Genetic and Molecular Basis of Human Hereditary Diseases

J. Edwin Seegmiller

A fresh view of some old and new diseases recognized by means of modern genetics and biochemistry.

An area of significant medical progress over the past decade has been the recognition and the biochemical characterization of an ever-increasing number of human hereditary diseases (1). Some of the practical consequences have been the development of objective chemical and biochemical methods for more precise diagnoses of a large number of these diseases (many of them newly recognized) and the opening of new approaches for treatment of clinical disorders including some types of mental retardation that have, in the past, been regarded as hopeless medical curiosities. The prospect of more widespread use in clinical laboratories of automated analytic systems for chemical determinations gives promise of the detection of an even larger number of genetically determined abnormalities of body chemistry in the years ahead.

Although many of these inherited diseases are relatively rare disorders, there are considerations that cause these disorders to assume a far greater importance to the medical and scientific community than is revealed by their statistical incidence.

Basic Concepts

Gene-Enzyme Relationship

These disorders are experiments of nature that present unique opportunities for expanding our knowledge of many basic biologic processes. Some of our fundamental concepts of the mechanism of gene action can be traced to basic studies of human hereditary diseases. From his studies of the rare human metabolic disease alcaptonuria, the Brit-
ish physician Archibald Garrod proposed the basic concept of the gene-enzyme relationship in 1908 (2), over 30 years before its full development by microbiologists (3, 4). With further refinements contributed largely by microbial genetics, this concept has become the basic premise for one of the fundamental mechanisms of gene action in which a single gene or cistron controls the amino acid sequence of a single polypeptide chain (5, 6).

Another inherited human disease, sickle cell anemia, has provided an even more basic example of the more detailed mechanism by which gene action can produce a disease. Substitution of a single amino acid—valine for glutamic acid—in the 6 position of the beta chain of the hemoglobin molecule gives rise to sickle cell hemoglobin which has different physical properties from normal hemoglobin (7, 8). The tendency of the sickle cell hemoglobin to form aggregates when deprived of oxygen is the primary cause of the red blood cell destruction and occlusion of the small blood vessels that characterize this disease to the clinician. Among the possible genetic codes for valine and glutamic acid (9), codes can be found that differ by only one base, suggesting the possibility that the mutation responsible for the appearance of this hemoglobin may have been a single substitution of one base in the DNA molecule directing synthesis of the beta chain at a position corresponding to the sixth amino acid.

Mutations

The above example provides a model for our thinking of one of the mechanisms by which genetic differences are expressed. In the simplest type of structural gene mutation, a relatively minor alteration in the base sequence of one small portion of the DNA molecule directing the assembly of a polypeptide chain could thereby be expected to produce an altered protein. If the protein product of the gene action happens to be an enzyme, and the substitution occurs at or near the active binding site for the substrate, the resulting protein may be expected to have a diminished or absent enzyme activity. A defect in a regulator gene that controls the activity of a structural gene can produce a similar deletion of an enzyme in bacterial systems (10). Comparable regulator genes in mammalian cells have been postulated but are yet to be demonstrated conclusively. The enzyme deletion, in turn, introduces a chemical aberration in cellular metabolism which may be reflected in bacterial mutants by alterations in growth or in nutritional requirements. In the human species, such biochemical mutations can produce a metabolic disease (1).

Genetic mutations produced in microorganisms at will have provided
powerful tools for increasing our knowledge of the operational details of basic genetic and biochemical processes in these organisms (11, 12). Only in the human species do we find any significant number of well-characterized genetic biochemical deletions in higher life forms that might serve comparable roles in aiding the study of these basic genetic processes in mammalian cells. It seems unlikely that this reflects a higher incidence of genetic mutation in man, but rather, more intensive study of our own species and, in particular, the interest of physicians in the diseases that have resulted from some of these processes.

**Diagnostic Procedures**

**Past**

As might be expected, the classic metabolic diseases, the so-called "inborn errors of metabolism" that were first recognized were brought to the attention of the physician by reason of the accumulation of abnormal chemical substances which the physician could detect with his five senses. One can feel and see the deposits of crystals of sodium urate that constitute the tophi located in and about the joints in patients with gouty arthritis, the clinical features of which were first described by Hippocrates around the 4th century, B.C.; and, of course, the sweet taste of glucose has provided an unaesthetic but, nevertheless, useful test of the urine for diabetes since the 17th century—long before chemical tests were available to the physician. In alcaptonuria, a rare disorder first definitely described a century ago, one can see the blackening of the infant’s urine on the diapers caused by the oxidation by air of homogentisic acid, a chemical relative of photographic developer, which is excreted in large quantities throughout the life of the individual.

