Clinical and Biochemical Observations in Galactosemia

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IT IS now well known that the disease, galactosemia, represents an inborn and hereditary error in the metabolism of galactose. More than 40 cases have been reported in the literature up to the present time† and many more unreported cases have been discovered in the course of investigations on this disease. Thus, although the condition is a rare one, it probably occurs not as infrequently as was heretofore believed.

It is the purpose of this communication to review the clinical aspects of galactosemia, to discuss in detail the recently elucidated biochemical defect considered to be the basic disturbance of this condition, to emphasize newer methods of diagnosis, and, finally, to comment on the factors possibly contributing to the toxic manifestations of the disease.

CLINICAL FEATURES

The symptoms which occur in galactosemia usually manifest themselves shortly after birth and are known to be causally related to the ingestion of milk or galactose. The main features include early nutritional failure, mental retardation, hepato-splenomegaly, osteoporosis and cataracts. Afflicted infants usually appear normal at birth, but after a few days of milk ingestion they readily develop vomiting and diarrhea together with lethargy and drowsiness. Jaundice is a very common finding as the disease progresses and is usually associated with enlargement of the liver and spleen. The hepatomegaly is initially due to fatty infiltration, but in the later stages cirrhosis ensues. In severe cases the disease is often fulminating and death may occur in the neonatal period or soon thereafter. If the clinical condition extends beyond a period of four to eight weeks, one can usually detect evidence of mental retardation and cataract formation. Both symptoms tend to become increasingly prominent as galactose ingestion continues. A frequent occurrence is the development of hypoglycemia when blood galactose levels are significantly elevated. This syndrome may readily escape detection if only total blood sugar determinations are performed.

The earliest clue to the diagnosis comes with the finding of a reducing sugar (galactose) in the urine. This sugar, unlike glucose, is not fermented by yeast and also is not a substrate for the enzyme glucose oxidase. The recently introduced clinical test papers (e.g. Clinistix®, Tes-Tape®) which utilize the glucose oxidase reaction will thus be negative. It is important to emphasize that the galactosuria is inconstant and can be absent on the routine admission urinalysis of a patient who, because of persistent vomiting, may not have been able to absorb much of the ingested milk. In contrast to the galactosuria, the tolerance of orally or intravenously administered galactose is always significantly impaired. For this reason the performance of the galactose tolerance test has often been considered necessary for the diagnosis of the disease. This test is not without hazard, however, because of the frequent occurrence of hypoglycemia as well as the pos-

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† Extensive bibliographies citing individual case reports may be found by consulting references 1 to 3.

sible deleterious effects of the additional galactose. More recently a newer and more specific enzymatic test has been described (*vide infra*) which obviates the need for the galactose tolerance test.

The other usual urine abnormalities consist of albuminuria and amino-aciduria.[6] The amino-acid pattern has been essentially similar in all cases, with a predominance of the neutral, simple aliphatic chain type; i.e. serine, glycine, alanine, threonine, glutamine, and valine; in addition phenylanine, lysine, cystine, glutamic acid, methyl histidine, tyrosine and amino-isobutyric acids have been detected in the urines of these patients.[4,5]

NUTRITIONAL THERAPY

Fortunately, one of the striking features of this disease is the remarkable improvement which occurs when these patients are placed on galactose-free diets. If the disease is not too advanced, all of the symptoms may regress and even disappear completely with dietary restrictions only. One readily observes rapid weight gain and cessation of nausea, vomiting and diarrhea. The liver and spleen return to normal size. Cirrhosis, if present, has been known to disappear completely.[1] The urinary abnormalities mentioned above likewise promptly cease. Cataracts, if present, often regress somewhat and in some instances they may disappear completely.[2] The major symptom which usually shows no improvement is the mental retardation. It has been observed by most authors that the central nervous system damage may not improve despite the most rigid adherence to a galactose-free diet. It is for this reason that early diagnosis and prompt institution of therapy are so important.

