this condition, studies in this Department revealed an apparently normal chromosome constitution (Kaufmann, B. N., Johnston, A. W., Personal communication).

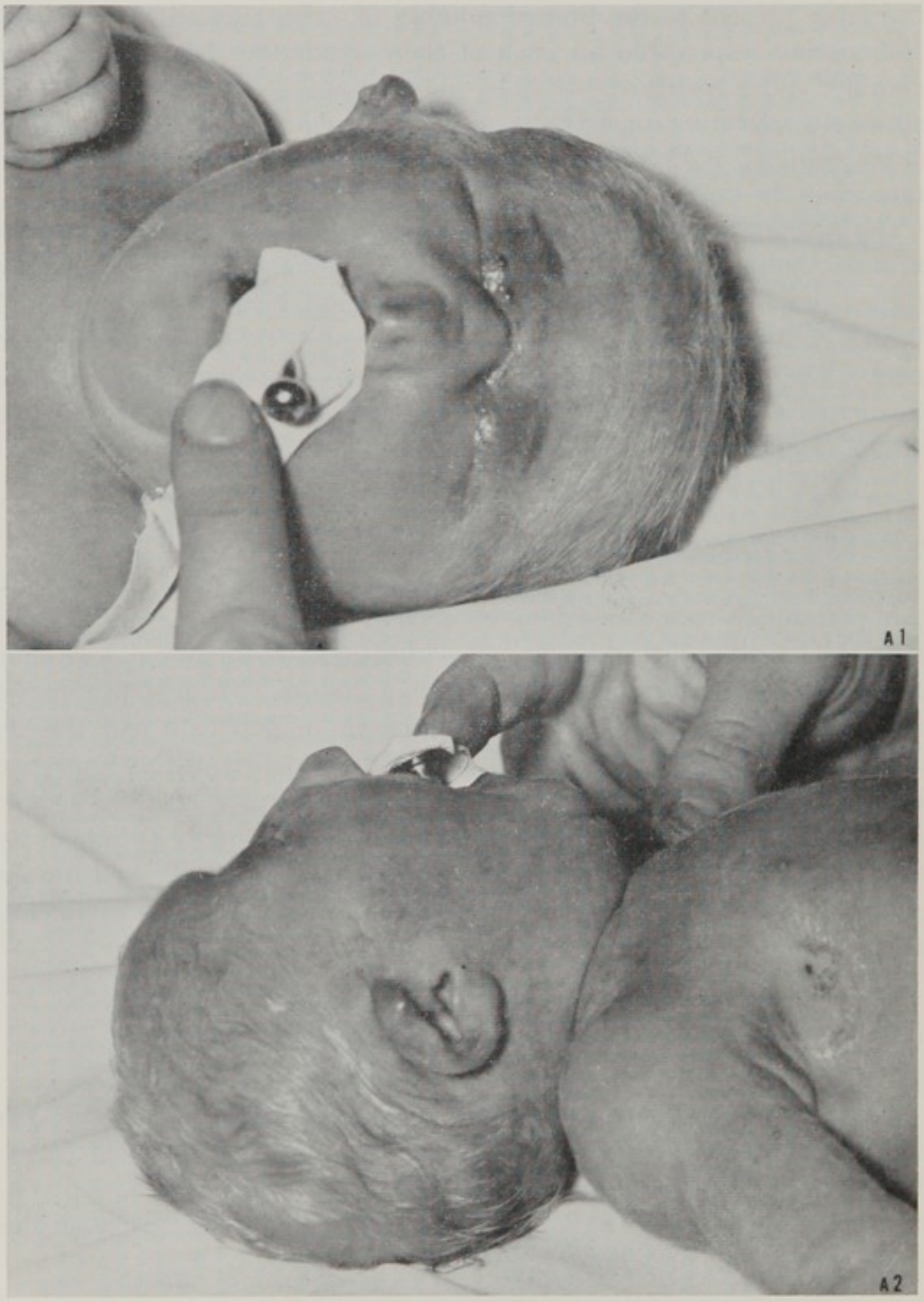

FIG. 7—(a) A case of Group IV trisomy. This newborn female infant (2355G.) shows microcephaly, anophthalmia, polydactyly, with gross anomalies of the nose and ears. She has a ventricular septal defect.

FIG. 7—(a) *Continued*

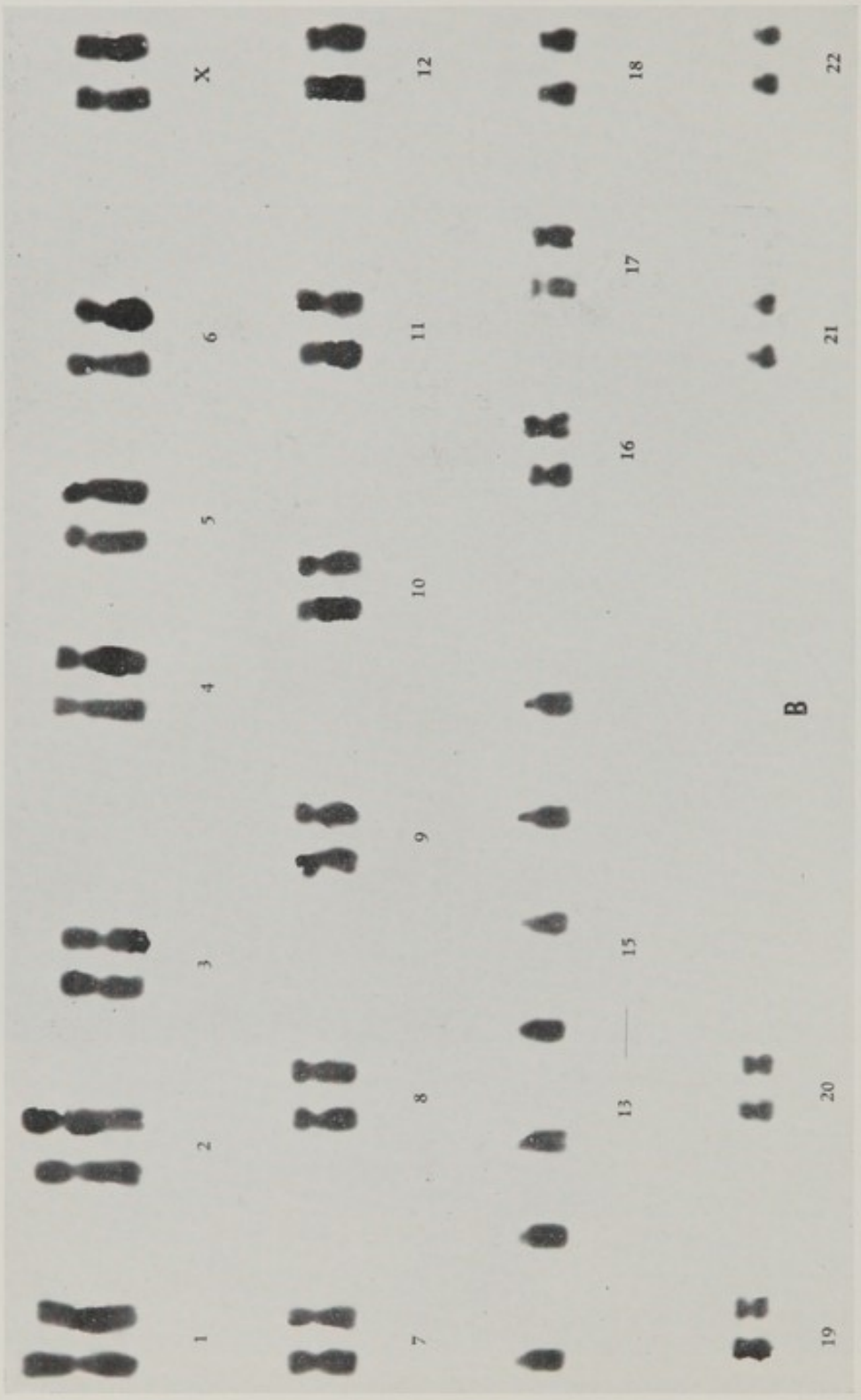

FIG. 7—(b) Karyotype of patient, 2n + 1 = 47 (XX). An extra Group IV chromosome is present.

