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Fifty Years of Medical Genetics

The union of biochemistry and genetics offers a rational approach to diagnosis, prevention, and therapy.

Laurence H. Snyder

Fifty years ago, in 1908, Sir Archibald E. Garrod presented in England a most remarkable set of the Croonian lectures (*1*), setting forth a new concept of human disease, which he called "inborn errors of metabolism." Garrod was far ahead of his time in this concept, and it took many years for geneticists to appreciate the full significance of his contribution. The rapid and widespread development of medical genetics at the present time owes its inception to the recently renewed interest of human geneticists in Garrod's demonstration that, through mutation, the dysfunction of a gene-controlled enzyme necessary for normal metabolism is a basic mechanism in the production of genetic disease.

The term "genetic disease" is used in this paper to apply broadly to any deviation from the usual or normal condition, for which a genetic basis can be established. I shall attempt to show that there are reasons for believing that genetics is involved in one way or another in the development of all disease.

Garrod illustrated his concepts with his own basic studies of four human anomalies: albinism, alcaptonuria, cystinuria, and pentosuria. Today, both in experimental organisms and in man himself, the identification of many enzyme dysfunctions with mutant genes has reached a high point of development. On this 50th anniversary of Garrod's basic contribution, it is with deep appreciation of his foresight and ability that I offer a survey and critique of some of the advances in medical genetics that we have been able to build on the foundation of his fundamental concepts. Through the expansion of these concepts, genetics and biochemistry are rapidly becoming facets of one and the same science, and the resulting mosaic is playing an indispensable role in the progress of medicine.

The reawakening of the interest of medical geneticists in the biochemical backgrounds of genetic diseases apparently had to await the occurrence of a number of other developments (*2*). Three main events brought into sharp focus the steps intervening between the presence of a gene in the cell and the appearance of a trait or disease in the individual, and thus led to a renewed interest in Garrod's suggestions. The first of these events was the firm establishment of the concept that all metabolism proceeds through series of small sequential steps, each step catalyzed by an enzyme. The second was the demonstration (*3*) that increase in radiation level can increase the mutation rate. The third event was the realization (*4*) that with the aid of radiation-induced mutations the biochemical activities of genes can be definitively studied in microorganisms which are peculiarly suited both to genetic and biochemical investigation. The researches undertaken all over the world along these lines have brilliantly demonstrated the facts that many enzyme dysfunctions are indeed referable to specific mutant genes and that it is reasonable to presume that the production of normal enzymes is dependent on the activity of the normal unmutated alleles of these mutant genes.

Metabolic Blocks

Theoretically, we may conceive of a number of ways in which the lack, or partial lack, or inhibition of an enzyme may lead to altered metabolism, and thus, perhaps, to pathological consequences. I shall present some of these possibilities in simplified form, adding examples from genetic pathology in man where they are known or suspected.

Consider the sequential reaction

$$P \rightarrow Q \rightarrow R \rightarrow S \rightarrow T$$

in which each arrow represents an enzyme catalyzing the conversion of one substance to another. The specificity of each enzyme is considered to be the result of the activity of a single gene.

As an illustrative example of what is meant by a conversion catalyzed by one enzyme, xanthine (one of the purines) is oxidized to uric acid by the enzyme xanthine oxidase. The uric acid is then excreted in the urine. In the reaction shown in Fig. 1, note the essential similarity of xanthine and uric acid, and the addition of an oxygen atom brought about by the activity of the enzyme.

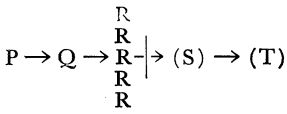
Returning to the sequential reaction

$$P \rightarrow Q \rightarrow R \rightarrow S \rightarrow T$$

consider now the gene that controls the enzyme that converts R to S. As a consequence of mutation, the mutant form of this gene may result in a somewhat different molecule, no longer enzymatically effective in facilitating the reaction, or at least no longer as effective as it formerly

Dr. Snyder, retiring president of the AAAS, is president of the University of Hawaii, Honolulu. This article is based on his AAAS presidential address, which was given on 28 December 1958, during the Washington, D.C., meeting.

was. One likely consequence of the metabolic block is that substance R will accumulate.