Galactose-free diets can readily be instituted in infancy by resorting to any of a number of commercially available milk substitutes such as Nutramigen®, Dextri-Maltose® or soy bean preparation. It has been amply demonstrated that these diets are compatible with normal growth and development. Although Platt[7] feels that galactose ingestion may be of special benefit and that galactose is poorly synthesized in the body, there is at present no definite clinical evidence that central nervous system function, or somatic development in general, is impaired by the prolonged use of galactose-free diets. There is ample evidence, as indicated herein, that patients with galactosemia possess the enzyme, UDP Galactose-4-epimerase, which would allow for endogenous synthesis of galactose from glucose and hence permit galactolipid formation in the central nervous system[8] and elsewhere.

It has been frequently observed that patients with galactosemia may in later years of life ingest varying quantities of galactose without experiencing any significant side effects.[1] This is probably related to two factors: (a) the improvement in the tolerance to galactose in these patients with increasing age; and (b) the fact that in later years the ratio of ingested galactose to body weight or surface area is far smaller than in the neonatal period when the symptoms are so pronounced.

BIOCHEMICAL STUDIES

(*A*) *Normal Galactose Metabolism*

Considerable knowledge has been gained in recent years concerning the metabolism of galactose and its utilization in the body. Most of the ingested dietary galactose is in the form of lactose, the main carbohydrate of milk, which in the intestine is split into its two component monosaccharides, galactose and glucose (Fig. 1). These sugars differ only in the orientation

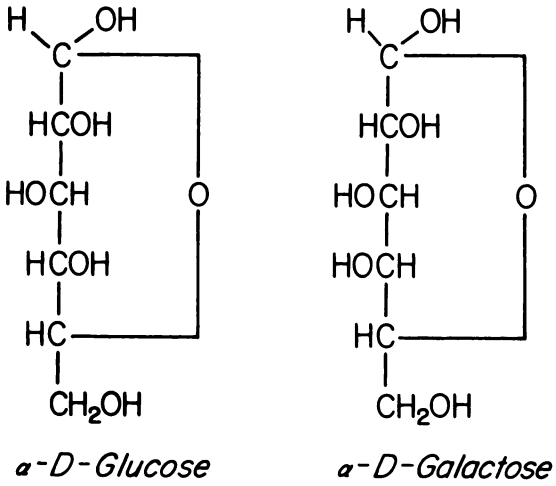

Fig. 1. Comparison of the structure of α-D-glucose and α-D-galactose.

of the hydrogen and hydroxyl groups about the fourth carbon atom. The galactose may be incorporated into galactolipids, and muco-polysaccharides (chondroitin sulfate) but most of it is converted to glucose derivatives which can then be utilized for energy. The exact manner whereby galactose enters the glucose "energy pool" has been greatly elucidated as a result of the investigations of Leloir, Kalckar and their associates.[8–11]

The first step in this direction consists in the phosphorylation of galactose α-galactose-1-phosphate (Reaction I). This requires adenosine triphosphate (ATP) and a specific enzyme, galactokinase.

(I) Galactose + ATP → Galactose-1-P + ADP

The galactose-1-phosphate is then converted to glucose-1-phosphate by a second reaction (II) which involves a specific nucleotide, uridine diphosphoglucose (UDPGlucose). In this reaction the galactose-1-phosphate is transferred to the nucleotide to yield uridine diphospho-galactose (UDPGalactose) while at the same time glucose-1-phosphate is liberated. The enzyme catalyzing this reaction has been called phospho-galactose uridyl transferase or, more simply, P-Gal transferase.