TABLE 3—*Numerical Abnormalities of the Sex Chromosomes*

Clinical Condition	Chromosome Constitution	Nuclear Sex	Phenotype
Gonadal Aplasia (Turner's syndrome)	2n − 1 = 45 XO	Negative or Positive	Usually sterile ♀
Chromatin-positive Klinefelter's syndrome	2n + 1 = 47 XXY	Positive	Sterile ♂
Triple X syndrome	2n + 1 = 47 XXX	Double-positive	Usually fertile ♀
Triple X/Y syndrome	2n + 2 = 48 XXXY	Double-positive	Sterile ♂
Double X and Y syndrome	2n + 2 = 48 XXYY	Positive	Sterile ♂
Tetra-X syndrome	2n + 2 = 48 XXXX	Triple-positive	? Fertile ♀
Tetra-X/Y syndrome	2n + 3 = 49 XXXXY	Triple-positive	? Sterile ♂

Gonadal Aplasia (Turner's Syndrome)

In Turner's syndrome,[135] or more specifically, in chromatin-negative gonadal aplasia, the main pathological defect is to be found in the reproductive system. There is no true gonad and in the ovarian site all that can be found is a streak of white tissue composed of ovarian stroma without germinal cells.[140] The remainder of the reproductive tract is that of a normal female, but after puberty these structures remain infantile. Menstruation does not occur, and with the exception of sex hair, secondary sex characteristics do not develop. Patients with this condition are invariably of short stature and may show a wide assortment of congenital abnormalities including webbing of the neck, shield chest, peripheral lymphedema, coarctation of the aorta, brachydactyly and hypoplasia of the nails.[58] Only the vaguest estimates of the frequency are known; one authority suggests a minimum frequency of 1 in 10,000 but it is probably higher than this.[115]

About 80 per cent of patients with Turner's syndrome have chromatin-negative nuclear sex,[56] and all cases of this type submitted to chromosome analysis have had a chromosome number of 45 with an XO sex chromosome constitution (Fig. 8).[42, 47, 70, 33] Very rarely cases of chromatin-negative Turner's syndrome have been reported to menstruate, and in others histological evidence of scantly primordial follicles has been demonstrated. Fertility is therefore a possibility, and in fact one XO case is known to have given birth to a male child with an apparently normal chromosome constitution.[4]

The chromatin-positive cases of gonadal aplasia that have been studied to date show a puzzling variety of X chromosome anomalies including XO/XX[46] and XO/XXX[72] mosaicisms, an apparent XO sex chromosome complement,[57] and large "translocations"[38] and deletions[72] of the X chromosome (Table 5).

Although there is at present no good evidence to suggest etiological factors other than abnormalities of the X chromosomes as a basis for either chromatin-negative or chromatin-positive gonadal aplasia, it has been widely held during the past ten years that environmental factors affecting the early embryo might produce such an individual. This evidence stems from the remarkable fetal castration experiments of Jost.[75] Jost found that if the gonads of a fetal rabbit are destroyed at a critical stage of differentiation, the genital ducts and external genitalia will develop along female lines irrespective of the genetic sex of the embryo. Male differentiation is thus dependant on the production of an organizer substance by the gonad at this stage of development. Environmental

FIG. 8—Karyotype of a patient with chromatin-negative gonadal aplasia. $2n + 1 = 45$ (XO).

factors affecting the undifferentiated human gonad are therefore theoretically possible causes of gonadal aplasia in a genetically normal XX or XY individual. The case of "pure gonadal dysgenesis" recently reported to have an XY sex chromosome complement[61] cannot be cited as an example of this, as it does not fulfill the diagnostic criteria for gonadal aplasia as the patient has none of the features of Turner's syndrome, and the internal genitalia were not examined to exclude male pseudohermaphroditism.

Klinefelter's Syndrome (Primary micro-orchidism)

Chromatin-positive Klinefelter's syndrome is characterised by small testes, sterility, a histologically distinct type of seminiferous tubule atrophy, chromatin-positive nuclear sex, and an increased excretion of gonadotrophin. Variable features include gynecomastia, eunuchoidism, and mental defect.[34, 126] Although the clinical manifestations are only apparent after puberty, a marked deficiency of germ cells is observed in testicular biopsy material from prepubertal cases.[29] An interesting feature is the frequent finding of disproportionate body measurements, readily apparent in their long-leggedness.[126] The high lower segment/upper segment ratio is not due to delay in epiphyseal fusion, as skeletal maturation follows the normal male pattern. In fact, skeletal development in these patients follows so closely that of the normal male that the well-recognized sex difference in bone maturation has been attributed to genes carried by the Y chromosome.[130] Individuals without a Y chromosome, that is normal females and patients with chromatin-negative gonadal aplasia, show more rapid bone maturation.

The frequency of chromatin-positive Klinefelter's syndrome is clearly much higher than that of gonadal aplasia, and has been estimated to occur about once in every 400 male births.[95] It is found in about 1 per cent of institutionalized mentally defective males,[28, 96, 116, 25] and appears to account for approximately 3 per cent of all male subfertility as seen at a male fertility clinic.[27]

Chromosome studies reveal a chromosome number of 47, the extra chromosome being one of the sex chromosomes, giving a sex chromosome constitution of XXY (Fig. 9).[71, 10, 100] Several remarkable cases are known which show the clinical features of both mongolism and Klinefelter's syndrome, these cases have 48 chromosomes with the XXY sex complement in addition to the autosomal trisomy described above.[43, 81, 82]

Patients with the clinical and endocrinological features of Klinefelter's syndrome but with chromatin-negative nuclear sex comprise two his-