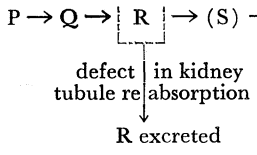


(The letters in parentheses indicate that the substance is no longer produced, or is produced in diminished amounts.)

The mere accumulation and storage of R may lead to pathological consequences. A probable example in man is Niemann-Pick disease, in which the genetic failure to degrade sphingomyelin causes this lipid to accumulate in the reticular and other cells, resulting in the manifold symptoms of the disease. A similar genetic failure to degrade one of the gangliosides leads to the storage of this lipid in the ganglion cells, with the resulting syndrome of infantile amaurotic idiocy (*5*). The recent discovery of a new phospholipid, malignolipin, found only in malignant tumors and never in normal tissues, may, if confirmed, eventually place cancer in this category (*6*).

Another possibility is that the accumulation of substance R may lead to its being excreted from the body. The excretion may be accompanied by symptoms, as stone formation, hematuria, and frequent micturition, in the disease xanthinuria, in which xanthine is not oxidized to uric acid (*7*). Conceivably the excretion could lead to pathological consequences by depletion of R or its precursors, or of other substances involved in the metabolism. In the last analysis, diabetes may well prove to belong in this general category.

It should be mentioned in passing that there is a genetic disturbance of quite a different sort that can also lead to excess excretion, in the urine, of a substance normally found there only in minute amounts, if at all. This disturbance is not a block in the direct metabolism of the substance excreted, but is rather a defect in the renal tubular reabsorption mechanism for that substance.



The precise genic action involved is as yet unspecified, but the phenomenon occurs in such diseases as glycinuria, cystinuria, and renal glycosuria (*8, 9*).

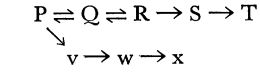
If the reaction P → Q → R is reversible, substance P rather than substance R may accumulate, leading to disease.

One type of glycogen disease of the liver is an excellent example, in which the genetic dysfunction of glucose-6-phosphatase prevents the reconversion of glycogen to glucose, but at a point several steps removed from glycogen itself; the reversible nature of the rest of the reaction results in the accumulation of glycogen (*10, 11*).

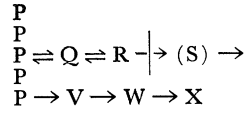
In some instances the mere absence of substance T may characterize the trait. Albinism, for example, is the result of the absence of melanin, due to the genetic dysfunction of tyrosinase. When this enzyme is lacking, tyrosine is not oxidized to dihydroxyphenylalanine, which latter substance normally proceeds through various conversions to melanin. Apparently the tyrosine is then metabolized through alternative pathways, with no further noticeable effects.

Alternative Pathways

Alternative metabolic pathways are not unusual in biochemical conversions, and this fact presents further possibilities in the production of genetic disease. It may be, for example, that although the bulk of substance P proceeds through Q, R, and S to T, some small amount of it normally passes through the steps P → v → w → x.

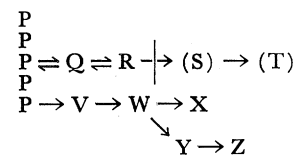


(The small letters are used to indicate that lesser amounts of substance P ordinarily follow this path.) If, then, the metabolic break occurs as before between R and S, and if substance P is thus accumulated, much more of it may be forced through this alternative pathway, and increased amounts of V, W, or X may cause pathologic effects through the altered patterns of substances in the cells.



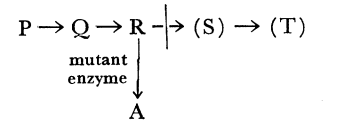
An example in human genetics is the severe mental disease phenylketonuria, in which phenylalanine cannot be oxidized to tyrosine, and as a result passes in large amounts through deaminating reactions which certainly derange the amino acid balance in the cells (*12*).

When a substance is forced through an alternative metabolic pathway, still other possibilities are opened up. When large amounts of W, for example, are available, some of this substance may in turn be diverted to a series of conversions not ordinarily followed: W → Y → Z.