It was only 30 years ago that the unusual odor of the urine of a mentally deficient child led the Swedish chemist Fölling to isolate phenylpyruvic and phenylacetic acid from the urine, thereby permitting the chemical detection of the disease we now know as phenylketonuria (13).

A by-product of the increasing use of chemical tests of biologic fluids to aid in the diagnosis of disease has been the detection of chemical abnormalities in a continually increasing number of clinical disorders and even in individuals without clinical symptoms. Many of these disorders have been detected because of the lack of specificity of many of the chemical tests in routine use. An ironic result of the "progress" in substitution of more specific tests is the inability to detect many of these chemical abnormalities. As an example, homogentisic acid gives a
strong reducing action with the alkaline copper solutions that have been in use in the past for detecting sugar in urine. In fact, some alcaptonuric patients have been treated with insulin for diabetes on the basis of this test. This erroneous diagnosis would have been avoided if the clinical chemist performing the test had been observant and had noted that the fluid overlying the red copper oxide was black instead of blue or green. With the use of more specific methods for the routine detection of glucose in urine, the ability to detect alcaptonuria has been lost and patients with this disorder have gone undetected by this specific chemical test.

Present and Future

There is good reason to expect that genetically determined differences in body chemistry will be detected at an increasing rate during the years immediately ahead of us. This should come about through the routine determination of an increasing number of chemical components of body fluids in a progressively larger portion of the patient population as an objective aid to the clinician in his detection of disease. Such widespread use of chemical determinations is being made possible by the use in clinical laboratories of automated analytic equipment for chemical determinations.

Once an abnormal substance is noted, its identification then permits intensive studies of not only the basic enzyme defect giving rise to this substance but also the way by which the chemical aberration produces the clinical manifestations of the metabolic disease. At the present time there is no way of effecting a true cure of these diseases by correcting the defect in the gene. Nevertheless, these findings have provided new understanding of the disease process and given new hope for successful treatment of a remarkable array of diseases that have, until recently, been completely untreatable.

Basic Metabolic Defects

A detailed catalog of the phenomenally large number of basic metabolic defects that have been characterized during the past decade among hereditary diseases is not possible in this presentation. However, a few examples of these diseases, the general ways by which the biochemical defect gives rise to the disease process, and the rationale that leads to the successful treatment of an increasing number of these diseases will be discussed.

The proteins formed by the cell serve one of two general functions—
either as units of structure or as units of biochemical function, mostly
in the form of enzymes which catalyze specific chemical reactions. These
reactions are performed in a detailed sequence within the cell, somewhat
analogous to a factory production line.

The failure of an enzyme in the metabolic sequence to perform its
function results in the accumulation of the unreacted metabolite. It
cannot proceed along that particular production line for further proc-
essing. The interruption of some of the metabolic pathways results in
a situation that is incompatible with life, and it is thought that this
situation may account for some of the spontaneous abortions or deaths
in the first few weeks or months of life. If the interruption of metabo-
limism is not a complete disaster to life, it can result in a wide variety of
clinical expressions ranging from simple failure of a child to thrive, to
production of disease late in adult life. In still other conditions, such
as pentosuria, the defect seems to have no detectable deleterious effect
whatever, and is called a disease only by courtesy.

Ochronotic Arthritis and Alcaptonuria

In some diseases, the pathologic effects result from the compound
that accumulates. Two types of arthritis can be explained by this
mechanism. The classic metabolic disease alcaptonuria already men-
tioned produces no discernible ill effects for the first 30 years or so of
a patient’s life. For many years it was regarded as a medical curiosity.
It was in 1904 that Sir William Osler at Johns Hopkins Hospital made
the first clinical association of this medical curiosity with a puzzling
type of arthritis. This disorder ochronotic arthritis begins in adult life
and usually involves the spine and other major joints; postmortem
examination reveals the deposition of a black pigment in and about the
bones and cartilage.

Undoubtedly, our ability to make the association of the arthritis with
the abnormal metabolite was greatly facilitated by the tendency of
homogentisic acid to form pigmented products that can be readily seen.
It is conceivable that other types of hereditary arthritis could arise
from metabolites with equally deleterious effects on joint cartilage
which do not announce their presence by pigment formation and so,
remain unrecognized.