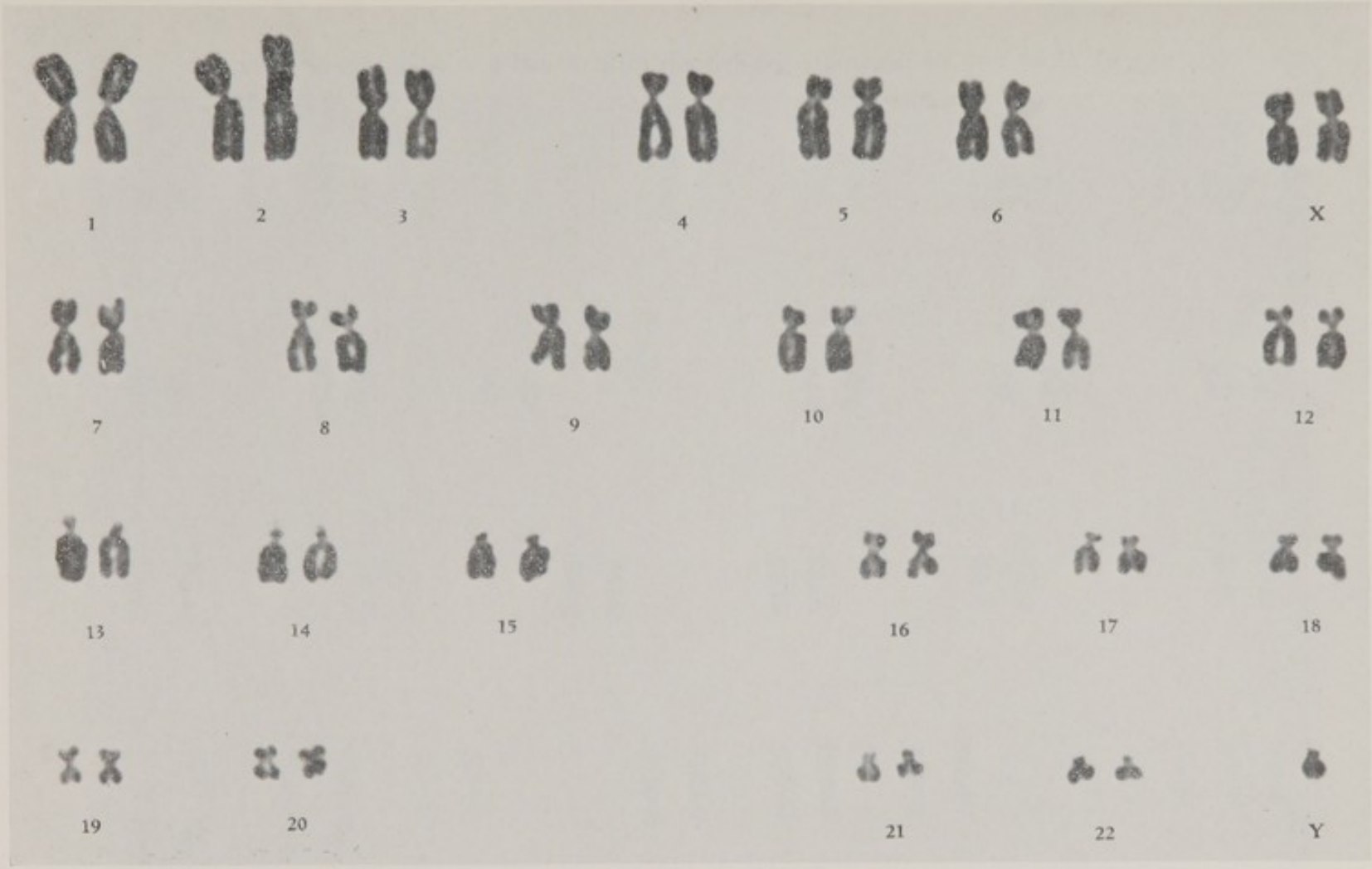

FIG. 9—Karyotype of a patient with chromatin-positive Klinefelter's syndrome. $2n + 1 = 47$ (XXY).

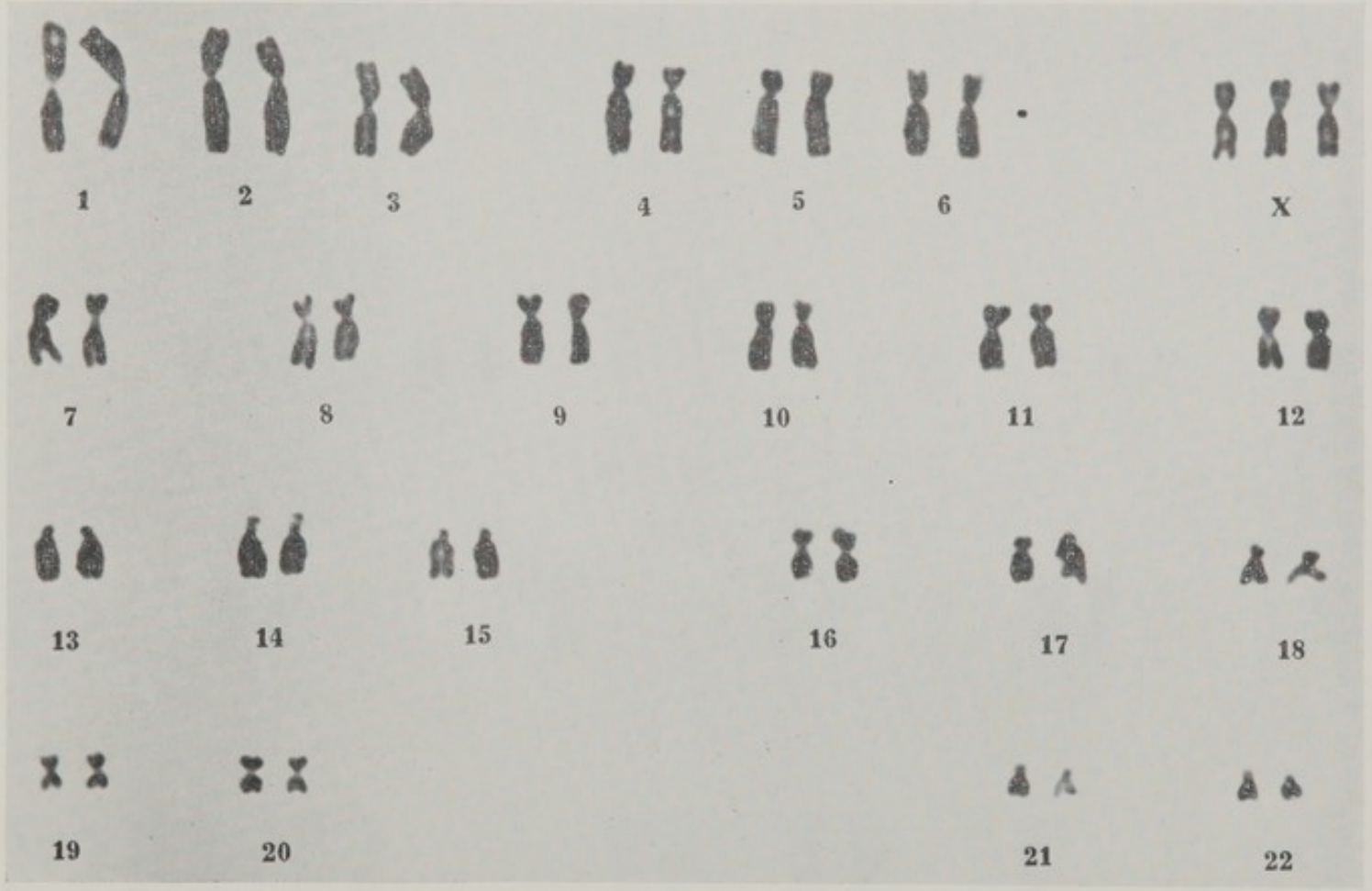

FIG. 10—Karyotype of a patient with the triple-X syndrome. $2n + 1 = 47$ (XXX).