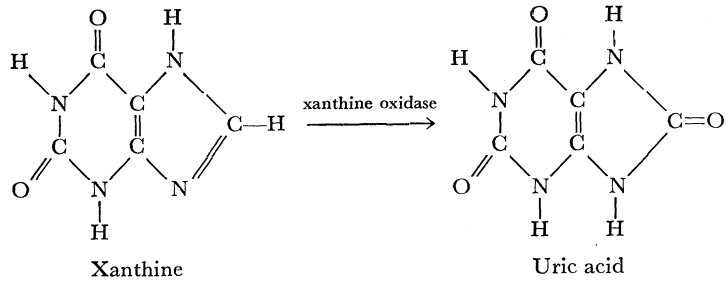


Under such circumstances, Z may conceivably cause pathological effects. No example in man has been clearly established, but the development of the abnormal form of the glycolipid kerasin in Gaucher's disease may be due to a reaction of this nature. The pigments laid down in ochronosis and causing degenerative osteoarthritis in some but not all patients suffering from alcaptonuria could well be examples of this type of genetic pathological development.

Finally, the mutant gene may result not merely in the lack of the enzyme formerly produced, but in the presence of a demonstrably functional but somewhat different enzyme. We have become aware of the fact that when the gene responsible for an *antigen* mutates, a specific but different antigen usually results from the activity of the mutant gene. It is probable that a similar phenomenon occurs in mutations involving enzymes.



If, for example, the enzyme which formerly converted R to S is changed through mutation in such a way that it now converts R to A (a new substance), the presence of A may give rise to pathological consequences. The ten or more variant forms of hemoglobin (*13*), each of which may result in a greater or lesser degree of anemia, are instances of this type of genetic disease. Sickle hemoglobin, for example, differs from normal hemoglobin in *only one* of the nearly 300 amino acid units of the half molecule, one of the glutamic acid residues of normal hemoglobin being replaced by a valine residue in the sickle form (*14*).



Xanthine Uric acid

Fig. 1. Oxidation of xanthine to uric acid.

In this instance the enzyme controlled by the mutant gene accounts for a very slight alteration of amino acid sequence in the polypeptide chain. Even this alteration, however, is sufficient to cause the illness and subsequent death of those individuals having only this type of hemoglobin.

Inhibitors

In addition to the foregoing suggestions, which deal primarily with enzyme lacks that are dependent on recessive mutations in genes which normally produce functional enzymes, one other possibility should be mentioned. It is known that enzymes may be interfered with by inhibition, and it seems likely that the inhibitor may at times be a substance produced by a mutant gene which is not itself directly concerned with the development of the enzyme inhibited. Since the inhibition appears to be caused by an active, effective substance, we tend to think of genetic inhibitors in terms of *dominant* mutations which result in specific substances causing metabolic blocks.

While it is certainly conceivable that the production of an effective substance could in some instances be contingent upon the homozygous state of a recessive gene, most genes seem to be capable in single dose of elaborating reasonably adequate amounts of the substances, and are thus in some degree dominant to their alleles which fail to produce the active agents.

In the reaction below, the action of the inhibitor is indicated by a box around the enyzme.

$$P \rightarrow Q \rightarrow R \boxed{\rightarrow} (S) \rightarrow (T)$$

A probable example in medical genetics is the inhibition of the enzyme enolase in glycolysis, resulting in the disease hereditary spherocytosis (*15*).

Many complications may be envisaged in regard to inhibitions of enzymes. To cite just one of these, it is known that excesses of certain normal metabolites may act as inhibitors. In the reaction below, the mere accumulation of substance R as a result of the genetic dysfunction of the enzyme converting R to S may cause inhibition of the enzyme converting B to C.

$$P \rightarrow Q \rightarrow \overset{R}{\underset{R}{\overset{|}{R}}}\!\!\rightarrow (S) \rightarrow (T)$$

$$B \boxed{-R\rightarrow} (C)$$

An example in man is the greatly reduced pigmentation which occurs incidentally in individuals suffering from the severe mental disease phenylketonuria. The excess of phenylalanine, which is found in those who have this disease, has been shown to act as an inhibitor of tyrosinase, thus diminishing the conversion of tyrosine to melanin (*16*).

The foregoing series of reactions portrays in very simplified form some of the possible paths by which mutations resulting in enzyme dysfunctions may lead to genetic disease. An important underlying principle in these concepts is that once a gene has mutated, the mutant allele is henceforth copied in its altered chemical form just as faithfully as the unmutated gene was formerly copied in its original chemical structure, thereby leading to the incorporation of the new allele into the pool of genes of the species.

