Detection of Human Inborn Errors of Metabolism by Examination of Urinary Metabolites

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An increasing number of recessively inherited human diseases are being explained in terms of a missing or defective enzyme. The resulting aberration of metabolism is reflected, in many cases, in an altered composition of metabolites in the urine and other body fluids. Progress in this field is dependent on making the initial association between a familial disease and an aberration of body chemistry. The development of comprehensive analytic systems for the detection of abnormal quantities or kinds of metabolites in biologic fluids obtained from patients with inherited diseases should greatly facilitate this process. Biochemical characterization, at an enzyme level, then becomes possible and is essential for improved diagnosis and rational approaches to treatment of the disease.

In recent years, remarkable progress has been made in our understanding of the genetic mechanisms involved in production of human disease states. An ever-increasing number of recessively inherited diseases are being explained in terms of a deficient or defective enzyme resulting from a defective pair of genes. The resulting disruption in a normal pathway of metabolism is often reflected in a change in composition of urinary metabolites. As a consequence of these advances, the clinical recognition and the biochemical characterization of many of these familial diseases have become more precise, and some types of disorders can now be treated successfully. This progress has been greatly facilitated by the remarkable advances in our knowledge of basic biochemistry made in the past two decades, as well as the increasing sophistication of the clinicians in the field of biochemistry. However, the rate of this progress in explaining hereditary diseases in terms of biochemical defects is seriously hampered by limitations that are, to a large extent, technical problems.

One of the most difficult problems that repeatedly confronts the
clinical investigator is that of finding a bridge that will serve as a link from a familial disease, recognized only by its clinical symptoms, to an aberration of body chemistry. The advances of modern laboratory science give promise of greatly expanding the frontiers attainable with the physician’s traditional tools—his own powers of observation.

Metabolic Disorders

It is worth noting that the chemical disorders that were first recognized—and so constitute the classic metabolic diseases—announced their presence by producing abnormalities that were detectable with the unaided senses. If the primary biochemical derangement produced an abnormal metabolite that either had a strong color or spontaneously formed a colored product visible in tissues or urine, the clinician could readily make the association with the disease state. Thus, the black pigment produced by the oxidation of homogentisic acid in alkaline urine of the alcaptonuric patient permitted the recognition of this biochemical disorder over a century ago (1). The recognition that homogentisic acid excretion was increased by ingestion of phenylalanine and tyrosine (2) provided the first indication of the metabolic pathway for their degradation and led, eventually, to the demonstration of a long-postulated absence of homogentisic acid oxidase (3).

The clinical consequence of this disorder—severe arthritis of the spine—which develops in adult life, has as its pathologic correlate a black pigment that accumulates in cartilage and connective tissue of these patients. Whether or not the cartilage degeneration of other familial forms of osteoarthritis may be mediated by an accumulation of other metabolites that do not so readily announce their presence is, of course, unknown at the present time. Since homogentisic acid is a chemical relative of photographic developer, its reducing properties permitted its detection in the copper reduction test routinely used to detect glucose in urine. Improvements in urinalysis by the substitution of the highly specific test using glucose oxidase has, at the same time, removed the ability of the routine clinical laboratory to detect this interesting metabolic abnormality unless a nonspecific reduction test is used.

The precipitation of a substance of limited solubility from solutions that have become supersaturated provides a relatively simple indication of its presence in abnormally high concentrations. Consequently, conditions associated with abnormally high concentrations of sparingly soluble metabolites have been among the earliest chemical abnormalities to be recognized in clinical medicine. The limited solubility of uric acid and its salts leads to crystalline deposits of monosodium urate in gouty tophi and free uric acid in renal calculi. The clinical consequences
of these events—the severe inflammatory reaction to urate crystals of acute gouty arthritis and the renal colic during passage of a kidney stone—give compelling notice to the physician of the presence of increased concentrations of this metabolite in serum or urine (4–6).

It is not surprising, therefore, that metabolic disorders giving rise to renal calculi composed of other sparingly soluble metabolites, also claimed the attention of physicians and chemists at an early stage of scientific development. Kidney and bladder stones provided the first source of the amino acid, cystine, in the early part of the nineteenth century, as well as a very crude way of identifying the human metabolic abnormality cystinuria. We now know that the increased concentration of cystine in the urine results from a defect in the reabsorption of cystine by the renal tubule. The use of more sophisticated analytic systems on the urine of these patients showed that they have a defect in renal transport of the amino acids lysine, arginine, and ornithine, as well as cystine (7). The deposition of cystine crystals, this time in body tissues, provided the first direct evidence of the biochemical abnormality in an entirely different, rare familial disease, cystinosis. Children affected with this disease invariably die of renal failure; however, a variant of the disorder in adults is entirely benign. In both conditions, the crystal formation is an indication of a high concentration of cystine within the cells (8).