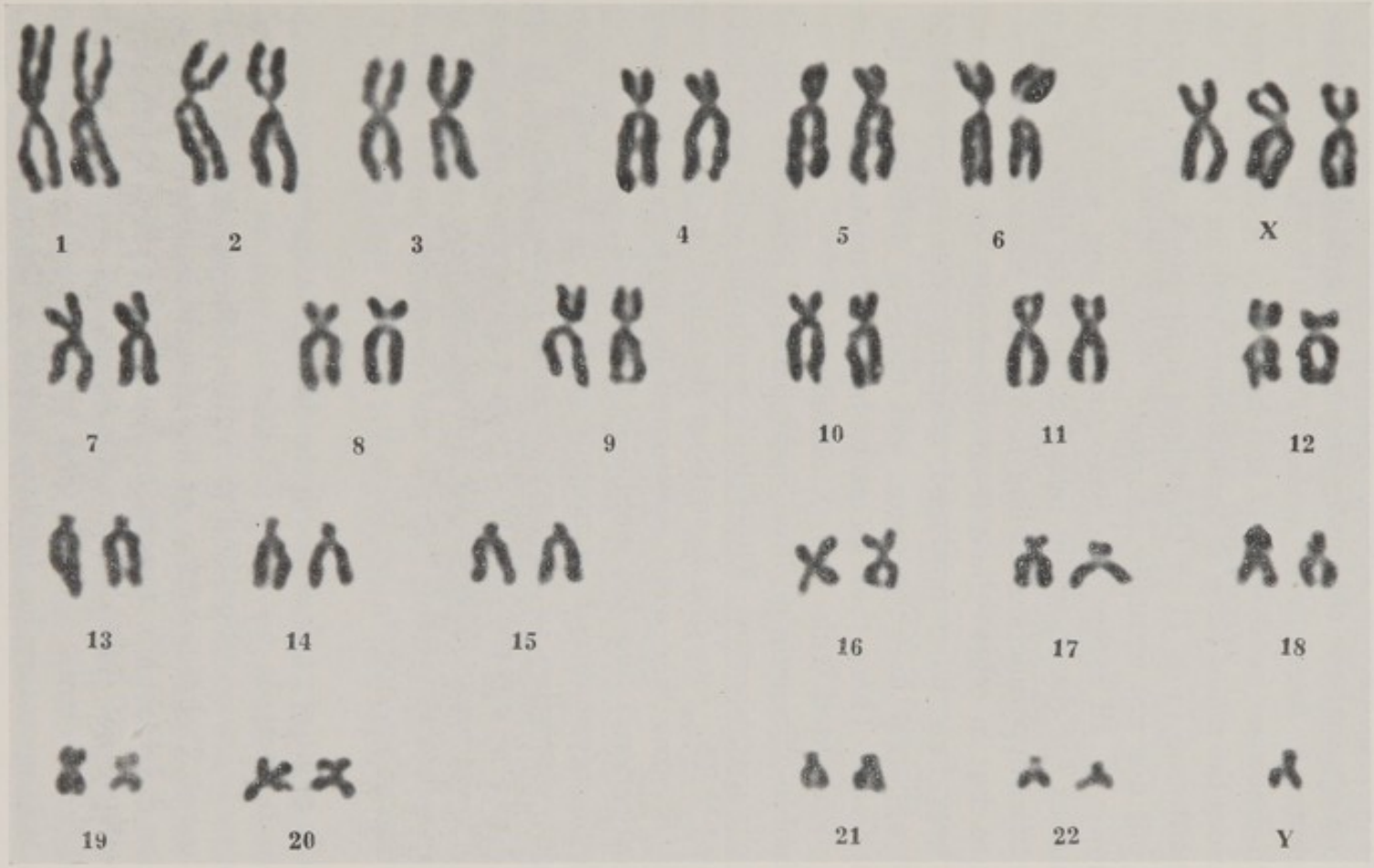

FIG. 11—Karyotype of a patient with the triple X/Y syndrome. $2n + 2 = 48$ (XXXY).

tologically distinct types, which may both be distinguished from the chromatin-positive variety.[34] The few chromatin-negative patients to be studied have shown a 46 (XY) genotype.[33, 46]

Nuclear sexing surveys among mentally defective individuals have revealed an unusual sex chromatin anomaly of a double complement of sex chromatin in intermitotic nuclei (Fig. 4).[8, 36] The female patients have a chromosome number of 47 and have three X chromosomes (Fig. 10), whereas the males have 48 chromosomes with an XXXY sex chromosome constitution (Fig. 11).[33] Of at least ten triple-X cases described to date all but one are mentally retarded.[67, 119, 72, 24, 52, 127, 9, 74] It should be noted, however, all except one were found during surveys of patients in institutions for the mentally retarded. In some there is evidence of slight ovarian hypofunction leading to irregular menstruation and early menopause, but in others there is no abnormality of sex differentiation and at least four have produced offspring. Certain mongoloid features are apparent in three cases, and there is webbing of the neck in another case. There thus seems to be no characteristic clinical abnormality associated with three X chromosomes.

The double-chromatin-positive male patients show the clinical features of chromatin-positive Klinefelter's syndrome with some additional anomalies.[9, 32] The few that have been described to date are mentally retarded (because they were ascertained by nuclear sexing surveys in mental institutions); one has webbing of the neck.

Very little information is at present available on the other three sex chromosome anomalies which have recently been reported. It appears that the XXYY[98, 9] and the XXXXY[49, 50] individuals show many features of Klinefelter's syndrome. The XXXX individuals have apparently normal female reproductive systems.[9] Triple sex chromatin masses are found in a proportion of intermitotic nuclei in the tetra-X and the tetra X/Y individuals.

From the point of view of sex determination in man these cases are of very great interest. It now appears that in man the presence of a Y chromosome is always associated with male differentiation irrespective of the number of X chromosomes. As has already been mentioned sex determination in Drosophila appears to depend on a strict balance between masculinizing genes carried on the autosomes and feminizing genes carried on X chromosomes: the Y is inert with respect to sex determination although it is necessary for fertility. Hence in Drosophila the XO is a sterile male, whereas the XXY is a fertile female. In man, and in the house mouse,[118] the XO is a female individual, and in man the XXY, the XXXY, and the XXXXY are males (the latter two have not been observed

in the mouse). The XXX and XXXX individuals are females in whom the additional X chromosomes do not appear to affect sex differentiation appreciably. The only condition in which a testis is formed in the absence of a Y chromosome, is in chromatin-positive true hermaphroditism. Thus the human Y chromosome must normally carry strong male determiners.

Chromosomal Mosaicism

At the time of writing, the reports of cases of chromosomal mosaicism implicate the sex chromosomes only (Table 4) although there would seem to be an equal possibility for the existence of autosomal mosaicism, for example involving the autosome trisomic in mongolism.

Table 4—*Chromosomal Mosaicism*

Clinical Condition	Sex Chromosome Constitution
Chromatin-positive Klinefelter's syndrome	XX/XXY
Chromatin-positive Turner's syndrome	XX/XO
Chromatin-positive Turner's syndrome	XO/XXX
Male Intersex	XO/XY

The sex chromosome mosaics do not seem to be associated with any new clinical syndromes. The XXY/XX individual[44, 21] is a chromatin-positive case of Klinefelter's syndrome, the XO/XX and the XO/XXX have chromatin-positive gonadal aplasia.[72, 46] The single report of the XY/XO mosaic involves a chromatin-negative intersex with undifferentiated gonads and female internal genital ducts.[63] In many ways this individual resembles certain cases of male pseudohermaphroditism.

Structural Abnormalities of the Chromosomes

At least four different types of reciprocal translocation between autosomes are known in man and these all involve satellited chromosomes (Table V). Turpin and his colleagues described the first example in a 4½ year old boy with multiple anomalies of the skeletal column to which they give the term *polydysspondylie*.[136] The clinical features include fusion of several vertebrae, absence of a hemivertebrum and rib, calcification of the interclinoid ligament over the sella turcica, slightly subnormal intelligence, and a general mild retardation in development. He has 45 chromosomes, an XY sex chromosome complement, and lacks one of the Group VII chromosomes. In addition, one of the Group IV chromosomes is remarkable in that it has very much larger short arms than its

homologue. This state of affairs is interpreted as a reciprocal translocation between chromosomes 22 and 15, although of course the difficulty in identifying the individual chromosomes within Groups IV and VII makes such an exact interpretation exceedingly difficult if not impossible.