Environmental Effects

The metabolic blocks thus far discussed have a direct relation to the production of genetic disease. The relation, however, may not always be so direct, and the pathological consequences of a gene may manifest themselves only in specific environmental situations. It has recently been shown, for example, that a genetic deficiency of the enzyme glucose-6-phosphate dehydrogenase may cause an alteration in glutathione metabolism, resulting in an instability of reduced glutathione (*17*). As a consequence of this instability, the red blood cells are liable to hemolysis following the ingestion of certain drugs such as naphthalene, primaquinine, sulfanilamide, and nitrofurantoin. Thus an induced hemolytic anemia develops, which, though appearing to be environmentally produced, has a definite genetic basis. From a practical standpoint, an assay of blood cells for this enzyme may make it possible to detect those individuals who are drug-sensitive and would be harmed by these drugs.

There are innumerable potential interactions between hereditary and environmental influences. It should be kept in mind that not all inhibitors are under genetic control. In addition it should be recalled that many enzymes have a coenzyme, or prosthetic group, as a necessary adjunct to the protein core. Although the protein core is determined by a gene, the coenzyme is often of vitamin origin, and is thus, in man, at least, environmentally conditioned. It is conceivable, therefore, that an enzyme which in one individual is inhibited by a genetically produced substance may in another individual be inhibited by an environmentally provided agent. Similarly, an enzyme which in one person is rendered dysfunctional by a genetic defect of the protein core, or apoenzyme, may in another person be inactivated by an environmental lack of the coenzyme. As a result of these and other exogenous effects, "phenocopies" may be produced, mimicking the genetic conditions. For example, nutritional siderosis appears to be a phenocopy of genetic hemochromatosis.

For many years I have predicted to my classes in medical genetics that the vitamin-deficiency diseases resulting from the nutritional lack of coenzymes would someday be found to be paralleled by similar diseases due to genetic deficiencies of the corresponding apoenzymes. This prediction has recently been fulfilled by the recognition of a genetic form of pellagra, designated as Hartnup disease (*18*). Classic pellagra is, of course, the result of a nutritional absence of the coenzyme containing niacinamide. In the genetic form there is apparently a dysfunction of the corresponding apoenzyme

involved in the metabolism of tryptophan. We may look forward to similar descriptions of genetic forms of scurvy, beriberi, and the rest. A genetic form of rickets, resistant to vitamin D and exhibiting hypophosphatasia, has been observed occasionally, and is the subject of a careful recent study (*19*). There is some evidence that it may prove to belong in the category of diseases resulting from a genetic defect in the renal tubular reabsorption mechanism, in this instance for phosphate.

Of course, the very fact that man requires vitamins in his diet in the first place is the result of gene-controlled enzyme dysfunctions apparently shared by all human beings. Most animals, for example, can synthesize L-ascorbic acid, by a series of steps, from D-glucose, and hence do not require ascorbic acid in their diets. Man and other primates, however, lack one enzyme in the series, and as a result are unable to accomplish the conversion of L-gulonolactone to L-ascorbic acid (*20*). Ascorbic acid thus becomes a vitamin for man: vitamin C.

In spite of the fact that genes are normally identical from cell to cell of the individual, the cytoplasmic constituents of the cells need not be equivalent. The unequal distribution, during mitosis, of mitochondria, microsomes, and other inclusions can result in the occurrence of identical genes in cytoplasmic environments which differ in concentrations of enzymes, substrates, and other materials from tissue to tissue. It is well known that the effect of a gene can be altered considerably by the environment, without any change taking place in the gene itself. In this way a given gene may produce an effect in one tissue and not in another.

In like manner, one gene may exert its influence at an early stage of the development of the individual while another may not be effective until a later stage. The consequences of the gene that conditions congenital ichthyosis, for example, are clearly detectable in the embryo, while the effects of the gene for Huntington's chorea are apparent only in later life. The explanation for this phenomenon appears to rest on a firm biochemical basis. Enzymes often compete for the same substrate. Equilibria in reversible reactions require time to be achieved, and the establishment of an equilibrium may free a substrate for a new conversion by a different enzyme. The accumulation of by-products may slowly reach critical levels at which the by-products may act as new substrates or as inhibitors.