We have already mentioned Archibald Garrod’s studies of this dis-
ease in which he formulated the first rational hypothesis to account for
what he named “inborn errors of metabolism” (2). He proposed that
homogentisic acid accumulates in these patients because they lack an
enzyme that normally degrades it. It was not until 1958 that there was
an opportunity to test Garrod's hypothesis. A patient with alcaptonuria required surgery for uncontrollable gastrointestinal bleeding. At operation, a small piece of his liver was removed for biochemical studies. The sequence of enzymes that are normally responsible for both the formation and the disposal of homogentisic acid were studied (14). Homogentisic acid oxidase was the only enzyme absent from the series of enzymes that degrade tyrosine, providing concrete evidence in this patient of the correctness of Garrod's hypothesis.

Phenylketonuria and Tyrosinemia

Other defects in this metabolic sequence are now known. Thus, the absence of the enzyme for formation of tyrosine from phenylalanine results in the metabolic disorder phenylketonuria, with its accompanying mental deficiency (1). Another disorder, tyrosinemia, has recently been characterized as a defect in the enzyme p-hydroxyphenylpyruvic acid oxidase responsible for the formation of homogentisic acid (15). The clinical manifestations of this disorder are entirely different from the other two and consist of renal tubular dysfunction and a severe cirrhosis of the liver, leading to early death in many patients unless special treatment is instituted (16, 17).

Gout

Another enzyme deletion, which is presumed to have occurred in a remote ancestor of both man and the higher apes, has left the whole human species heir to the gout. In all other mammals, the enzyme uricase is present and degrades uric acid to its much more soluble end product, allantoin, for excretion. In addition, the human kidney excretes uric acid inefficiently. As a result, the mean concentration of urate in human serum is quite near the theoretical limit of solubility of urate (18). A portion of the hyperuricemic individuals deposit the sparingly soluble urate crystals in and about the joints which can give rise to erosion of joints and episodes of inflammatory reaction characteristics of the acute attack of gouty arthritis (19).

Glycogen Storage Disease

Disease in the human can also result from failure of the enzyme to produce a needed product, as is the situation in Type I glycogen storage disease. Here, there is absence of the liver enzyme glucose-6-phosphatase, which is responsible for the production of most of the blood sugar from glycogen (20). As a result, in childhood these patients have severe episodes of hypoglycemia, producing weakness, fainting, and severe
episodes of ketosis. If they do live to adult life, a surprising number have been found to develop gouty arthritis (21). The precise mechanism by which this defect in carbohydrate metabolism causes hyperuricemia and gout is another example of insights derived by studying these disorders. These patients have a lacticacidemia and ketonemia—both of which can cause a renal retention of uric acid. In addition, they have an excessive purine biosynthesis (22). A biochemical link between carbohydrate metabolism and purine metabolism can be made in the formation of 5-phosphoribosyl-1-pyrophosphate (PRPP), which is one of the two reactants of the first enzymatic reaction uniquely committed to purine synthesis:

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\text{Phosphoribosylpyrophosphate + glutamine} \rightarrow \text{phosphoribosylamine + pyrophosphate}
\]

This reaction is rate-limiting in the control of purine biosynthesis, but an increased quantity of PRPP in cells of patients with Type I glycogen storage disease has not yet been demonstrated.

**Nutritional Diseases**

A similar type of enzyme deletion can also be used to explain some of the nutritional diseases. Both the human and the guinea pig lack the enzyme which, in all other mammals, catalyzes the conversion of ketogulonic acid to ascorbic acid, Vitamin C (23). As a result, both the human and the guinea pig must be supplied with Vitamin C in their diet to avoid the development of scurvy. Scurvy can, therefore, be regarded as a metabolic disease shared by the entire human race which is kept in remission by the adequate intake of Vitamin C. Such a consideration provides the key to one of the therapeutic approaches used in treatment of metabolic diseases: some individuals with metabolic disorders will have unusual nutritional requirements for maintenance of health that are different from that of the general population.

**Histidenemia**

In some cases, the biochemical and clinical features of disease are the result of the accumulation of a compound that is a remote precursor of the blocked reaction. This occurs particularly if the reaction preceding the block is freely reversible. The accumulation of liver glycogen in excessive amounts in Type I glycogen storage disease that we have already mentioned is an example of such an accumulation.