TABLE 5—*Structural Abnormalities of the Autosomes*

Clinical Condition	Chromosome Constitution
Polydysspondylie	2n − 1 = 45 translocation 22/15
Mongol family type A.	2n = 46 translocation 22/15
Mongol family type B.	2n = 46 translocation 22/15
Chromatin-positive Klinefelter case	2n = 46 (XXY) translocation 14/14

The Paris group reports more recently a reciprocal translocation between two chromosomes in Group IV in a case of chromatin-positive Klinefelter's syndrome who has a chromosome count of 46 and an XXY sex chromosome constitution.[85]

Translocations involving one of the trisomic autosomes in several cases of mongolism are now reported. In one type (Table 5 type A) the two chromosomes taking part were interpreted as numbers 15 and 21,[114] and in the other type (Table 5 Type B) it appears that two of the three number 21 chromosomes are involved.[48] A most remarkable feature in one of the type B cases was the finding of 47 chromosomes in the father who was clinically normal—the extra chromosome being similar in appearance to number 19.[48]

Since these reports, two families are reported in which abnormal translocation chromosomes (type A) have been transmitted through three generations of normal females and is responsible for multiple cases of mongolism within the family.[18, 109] For example, in one pedigree, the "normal" grandmother had a chromosome count of 45, was monosomic for chromosome 15 and 21, and had an abnormal type A chromosome.[18] Three of her grandchildren were mongoloid and were found to have chromosome counts of 46, including the abnormal type A chromosome and two number 21 chromosomes; for practical purposes they were thus trisomic for chromosome 21. The grandmother and her two "normal" daughters, the mothers of the mongol children, are translocation heterozygotes in that they have 45 chromosomes and are monosomic for numbers 12 and 21. They must possess all the necessary genetic material of the missing chromosomes in the abnormal translocation chromosome for they are clinically normal.

At least one family is known in which there are several mongols in different generations with the straightforward trisomic condition indi-

cating that reciprocal translocation does not account for all cases of familial mongolism.[73] The inheritance of a mutant gene favoring nondisjunction of the chromosome trisomic in mongolism is a possibility in these families.

TABLE 6—*Structural Abnormalities of the Sex Chromosomes*

Clinical Condition	Chromosome Constitution	Nuclear Sex
Chromatin-positive Gonadal Aplasia	2n = 46 Deletion X	Diminished positive
Chromatin-positive Gonadal Aplasia	2n = 46 Translocation X (or isochromosome X)	Increased positive

Structural abnormalities involving the sex chromosomes are as yet known only in cases of chromatin-positive gonadal aplasia (Table 6). Jacobs et al.,[72] report one case in which equivocal nuclear sex findings were known before the demonstration of a large deletion of one of the X chromosomes. Only 7 per cent of oral mucosal cells were chromatin-positive and the sex chromatin was noted to be smaller than usual. It is possible that the case of Edwards et al.,[26] interpreted as an example of trisomy of chromosome number 18 is similar to this case as the clinical features show so many of the anomalies found in Turner's syndrome.

The case of chromatin-positive gonadal aplasia, whose karyotype is shown in Fig. 12, appears to have a larger sex chromatin mass than usual.[38] Analysis of the chromosome complement at first suggests that the patient is trisomic for chromosome number 3. However, in view of the nuclear sex findings and the associated clinical syndrome, it would appear more likely that the extra chromosome is an abnormal X. The abnormality may represent a reciprocal translocation, but the fact that the centromere is in the medial position, and several similar cases have now been reported,[51] makes it seem more likely that the abnormal chromosome is an isochromosome.

CHROMOSOME ABNORMALITIES IN OTHER TYPES OF HUMAN DISEASE

It is not the intention to discuss here the studies of chromosomes in cancer except to note that chromosomal abnormalities have been identified in cases of leukemia.[45, 5, 101] The association of mongolism and leukemia suggests that aneuploidy may predispose to neoplasia, and that this may be a profitable line of study in the future. However, there have been no reports of consistent chromosome aberrations in other forms of cancer.

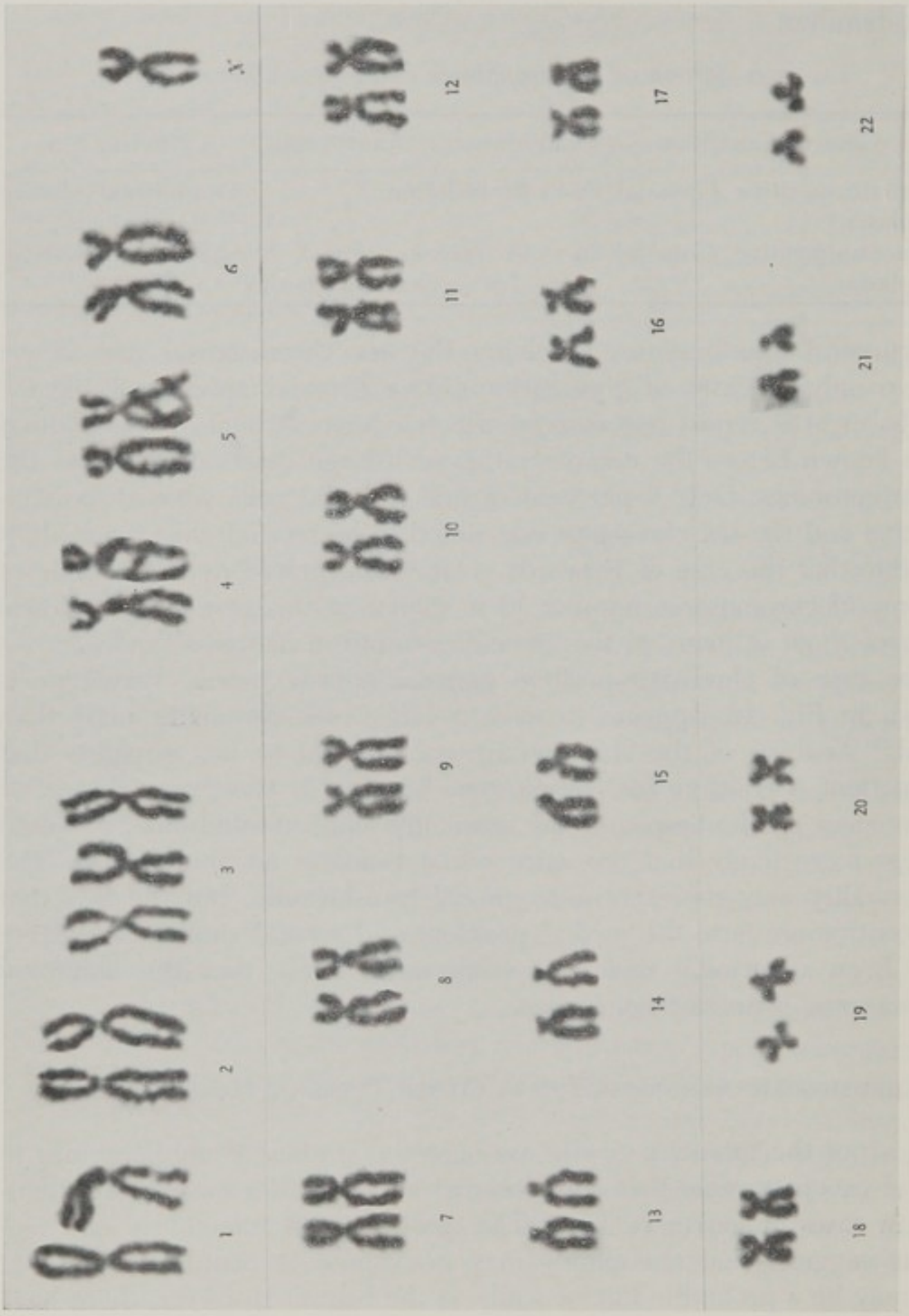

FIG. 12—Karyotype of a patient with a chromatin-positive gonadal aplasia. 2n = 46 (XX).

Chromosomes have also been studied in ovarian teratomata[53] and in hydatidiform moles,[128] but owing largely to the technical difficulties involved in these studies, few conclusions can be drawn from them at this stage.

