Progress in

MEDICAL GENETICS

VOLUME I

Edited by

ARTHUR G. STEINBERG

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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

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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[25] 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