In still other disorders there will be production of increased quantities of a substance which normally is present in small amounts. The accumulation of phenylpyruvic acid and phenylactic acid in the urine of the patients with phenylketonuria is an example of the way in which a
basic biochemical abnormality results in a metabolic diversion. Another example is histidinemia, a disorder of amino acid metabolism for which the enzyme defect was first demonstrated by Dr. LaDu et al. (24). The chemical abnormality for this disorder was detected as a result of a positive ferric chloride test for phenylketonuria found in the urine of a child attending a speech clinic. Unlike most patients with phenylketonuria, this child was of normal intelligence and had a normal concentration of phenylalanine in her plasma. The diagnosis of histidinemia was proven when it was demonstrated that imidazole pyruvic acid derived from histidine was the substance responsible for the positive ferric chloride test. Increased quantities of histidine were also present in plasma and urine of both this girl and her younger brother. The absence of the enzyme, histidase, was demonstrated readily by use of hardened skin removed from around the edge of the nail with fingernail clippers. Such cornified epithelium from normal individuals contains a measurable amount of the enzyme histidase which breaks down histidine to urocanic acid; there was none demonstrated in samples obtained from these children. At the present time there have been at least 15 cases of histidenemia identified. All but 2 of these patients are reported to have abnormalities of speech and some have mental retardation (4). The way in which an enzyme defect could result in a specific disorder of speech opens new realms in the understanding of the role of biochemical processes in brain function. Our speech experts believe that these patients have a shortened auditory memory and so are unable to learn by their sense of hearing as effectively as can normal individuals. Yet the intelligence of our two patients and their ability to learn by the visual route is entirely normal. Since the disorder has not yet been identified in individuals over 13 years of age, the clinical features of this disorder that might develop in adult life are not known.

**Brain Function Abnormalities**

In recent years, we have become aware of an increasing number of specific defects of metabolism that are associated with abnormalities of brain function. One of these is known as branched-chain ketonuria or Maple Syrup Urine Disease. This was first described in 1954 in a family in whom 4 of the 6 children had died within the first few weeks of life from severe brain damage (25). The mother brought to the attention of the doctors the fact that the urine of the affected children had a characteristic odor similar to that of maple syrup. On the basis of this observation, the urine was examined in greater detail and an abnormal concentration of organic acids was detected which were eventually found to be the keto acids derived from the branched-chain amino acids
leucine, valine, and isoleucine, which accumulate as a result of a deficiency of the decarboxylase that normally degrades them (1).

The severe brain damage in such instances can be prevented by treatment with a synthetic diet low in branched-chain amino acids. This disease was diagnosed in a baby 5 days old; the special diet was begun immediately and has been continued ever since (26). The child is now 5 years of age, has grown well, and has a normal intelligence. Other patients with this disorder have been similarly treated (1).

Another inherited biochemical disorder which gives rise to neurologic symptoms was first described nearly three years ago (27). The patients, two young brothers, had spasticity, choreoathetosis, mental retardation, and a most bizarre behavior which consisted of a compulsive biting resulting in the ends of the fingers and the lips being chewed away. It was associated with hyperuricemia and with the largest production of uric acid per kilogram of body weight that has yet been found. The development of gouty arthritis has been a relatively late manifestation of the disease. However, gouty nephropathy has contributed to the death of other patients by the age of puberty (28). The disorder affects only males and shows a pattern of inheritance carried by the females over at least 3 generations, thereby suggesting that the gene controlling the biochemical defect resides on the X chromosome (29). The recent discovery that these children are lacking an enzyme of purine metabolism (hypoxanthine-guanine phosphoribosyltransferase) provides evidence that function of this enzyme is involved in the normal regulation of purine biosynthesis (30). In addition, it provides a biochemical basis for not only another type of neurologic disease but also a link between a characteristic abnormality of behavior and a genetically determined biochemical disorder. The precise mechanism by which a single genetic defect gives rise to this clinical syndrome can provide a powerful wedge in our search for knowledge that might be used in more effectively controlling such disorders.

**Summary**

Modern genetics and biochemistry provide new insight into some old and new hereditary diseases which in turn enlarge the future possibilities for recognition and treatment of these disorders. There is a great potential value of mammalian mutants—these experiments of nature—as tools for extending our knowledge of the importance of specific biochemical reactions in the normal physiology and biochemistry of the human. The immediate future holds the prospect that an even larger number of genetically controlled individual chemical differences will be recognized in the human species. This should result from the
performance of a larger number of routine chemical determinations on an increasing segment of the patient population made possible by the widespread use of automated analytic equipment in clinical laboratories.

References