There remain the increasingly large list of conditions in which chromosome analysis has failed to reveal any abnormality. These include cases of human intersexuality such as true hermaphroditism,[60, 65, 31, 23, 55] male pseudohermaphroditism with testicular feminisation,[69, 117, 20, 2] and male pseudohermaphroditism with ambiguous sex differentiation.[2]

Ford[46] quotes a list of hereditary conditions in which examination has shown no chromosome abnormality:

Acrocephalosyndactyly
Anencephaly
Arachnodactyly (Marfan's syndrome)
Chondrodystrophy
Crouzon's disease
Epiloia
Gargoylism
Gaucher's disease
Hypopituitary dwarfism
Juvenile amaurotic idiocy
Laurence-Moon-Biedl syndrome
Little's disease
Osteogenesis imperfecta
Phenylketonuria

Despite the negative findings of several investigators, a recent report of chromosome studies in Marfan's syndrome describes abnormally enlarged satellites in two out of three cases examined.[133] The authors suggest that this observation may be the basic defect in the syndrome. However from the clinical information available, it is not certain that the diagnosis is acceptable in the two cases which show the chromosome abnormality, while the other case which does not show satellite enlargement has the typical features of the Marfan syndrome.[93] Experience with this and other syndromes suggests that the size of satellites varies in different individuals without being necessarily associated with congenital disease.[37]

The Etiology of Chromosomal Aberrations

Numerical abnormalities of the human chromosomes may best be explained by a mechanism known as non-disjunction. This term refers to an abnormality of cell division in which newly divided chromosomes fail to proceed to opposite poles of the spindle, and instead remain attached to one another and pass together to one pole. The resultant daughter cells thus contain unequal numbers of chromosomes. For a person to show the same abnormal chromosome number in all his cells, non-disjunction must occur either during the early division of the zygote or during meiosis in the parental germ cells.

I. *Mitotic non-disjunction during the early division of the zygote:* If mitotic non-disjunction of the sex chromosomes occurred at the first cleavage division of a female (XX) zygote, then one daughter cell would contain three X chromosomes and the other daughter cell only one X chromosome. If one cell was eliminated, then either the XO individual with gonadal aplasia, or the XXX individual with the triple-X syndrome would result. If both these daughter cells proliferated normally, the individual would develop into an XIO/XXX mosaic. The actual demonstration of this type of human sex chromosome anomaly[72] is to date the best evidence in favor of mitotic non-disjunction in man. Gynandromorphism in Drosophila[16] may be due to this type of mechanism, but further supporting evidence in mammals is at present negative and is based on the failure to detect cytological evidence in favor of non-disjunction of the sex chromosomes in male meiotic figures in the mouse.[102]

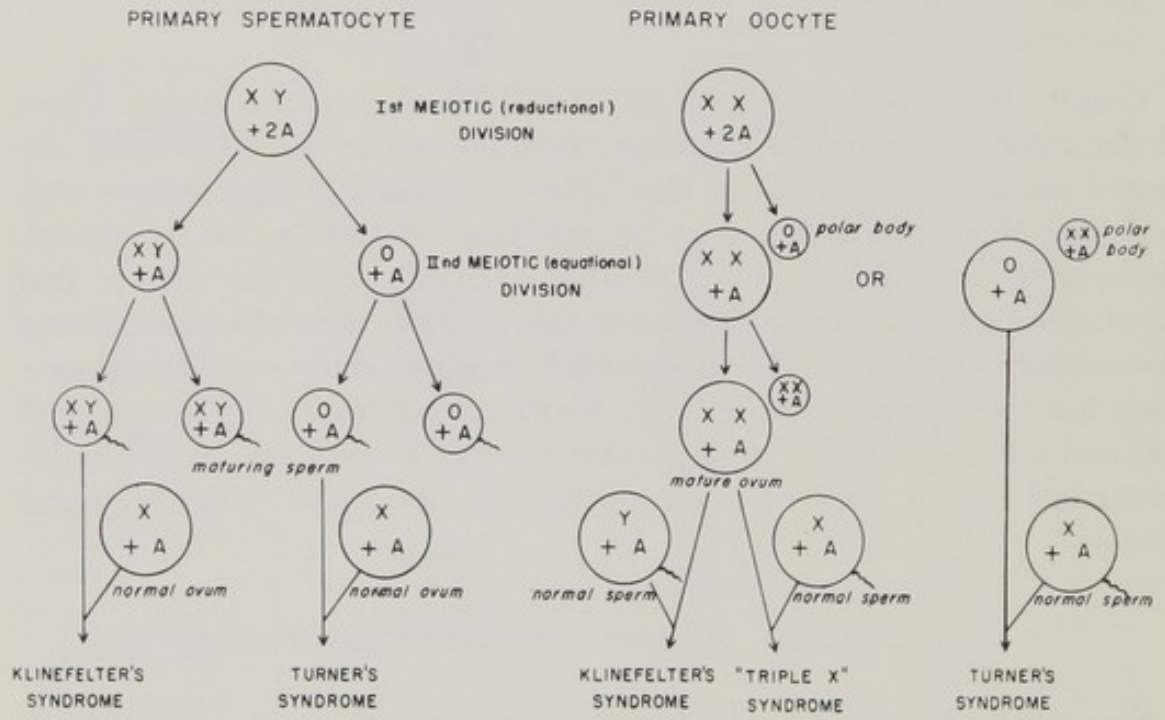

FIG. 13—Diagrammatic representation of meiotic non-disjunction of the sex chromosomes.

II. *Meiotic non-disjunction in the parental germ cells:* Non-disjunction during gametogensis offers a simple explanation for many of the numerical chromosomal abnormalities except mosaicism. As shown diagrammatically in Fig. 13, occuring in the sex chromosome pair during the first meiotic (reduction) division, it leads to the formation of abnormal gametes bearing either no sex chromosomes, two X chromosomes, or an X and a Y chromosome. When these abnormal gametes take part in fer-

tilization with normal gametes, then XO, YO, XXY and XXX are the possible sex chromosome complements of the zygotes. When abnormal gametes from *both* parents take part in fertilization then two new combinations are formed—the OO (with no sex chromosomes) and the XXXY. Non-disjunction at *both* first and second meiotic divisions in *one* of the parents with fertilization by a normal gamete from the other parent could give rise to XXXY, XXXX, XXYY, XXXYY, XXXXY, or XXXXX zygotes. Continuing on this rather näive line of reasoning, and increasing the number of non-disjunctional events, one could propose almost unlimited permutations of sex chromosomes. With the exception of the XO, the XXY, the XXX, and the YO (which has not been reported and may prove to be inviable), an explanation of the origin of the other four known sex chromosome anomalies requires more than one abnormal cell division.

It is of very considerable interest that a similar mechanism was demonstrated by Calvin Bridges and others over 40 years ago in Drosophila.[15] Comparison of this work with the human chromosome studies shows some remarkable correlations. Bridges showed that certain exceptional female flies were occasionally produced during his Drosophila breeding experiments. These exceptional females resembled the mother in all sex-linked characters and apparently carried no sex-linked characters from the father. This suggested that both X chromosomes were inherited from the mother, and this hypothesis was later confirmed when an XXY sex chromosome constitution was demonstrated cytologically. Bridges was also able to show that exceptional males bearing sex-linked characters from the father had an XO sex chromosome complement; these males were sterile. These findings were explained by non-disjunction of the sex chromosomes at the first meiotic division during oogenesis in the mother, leading to abnormal ova containing either both the sex chromosomes or no sex chromosomes. In addition to the XO and XXY flies, another exception was found, the XXX or super-female, which is sterile and usually non-viable. As the XXY fly is fertile, it was possible to breed from it other exceptional flies. These include the XYY fly which is a fertile male. Breeding the XXY female with the XYY male, produced a further viable exception with the XXYY constitution. There are no records of a triple-X/Y fly, although such is theoretically possible.