Moreover, a biological protein is a mixed population of molecules of very different ages, some being very new, while others may be months old. Correlated with these differences in age are differences in the conformation of the molecules, involving such things as the formation of dithio bonds and the substitution of amino acids (*21*).

The descendents of a gene, then, may well be located in environments in the mature individual which are biochemically different from those in which the gene occurred in the embryo or the young organism. The effects of genic action could thus be quite understandably different at various times in the life history of a given individual.

It would seem to be a reasonable inference that genes do not exert absolute control over the presence or absence of specific enzymes but rather determine the potential development of particular enzymes and enzyme systems in particular environmental situations (*22*).

Thus the interlocking network of hereditary and environmental influences, which has long been obvious in the overt manifestations of traits and characteristics, is now seen to be equally operative in the basic biochemical and physiological processes of the cell itself.

Dominance

The concepts which I have discussed up to this point have an important bearing on our understanding of many Mendelian phenomena which were originally but vaguely understood. In particular, such terms as dominance, recessiveness, and epistasis have become clarified in the light of biochemical considerations (*22*).

In Mendel's original paper appears the statement (translated) ". . . those characters which are transmitted entire, or almost unchanged in the hybridization, and therefore in themselves constitute the characters of the hybrid, are termed the *dominant,* and those which become latent in the process *recessive.*" Since Mendel's day these terms have been applied to the genes (Mendel's *elements*) themselves as well as to the characters.

For many years the fact that one gene of a pair may be dominant to the other was merely stated, not explained. Today a simple, reasonable explanation is possible in terms of biochemical activity. The primary action of a gene appears to be the control of the specificity of a substance such as an enzyme which is responsible for the catalysis of a particular step in the synthesis or degradation of some compound. A recessive mutation in a gene often results in the failure of the mutant gene to develop the enzyme.

Albinism in man has been shown to be dependent upon the homozygous condition of a gene which has been named *c*. Thus *cc* individuals are albinos, but both *CC* and *Cc* persons are normally pigmented. Gene *C* is thus said to be dominant to its allele *c*.

The enzyme tyrosinase is always identifiable in pigmented individuals, who have the gene *C,* but is not demonstrable in albinos, who lack this gene (*23*). It would appear that *C* is responsible for the elaboration of tyrosinase, while its mutant allele *c* fails to develop this enzyme. It is equally apparent that one dose of *C* is grossly as effective as two. This latter fact is not surprising when it is realized that enzymes function by facilitating biochemical conversions all out of proportion to the amount of enzyme present. Since a particle of enzyme, once its quantum of catalytic action has been accomplished, dissociates itself from the resulting compound and is available for further catalytic activity, it is apparent that a small amount of enzyme may be sufficient to accomplish complete or nearly complete conversion.

It is logical to infer, then, that within a given genetic milieu one dose of *C* in a heterozygous (*Cc*) individual can elaborate sufficient tyrosinase to convert the available tyrosine to melanin. Since two doses of the gene (*CC*) could accomplish no more than this, the genotype *Cc* is as effective as the genotype *CC* in the development of melanin, and *C* is said to be "dominant" to *c*.

Consider your vitamin intake as an analogy. If, in your childhood, each of your two parents had provided you every day with your vitamin requirements, you might have had more than your minimum needs, but you would not have suffered from vitamin-deficiency diseases. If only one of your parents had supplied your needs, you would also have been free from deficiency symptoms. But if neither parent had provided any vitamins, the effects of the deprivation would have become manifest.

In man it has been demonstrated by means of phenylalanine tolerance tests that about half of the phenylalanine-oxidizing enzyme is in an inactive or ineffective form in those who are heterozygous for the gene for phenylketonuria (*24*). Thus those who are carriers of the gene may be identified by a chemical

test, even though outwardly they are quite normal. Other instances of genetic disease must be studied along these lines in order to test the general validity of this principle. Among the diseases, in addition to those already mentioned, in which the enzyme lack or deficiency has been specifically identified, and which are available for such study, are acatalasemia, alcaptonuria, constitutional hepatic dysfunction, at least one form of cretinism, galactosemia, several forms of glycogen disease, hypophosphatasia, and one form of methemoglobinuria (*11, 25*).

In addition to behaving as obvious enzymes, the substances controlled by genes may in some instances act as antigens or inhibitors or hormones. It is tempting to postulate that the activities of even these substances may be but specialized forms of enzymatic activity.