(II) Galactose-1-phosphate + UDPGlucose ⇄ UDPGalactose + Glucose-1-phosphate

In a third reaction (III) the two uridine nucleotides are interconverted in a manner which is still incompletely understood at the present time. It has recently been observed that diphosphopyridine nucleotide (DPN) is a cofactor for the reaction and it is believed that a 4-keto sugar may be an intermediate.[12] The enzyme has previously been called galactowaldenase but in view of the recent observations on the probable mechanism of the reaction it is perhaps better called UDPGalactose-4-epimerase.[8]

(III) UDPGalactose $\overset{\text{DPN}}{\rightleftarrows}$ UDPGlucose

(B) *The Enzymatic Defect in Galactosemia*

In studies on the possible causes of galactosemia it seemed reasonable to presume that the defect might involve one or more of the above reactions. A clue in this direction came from the observations of Schwarz *et al.*,[13] who demonstrated that galactose-1-phosphate accumulated in the red blood cells of galactosemic subjects upon the ingestion of milk or of galactose. We were able to confirm these observations with *in vitro* experiments in which it was shown the galactosemic erythrocytes when incubated in a medium containing galactose showed a significant accumulation of galactose-1-phosphate, in contrast to normal red cell similarly incubated.[14] This suggested that the defect in galactosemia could be due to a block in the conversion of galactose-1-phosphate to glucose-1-phosphate, and that such a block might reasonably involve the enzyme P-Gal transferase.

In further studies it was observed that the normal red cell is able to catalyze all three reactions referred to above.[15] In contrast, erythrocytes from galactosemic patients, while normal with respect to their content of enzymes I and III, showed a striking absence of P-Gal transferase activity. This observation has been confirmed in over 20 cases of galactosemia studied thus far. These galactosemic red cells did not lack any co-factors nor did they contain demonstrable inhibitors to account for the lack of transferase activity. It was initially reasoned that the absence of the enzyme might simply be due to an adaptive phenomenon; that is, since many of the galactosemic patients tested had been off galactose for extended periods of time, the red cells might gradually have lost their transferase activity. However, when erythrocytes of patients who had been off galactose for as long as 18 months were examined they were found to be normal with respect to all three enzymes. Also, more recently, it was shown in one patient that the transferase deficiency existed at birth by demonstrating the absence of the transferase in the cord blood of an infant afflicted with the disease.[16] The enzymatic defect noted in the red cells has also been investigated in the liver, which normally contains significant amounts of the enzymes. In two galactosemic patients analysis of liver tissue obtained by biopsy revealed only traces of transferase activity compared to normal liver obtained at biopsy.[16]

(C) *Possible Alternate Routes of Galactose Metabolism*

Observations have been made by several groups of investigators to suggest that administered galactose can be metabolized to some degree in galactosemic patients.[3] These conclusions have been based on the fact that anywhere from 30 to 80 per cent of the ingested galactose is retained and not accounted for by urinary excretion of the sugar. From the observations of Schwarz *et al.*[13] one would expect of course that some of this retained galactose would be in the form of galactose-1-phosphate. Recent studies performed with C^{14}-labeled galactose in a 24-year-old male with galactosemia have permitted a more definite investigation of this problem.[17] Although this patient's ability to metabolize galactose was only one per cent compared to the normal, it was shown that 3 per cent of the administered galactose could be accounted for in his urine as a glucosiduronic acid. In view of our knowledge of the mechanism of glucosiduronic acid formation, this suggests very strongly that at least 3 per cent of the galactose must have been converted to a glucose metabolite (via the urine nucleotides). These studies would indicate that either the observed enzymatic defect in galactosemia is incomplete or that there exists a possible alternate or accessory pathway for galactose metabolism. Any such pathway probably could not be a major route for galactose metabolism, for otherwise one would never see the pronounced clinical manifestations in this disease subsequent to galactose ingestion.*

(D) *New Spectrophotometric Test for Galactosemia*

It has been customary to confirm the diagnosis of galactosemia by means of the oral or intravenous galactose tolerance test. As mentioned above, this test is not without hazard in an infant in whom there is a marked impairment of galactose metabolism. As a result of the discovery of the enzymatic defect in galactosemia a relatively simple spectrophotometric test has been developed which can readily be performed on blood specimens. This test, the details of which have been described elsewhere,[18] is both specific and sensitive. Thus far no false positives have been detected. Errors may occur, however, if the patient has been transfused with normal blood within three months prior to the assay. In such a situation, the transferase content of the donor cells may give a false negative result.[16]

In a newborn suspected of having galactosemia, the enzymatic method permits a diagnosis to be made (on cord blood) without exposing the infant to the possible hazards of galactose administration in order to arrive at the diagnosis. Traces of activity might be ascribed to admixtures of maternal blood and warrants therefore a second test one month later. The enzymatic method may in addition prove more useful than the galactose tolerance test in elucidating the hereditary aspects of galactosemia.