Color-blindness Studies

Owing to a lack of suitable genetic markers it is at present not possible to trace human chromosomes through a pedigree, and so determine the origin of the non-disjunction in the way that it has been accom-

plished in Drosophila. However we have one marker, the mutant gene for color blindness which is inherited as a sex-linked recessive characteristic. Thus, in man the most useful example of the exceptional female is the chromatin-negative case of Turner's syndrome who is color blind. These XO cases show a similar frequency of color blindness as normal XY males (8 per cent).[112] The frequency is much lower in normal XX females (0.65 per cent) and in XXY cases of Klinefelter's syndrome[113] since these individuals have to be homozygous for the mutant gene to manifest color blindness. At least four color-blind patients with gonadal aplasia are described in which both parents have normal color vision.[42] This indicates that the mothers are heterozygous carriers of the mutant gene, and have transmitted the affected X chromosome in their ova to their chromatin-negative color-blind daughters. This may be taken as evidence that the non-disjunctional event has occurred during spermatogenesis in the father, and that a sperm bearing no sex chromosome is responsible for the XO condition in the offspring. There is a single report of a color-blind case of gonadal aplasia whose father was color-blind, but this is not conclusive enough to implicate non-disjunction during oogenesis, as the mother could also have been a heterozygous carrier.[137]

Three color-blind XXY cases of Klinefelter's syndrome are known.[99] The mother, but not the father, of one patient was color-blind and was therefore homozygous for the gene. The parents of the other two cases had normal color vision, suggesting that the mother in each was a heterozygous carrier. Non-disjunction at either first or second meiotic division during oogenesis in the mother would explain the origin of the first color-blind patient, as both maternal X chromosomes carry the mutant gene. In the other two cases, meiotic non-disjunction must have occurred at the second (equational) meiotic division, as only one maternal X chromosome carries the mutant gene. Non-disjunction at the first meiotic division preceded by crossing over between the X chromosomes is an alternative explanation.

Studies on color blindness in chromatin-positive Klinefelter's syndrome suggested at first that the observed frequency (0 in 72) was compatible with the expected frequency in a comparable sample of normal females, and at the time this was considered good evidence for the presence of two X chromosomes.[113] Addition of more recent data, which includes the three color-blind cases mentioned above, raises the total number of cases tested to 103 and the number affected to three, which is nearly five times greater than that expected in a female population, but less than half that expected in a male population.[124] Non-disjunction at the second meiotic division provides one answer for this unusual frequency by in-

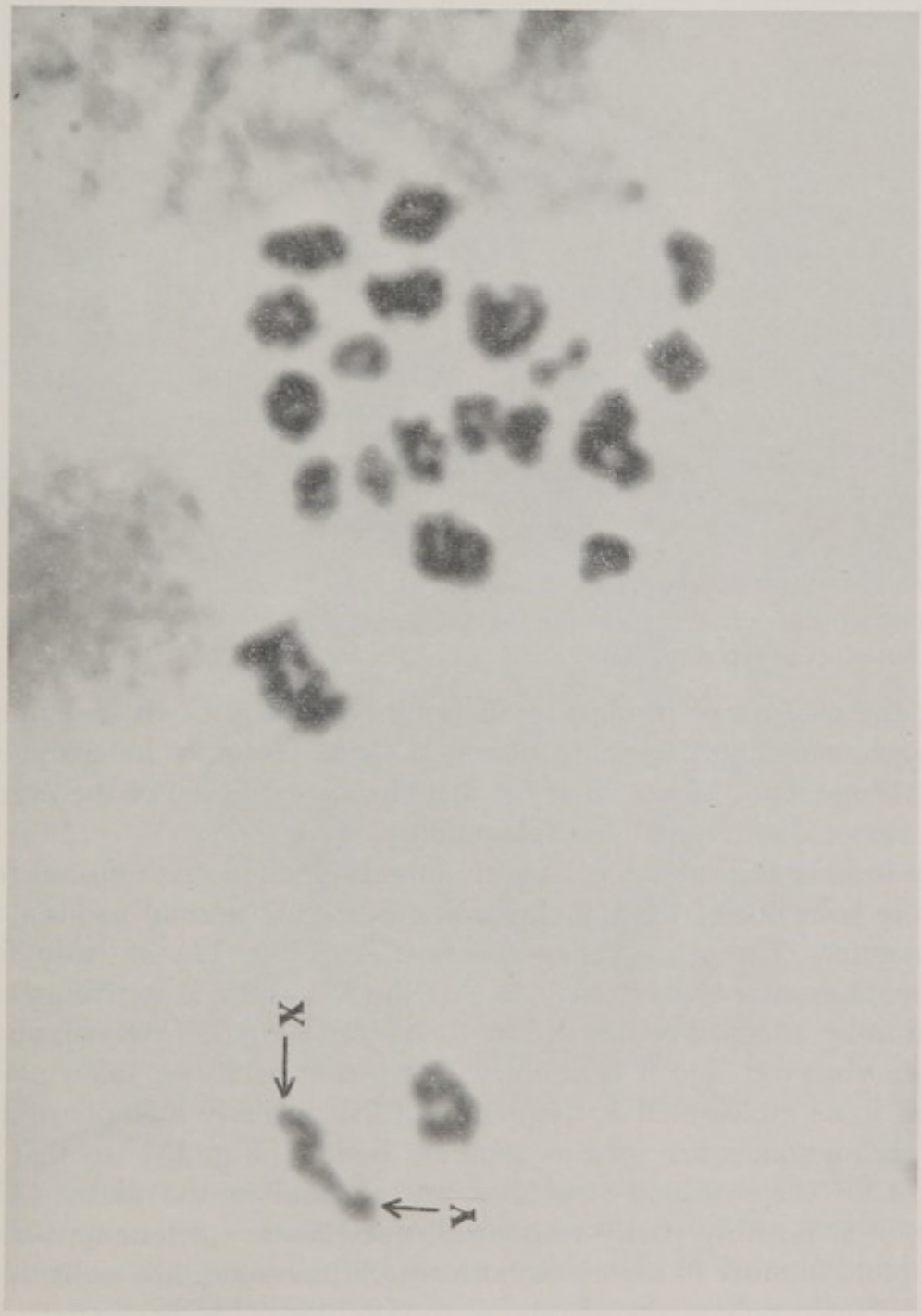

FIG. 14—The XY sex bivalent at first meiotic metaphase in a normal male (one autosomal bivalent is not seen in this plate). (Feulgen × 4,000.)