In the case of antigens, where identification is made relatively simple by the ability of the substance to provoke the production of specific antibodies, the mutation of a gene responsible for an antigen nearly always results in the development of a different but equally specific antigen. Heterozygotes are thus readily recognizable by the use of appropriate antiserums, and dominance disappears.

Between instances with no dominance and those with apparently complete dominance are all grades of the phenomenon. By means of various biochemical or immunological tests, and sometimes merely by keen observation, it is becoming increasingly possible to identify heterozygotes (*23, 26*) and thus to move both genetic prognosis and preventive medicine from a statistical to an individual basis. It is reasonable to presume that eventually, by appropriate, though often subtle tests, all heterozygotes will be identifiable. This, too, is an area of research that offers many challenges.

Sequential Reactions

The occurrence of mutant individuals and the genetic study of these individuals and their families have on several occasions indicated the complex sequential enzymatic nature of a reaction originally supposed to be simple and direct. Conversely, the prior biochemical demonstration that a conversion involves several enzymes acting sequentially has suggested the possibility of several genetic types of a particular disease.

As an example of the first of these two principles I may call attention to the recent work on the blood-clotting mechanism (*27*). No longer can we accept the simple Morawitz theory of blood coagulation, in which it was assumed that prothrombin plus thromboplastin plus calcium ions results in thrombin, and that thrombin plus fibrinogen results in fibrin, which produces the clot. No longer can we automatically classify as hemophilia any genetic hemorrhagic disease with prolonged clotting time. The study of mutant individuals with bleeding disease has indicated that at least nine gene-controlled substances (presumably enzymes) are required for the thromboplastic conversion of prothrombin to thrombin, and that each of these trace proteins may become dysfunctional through mutation.

The superficially similar diseases involving the thromboplastic activity of the blood-clotting mechanism now include, in addition to classical hemophilia, Christmas disease, parahemophilia, deuterohemophilia, tetartohemophilia, SPCA deficiency disease, Hageman deficiency disease, Stuart deficiency disease, and factor X deficiency disease.

The second principle, involving the indication on biochemical grounds that genetically diverse forms of a disease may be expected to occur, is well illustrated in nonendemic cretinism with goiter. When the complex sequential enzymatic steps in iodine metabolism leading to the synthesis of thyroid hormones were worked out, it was apparent that the failure of any one of these steps was genetically possible. Careful investigation with this in mind (*28*) has revealed at least three types of the disease, and more may be expected to occur. In one of the described types, iodotyrosines cannot be produced from tyrosyl residues and iodide, apparently because of a dysfunction of an oxidative enzyme. In another type, iodotyrosines cannot be coupled into iodothyronines with sufficient speed. In the third type, a dysfunction of the enzyme dehalogenase results in a failure of iodotyrosines to deiodinate.

Similarly, at least four genetic dysfunctions are now known among the six enzymes necessary for the interconversion of glucose and glycogen, and these result in four distinct forms of glycogen disease (*11*).

Other examples could be cited. It has in fact become very important to search for genetic heterogeneity in all diseases by appropriate methods (*29*). Therapeutic measures which may be of value in one genetic form of a disease are not necessarily successful in another, even though the overt symptoms of the two may sometimes be quite indistinguishable.

Studies of clinical features, age of onset, genetic mode of transmission, immunologic and biochemical patterns, and other techniques may aid in discovering genetic heterogeneity. The use of paper chromatography and electrophoresis has become of paramount importance in studies of this nature. Most recently, the development of techniques for separating individual human cells and growing them as pure clones in tissue culture has opened up new possibilities along these lines, as well as in the important area of the analysis of mutation rates (*30*).

Structural Anomalies

It has long been my contention (*31*) that structural anomalies are just as subject to interpretation on the basis of enzyme dysfunctions as are the storage diseases and other obvious metabolic disorders. It should be possible by appropriate biochemical methods to discover errors of metabolism in the development of such conditions as lobster claw, polydactyly, achondroplastic dwarfism, and multiple exostoses, even though, apart from the morphologic aberrations, the subjects appear to be in good health. This conviction is shared by others (*8, 32*), and it is now possible to document it in some instances.