Oxaluria was similarly recognized from the limited solubility of calcium oxalate in fluids of the urinary tract and the resulting renal stone formation. As with cystinuria, the application of more sophisticated analytic technics to oxaluria has shown an increased excretion of glycolic acid in some patients (9) and of L-glyceric acid in other patients (10).

Crystalluria found in a child with severe megaloblastic anemia, without symptoms directly attributable to the crystal deposition per se, has also been helpful in the recognition of another abnormality of metabolism. The crystals were identified as orotic acid, a precursor of pyrimidine nucleotides and were indicative of the excretion of large amounts of orotic acid in the urine (11). This characterization led to the rational and effective treatment of the child’s anemia with uridine. Uridine treatment also diminished the orotic acid excretion, providing good evidence of the existence of a regulatory mechanism in the human species for control of pyrimidine synthesis.

A similar degree of curiosity regarding the cause of a crystalluria, found in children with a familial neurologic disease classed as cerebral palsy, resulted in the discovery that these children produce more uric acid per unit body weight than has been found in any other human dis-
ease (12). This, in turn, led eventually to the recognition that an enzyme of purine metabolism, hypoxanthine-guanine phosphoribosyltransferase, was virtually absent in children with this particular type of X-linked cerebral palsy (13). A partial deficiency of the same enzyme was found in some patients with gouty arthritis who also produced excessive quantities of uric acid (14). In addition, a mild neurologic disease was found in some of the gouty patients who had the most severe degrees of enzyme deficiency.

An unusual odor to the urine was the first indication of the underlying biochemical disorders responsible for two different, genetically determined diseases that cause damage to the central nervous system. The subsequent demonstration of (1) the accumulation of phenylpyruvic and phenylacetic acids in the urine of patients with phenylketonuria (15) resulting from deficiency of the enzyme phenylalanine hydroxylase; and (2) the accumulation of the keto acids derived from the branched-chain amino acids leucine, isoleucine, and valine in the urine of patients with maple syrup urine disease (16) resulting from a deficiency of a decarboxylating enzyme, has led to the successful treatment of these disorders with special diets that are largely synthetic (17, 18).

Discussion

With the routine use of chemical tests applied to blood and urine, additional biochemical defects were detected as a result of the lack of specificity of these chemical tests. This type of serendipity has, in many instances, provided the biochemical lead for elucidating a new genetic disorder. With acceleration in the application of automated analytic systems to tests performed routinely by clinical laboratories, more tests can be performed on a larger segment of the population. In the course of operation of such a facility, there will undoubtedly be more opportunity for the alert clinician to happen upon more of these biochemical variants.

Nevertheless, in an era in which highly sophisticated, automated analytic systems are available for specialized purposes, it is ironic that we still lack any well-systematized, comprehensive procedure for examining, in depth, the composition of urine or other biologic fluids from patients with familial disease. Attempts that are made, usually, are somewhat haphazard and dependent upon the particular analytic system in use in that particular laboratory. A systematic scheme for analysis, similar to those used for identification of unknown chemical compounds by qualitative inorganic or organic analysis, is greatly needed. An analogous, systematic approach for detection of any and all abnormal quantities of metabolites in urine, plasma, or cells of
patients with a disease that is obviously familial, should provide the biochemical "handles" needed to accelerate our progress in understanding the biochemical basis of many more of the inherited diseases. The start that has been made in the development of screening tests and their application to urine and blood for detection of specific hereditary disorders in selected population groups, has already proven to be helpful in detecting new biochemical disorders (19).

The demonstration that some of these biochemical defects can be detected in fibroblasts cultures in vitro (13, 20) from skin explants suggests additional approaches. Development of systems for analysis of intracellular metabolites, as well as simple tests of the function of whole groups of enzymes, could be useful for detection of biochemical variations associated with genetically determined disease states.

There are very practical consequences of the ability to recognize the biochemical basis for some of these disease states. Such knowledge provides, in some patients, a rational approach to treatment. Specific assay for the biochemical lesion permits early diagnosis in offspring of susceptible families—in some cases before clinical evidence of the disease is apparent—and allows the start of appropriate treatment (18). In some instances the biochemical abnormality can provide a link to show an association of clinical disorders that had not previously been suspected (14). If the biochemical defect can be detected in cells grown from amniotic fluids (21) these disorders may then be diagnosed at a very early stage of fetal development, thus allowing genetic counseling to assume a more dynamic role in the future.

References