(E) *Genetic Factors*

It is quite evident that galactosemia is a hereditary disease but the exact genetic pattern has not been established. Although the disease occurs frequently in siblings, their parents never show any clinical manifestations. By means of the galactose tolerance test Holzel and Komrower[19] demonstrated that in the majority of cases usually one of the parents showed a lowering of the galactose tolerance test. It is conceivable that with a refinement of the transferase assay one may be able to pick up traits of this disease more readily. However, Dr. Richard Post of the Institute for Human Variation (Columbia University) has suggested[20] that the inheritance of the disease might be based on two multiple alleles, in which case one would have to look for still another deranged metabolic pattern that has thus far escaped our attention as a trait.

(F) *Possible Factors in the Toxicity of Galactosemia*

Recent animal studies have shed considerable light on factors possibly contributing to the pronounced physiologic disturbances which occur in galactosemia. It has been observed repeatedly that when animals (rats or chicks)

* Since the submission of this manuscript, evidence for such an accessory pathway of galactose metabolism has been obtained in this laboratory (Isselbacher, K. J.: *Science* (in press)).

are placed on 30 per cent galactose diets they frequently show a typical quivering syndrome and uniformly develop cataracts within a period of 14 to 21 days.[21,23] Examination of the lenticular[23] and hepatic tissues[24] of such animals has demonstrated the accumulation of galactose-1-phosphate, just as is the case in the galactosemic red cells. It is tempting to speculate that this hexose-phosphate accumulation might interfere with or inhibit one or more enzyme systems which in turn could lead to the physiologic disturbances seen experimentally and clinically. In line with this reasoning Schwarz *et al.*[13] have shown that the accumulation of galactose-1-phosphate in the red cells is associated with a reduced oxygen consumption of these cells.

Hansen *et al.* have studied the uridine nucleotide patterns in the livers of galactose-fed chicks.[21] They found the UDPglucose levels greatly reduced and the UDPgalactose content increased. In similar studies on galactose-fed rats and using specific enzymatic methods we have not been able to find any reduction in the hepatic concentration of UPD-glucose.[22]

The hypoglycemia which often occurs in galactosemia has also been considered by many as being causally related to the clinical manifestations of this disease. However it should be recalled that most of the symptoms found in galactosemia do not occur in diseases associated with more pronounced hypoglycemia, as in von Gierke's disease.

SUMMARY AND CONCLUSIONS

Galactosemia constitutes a hereditary disease in which galactose cannot be metabolized normally. The outstanding features are nutritional failure, hepato-splenomegaly with cirrhosis, mental retardation and cataract formation. Laboratory studies reveal a decreased galactose tolerance, galactosuria, albuminuria and amino-aciduria.

Effective treatment is possible by the institution of a galactose-free diet. Complete disappearance of symptoms may occur if the diagnosis is made early and the dietary regimen is promptly instituted.

The disease results from the congenital deficiency of a specific enzyme, P-Gal transferase (phospho-galactose uridyl transferase). As a consequence, galactose cannot be properly utilized and galactose-1-phosphate accumulates in the tissues. The latter accumulation may be causally related to the toxic manifestations observed clinically.

In spite of the known enzymatic defect some galactose utilization has been shown to occur in these patients. Reference is made to the use of a new and specific enzymatic blood assay for the diagnosis of galactosemia. The advantages of this procedure over the galactose tolerance test are presented.

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