The first clear indication that a simple, gene-controlled enzyme dysfunction can be the basis for a structural anomaly came when evidence was found for a defect in glycolysis in the red cells in hereditary spherocytosis (*15, 33*). In this disease the red blood cells develop as spherocytes, lacking the expandable biconcave surfaces of the normal red cells. As a result, the cells are osmotically and mechanically fragile, and rupture easily as they move sluggishly into the spleen and are held there.

In the presence of the appropriate gene, one of the enzymes responsible for glycolytic metabolism fails to function properly. Since the gene involved is dominant, the basic defect in spherocytosis may well be the result of an inhibition rather than an absence of the enzyme. The enzyme affected appears most likely to be enolase, which in normal glycolysis converts 2-phosphoglyceric acid to 2-phosphoenolpyruvic acid. The latter substance in its subsequent metabolism provides energy in the form of adenosine triphosphate (ATP).

When enolase dysfunction occurs as a

result of the activity of the implicated gene, the red cell can no longer build up adenosine triphosphate and maintain its usual store of chemical energy—energy necessary, among other things, for the maintenance of the integrity of the framework and the membrane of the cell.

Since the mature red cell appears to be the only cell of the body entirely dependent on glycolysis (*15*), the defect would be most noticeable in this kind of cell, although the mutant gene must, of course, be present in all the cells of the body. Moreover, the red blood cell, being readily accessible for study, is of paramount importance as a tool for the analysis of many metabolic errors, even though the clinical effect is most noticeable in other tissues. For example, phosphogalactose-uridyl-transferase was implicated as the enzyme deficient or nonfunctioning in galactosemia by studies of the red blood cells of children suffering from the disease, and this was only later confirmed in regard to the cells of the liver, where most of the damage is actually done (*34–36*).

Metabolic Interrelationships

Incidentally, biochemistry makes strange bedfellows of genetic diseases. Hereditary spherocytosis is now seen to be intimately related biochemically to galactosemia and the glycogen diseases through glycolytic metabolism, just as albinism, phenylketonuria, tyrosinosis, and alcaptonuria are all, surprisingly, biochemically related to one another through the metabolism of phenylalanine, one of the amino acids. In spite of the close biochemical relationships, however, the inheritance of such related disorders is quite independent and specific.

Since, of course, the metabolism of carbohydrates, fats, and proteins is closely integrated through the tricarboxylic acid cycle, it is well within the range of possibility that before too long the entire metabolic processes of man may be diagrammed as a single, elaborate biochemical pattern. Through the continuing study of more and more mutant individuals, each enzyme in the pattern will become identifiable in terms of the activity of a specific gene (a specific portion of a deoxyribonucleic acid molecule), and each genetic enzyme dysfunction will be related either to a mutation of that gene, resulting in a lack or modification of the enzyme, or to a mutation of a different gene, acting in such a way as to inhibit the activity of the enzyme.

The specification of the precise sequence of the steps in any reaction and the identification of precursors are enormously facilitated by the availability of mutants which block the various steps, since in the absence of such blocks the intermediary products are generally converted in the cell as rapidly as they are formed, and are thus difficult to detect. For example, the conjecture that phosphorylethanolamine is one of the long-sought, naturally occurring substrates of alkaline phosphatase was made possible by the availability of genetic instances of hypophosphatasia (*37*).

Woven into the over-all pattern there will be, of course, environmental threads in the form of vitamins and other coenzymes, and of such things as hydrogen ion concentration, inorganic ion strength, temperature, substrate concentration, drugs, and infections.

Moreover, such a biochemical pattern of genetic health and disease will not be restricted to "physical" traits. Already several "mental" anomalies have yielded to biochemical analyses. Phenylketonuria and infantile amaurosis, among others, occur as the result of single-gene enzyme dysfunctions, as already mentioned. Other mental disorders are at present being actively investigated from this standpoint.

It should perhaps be pointed out here that the metabolism of metallic elements is also involved in human disease and may be genetically interfered with. Examples are the disturbances of iron metabolism in hemochromatosis and in methemoglobinuria, of copper metabolism in hepatolenticular degeneration, and of potassium metabolism in family periodic paralysis (*11, 38*).