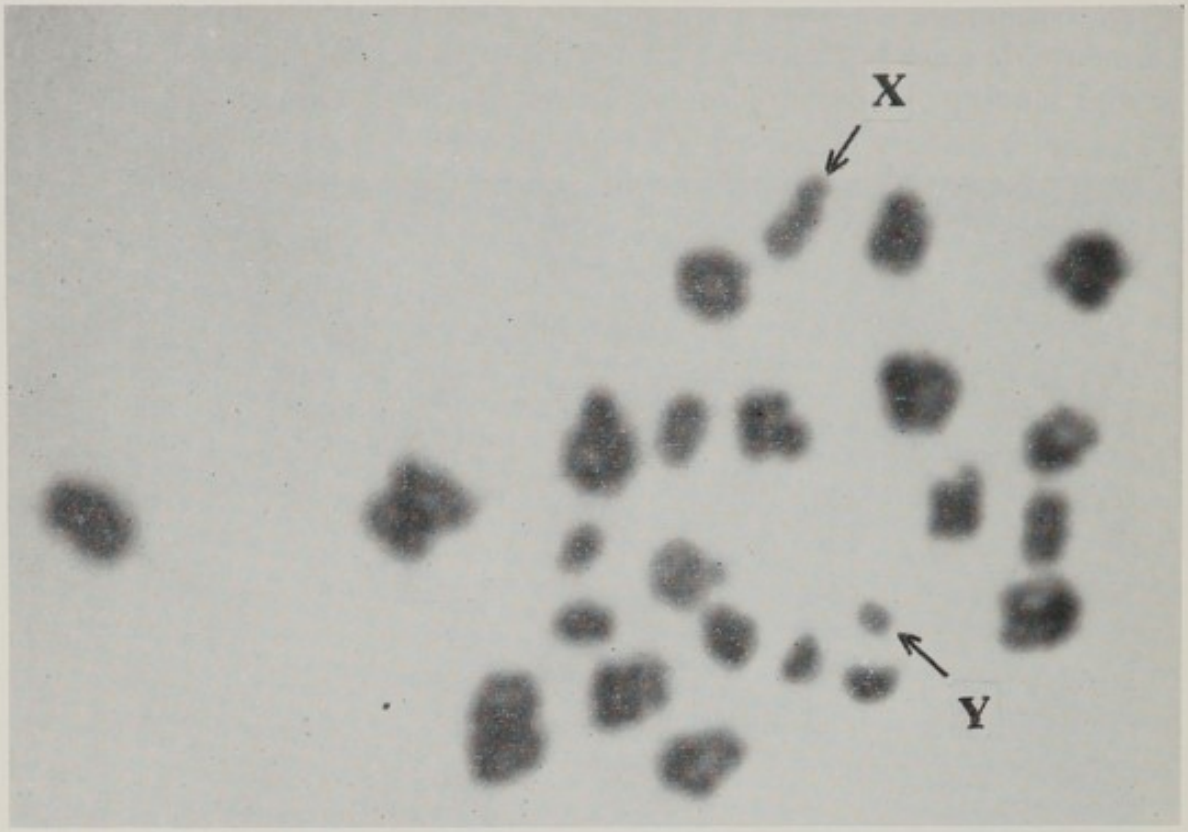

FIG. 15—Precocious disjunction of the sex chromosomes at first meiotic metaphase in a normal male. (Feulgen × 4,000.)

creasing the chances of producing offspring which have two X chromosomes each carrying the color blindness gene, from a heterozygous carrier. Mitotic non-disjunction at the first cleavage division of the zygote is still a reasonable alternative explanation.[124]

Before leaving the subject of meiotic non-disjunction one unusual feature of the behavior of the sex chromosomes during normal meiosis deserves mention. The sex chromosome bivalent (Fig. 14) at early first meiotic metaphase is characteristic in that the X and the Y are frequently observed to be attached terminally by their short arms.[40, 38] No chiasmata have been observed, and it is doubtful if crossing over ever takes place. Even this loose attachment is frequently absent in early metaphase and the sex chromosomes are seen as separate bodies (Fig. 15), so that 24 instead of 23 chromosomal structures are counted in the plate. (This observation is possibly partly responsible for Painter's estimation of 48 as the diploid number in man.) As both sex chromosomes are as likely to pass to the same pole of the spindle as to opposite poles, precocious disjunction has the same effect as non-disjunction.

The numerical chromosomal abnormalities produced by non-disjunction are transmissible if the aneuploid individual is fertile, for half of the gametes will be euploid and half will be aneuploid. This type of segre-

gation is possible but unknown in the triple-X individual, but is well known in mongolism. At least eight mongoloid females are reported as having had children, and of these five were normal, and five were mongoloid.[120, 84]

The Etiology of Structural Chromosomal Anomalies

Very little data has been collected on the factors which could be responsible for structural chromosomal abnormalities in man. Chromosome breakage is well-known following irradiation and possibly occurs during treatment with antimetabolites and cytotoxic drugs. Therapeutic doses of X-rays have been found to produce typical chromosome breakage and fusion anomalies in white cells from the peripheral blood,[134] and a history of extensive irradiation before conception is reported in a case of mongolism with a translocation abnormality.[114]

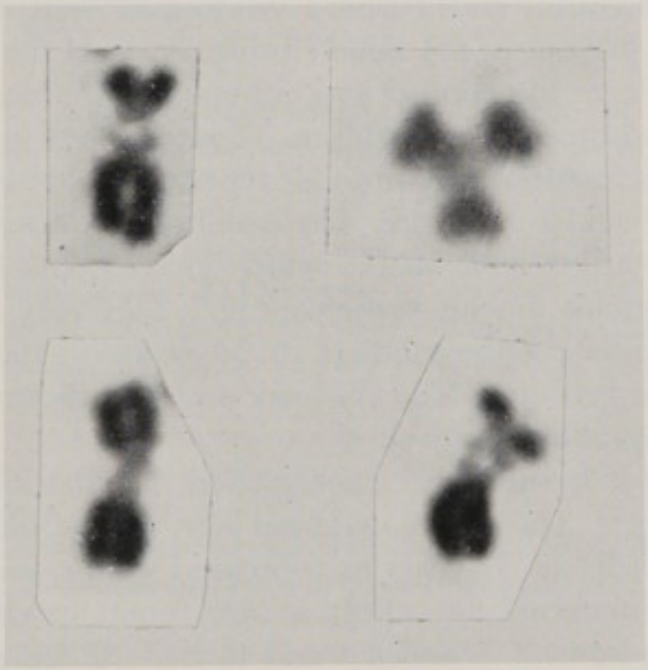

Fig. 16—Four instances of satellite association selected from different cells (Aceto-orcein × 4,000.)

As has been already mentioned, all the examples of structural chromosomal anomalies in man have involved satellited chromosomes. It may be that satellited chromosomes are more liable to chromosome breakage and translocation than the other chromosomes and in favor of this is the phenomenon of *satellite association*.[37] This phenomenon is observed frequently in human chromosome preparations from all tissues but particularly from peripheral blood where it may be present in as many as 60 per cent of cells. Satellite association is shown by chromosomes in

Groups IV and VII, several of which may appear attached to one another by their satellites (Fig. 16). Most frequently two chromosomes are associated together in this way but sometimes three or four are seen linked together as if holding hands in a circle. The presence of nuclear material in the region of the satellite possibly make the satellited chromosomes stick to one another. Chromosomes associated in this way at meiosis may be more liable to reciprocal translocation than non-satellited chromosomes. Similarly, satellite association may be a factor in the production of aneuploidy by non-disjunction, for of the three types of human autosomal trisomy that have been described, two involve satellited chromosomes.

Other Important Predisposing Factors in Human Chromosomal Anomalies

Several families are now known in which mongolism and Klinefelter's syndrome,[96] mongolism and Turner's syndrome,[73] and multiple cases of mongolism, are present in the same sibship. At least four individuals are described who have the clinical and chromosomal abnormalities of both Klinefelter's syndrome and mongolism. These findings indicate that there are predisposing factors common to all types of aneuploidy.

If one goes back to the early Drosophila breeding experiments one finds that there are several factors which increase the frequency of exceptional individuals among the offspring.[97] This may be done experimentally by irradiating the mothers or by exposing them to conditions of anoxia before breeding. The frequency of non-disjunction is also increased by using older virgin flies.

In mongolism it has long been recognized that the mother's age is an important factor.[107] The mean maternal age at the birth of a mongol child is in the order of 37 years as compared with a mean age of 29 in the controls. A maternal age effect is also found in chromatin-positive Klinefelter's syndrome,[30, 88] and a trend in that direction is seen in the limited number of triple-X and triple-X/Y cases that have been studied up to this time. There is however no evidence of any parental age effect in gonadal aplasia,[13] and this together with the genetic evidence from the color vision studies pointing to non-disjunction during spermatogenesis, suggests that other factors may be more important.

As prevention of these human chromosome abnormalities is our ultimate goal, careful investigation along these and other lines into the background of every case is essential. Accumulation of this type of data will lead to a knowledge of significant predisposing factors and perhaps in the future to methods by which they may be controlled.

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