The delineation of the complete genetic and biochemical pattern of man that I have envisaged involves, of course, many difficulties; but it also offers many challenges and promises important rewards. To give just one example, it would be of the utmost value in the elucidation of the basic mechanisms underlying heart disease to be able to formulate the metabolic interrelationships that must exist between the lipids and the purines. The relative levels of uric acid, cholesterol, and phospholipids have been shown to be related to the development of atherosclerosis and coronary artery disease (*39*). Although the metabolism of each of these substances may be independently controlled by the activities of known genes, as in gout, xanthomatosis, and the lipidoses, respectively, some basic, underlying, genetic mechanism must surely exist which enzymatically conditions their interacting biochemical activities. A step in this direction may have been taken with the recent demonstration that familial amyloidosis represents an inherited aberrancy in lipoprotein metabolism (*40*).

The most promising basis for the ultimate formulation of a complete chemical-genetic pattern for human beings lies in the now apparent principle of the unity of biochemistry—a principle which states that the fundamental biochemical reactions are identical for all organisms thus far studied, from microbes to man.

Practical Applications

The growing awareness that the primary activity of a gene is the control of the specificity of a substance such as an enzyme is adding immeasurably to the precision of the various practical applications of medical genetics. I have discussed these applications (diagnosis, treatment, prevention, and genetic prognosis) elsewhere in detail (*41, 42*) and will add here only some suggestions regarding the emerging values of the principles discussed in this presentation.

It is clear that an understanding of the basic mechanisms of pathology is necessary for accurate differential diagnosis. In particular, the knowledge of the *basic genic action* involved will facilitate diagnosis, which is all too difficult in many diseases and aberrations at the present time. In those instances in which the biochemical steps between the primary activity of the gene and the resulting manifestation of the trait are few, the trait is likely to be readily diagnosable, and the primary activity may be subject to comparatively easy identification. But where the biochemical steps are numerous, with alternative pathways available, and with many opportunities for the impact of subtle or overt environmental influences, the resulting clinical picture may be complex and confused, and the basic genic action may be difficult to specify.

Another difficulty in the delineation of specific genic actions is to be found in those instances in which the effect of an individual gene is very slight. The effects which I have thus far discussed have been evident as marked phenotypic discontinuities. There are, however, genic activities in which the phenotypic effect of an individual gene is not readily discernible, consisting of only a very slight alteration of structure or function.

Where several or many such genes

affect the efficiency of the same enzyme or process, however, their cumulative effects may be quite appreciable. Groups of genes with small but similar cumulative effects are referred to as polygenes, and they appear to be of importance in the genetically determined portions of quantitative variation, both normal and pathologic. Special methods are necessary for the analysis of the genetic and biochemical activities of polygenes (*42*).

Each individual has a unique assembly of genes and will have his own mode of reaction to disease, whether it be presented through infection, trauma, stress, or malnutrition, or wholly from within through biochemical error. The conviction that the genetic constitution is involved to a greater or lesser extent in all disease will serve as a stimulant to look beyond the secondary aspects of pathology and to search for the primary genic action in each case. Once this has been determined, both diagnosis and treatment will be facilitated. In this connection care must be taken that in assaying correlations between biochemical observations and clinical manifestations, cause is not confused with effect.

It is well to recognize that there is at present one apparent limit to potential therapy in genetic diseases. Although inhibitors, whether genetically determined or not, may be subject to environmental control, and although coenzymes and substrates may be provided nutritionally and in other ways, the basic protein enzymes can be gotten into a cell only by building them there. The building process is determined by the presence of appropriate genes.

As a consequence there would appear to be a residue of diseases, resulting from mutant gene-controlled absences of requisite, potent enzymes and apoenzymes, for which there is at present no apparent "cure" in the usual sense. The ingenuity of biochemists and physiologists may in the future, however, make it possible to devise alleviation even for these fundamental errors.

The increasing ability to identify carriers of mutant genes will add to the precision of practical applications in prevention and in genetic prognosis, by bringing to light precursory, preclinical and constitutional stigmata which can be employed in these applications. The elucidation of indices of predisposition, whatever their origin, can be of service in identifying those environmentatl factors which may act as precipitating causes of clinical manifestations. Such identification may well prove to be of primary value in the ultimate control of disease (*43*).

The prospects for the eventual understanding of human health and well-being grow ever brighter, and to no one is more appreciation due for his fundamental contributions to this field than to Sir Archibald E. Garrod.

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