Galactose Metabolism and Its Regulation

By Robert M. Cohn and Stanton Segal

Galactose, a constituent of milk sugar, is a major source of calories in the young as well as an important component of glycolipids and glycoproteins. The conversion of galactose to glucose is mediated by a series of four enzymes. Deficiency of the first two enzymes of the Leloir pathway are associated with two clinical entities: galactokinase deficiency galactosemia and uridyl transferase deficiency galactosemia. The latter condition in which phosphorylation by the galactokinase takes place is the more severe clinical state. Regulation of galactose metabolism in the higher organism has been examined from the point of view of genetic, developmental, and enzymatic considerations. The genetic heterogeneity encountered with both the galactokinase and unridyl transferase is explored. The developmental pattern of the enzymes involved in galactose metabolism are reviewed along with studies involving excretion of ^{14}C-galactose, which negate the impression that patients with transferase deficiency demonstrate increasing ability to dispose a galactose with advancing age. Four alternate pathways of galactose utilization are discussed, with particular emphasis on explaining the ability of Caucasion galactosemics, who have no detectable transferase activity to excrete small amounts of CO_2 after galactose administration. The control characteristics of the enzymes of the Leloir pathway are examined, with reference to the relationship of substrate and cofactor levels acting as constraints on this pathway. Finally, animal models of galactosemia are discussed and their heuristic value is considered.

GALACTOSE, the C-4 epimer of glucose, was discovered in milk sugar by Pasteur in 1856. This sugar, a component of lactose, is present in most animal milks and is a major source of calories in the young. While its existence has been termed a "freak" of evolution by Kalckar,[1] it is nonetheless a major constituent of glycolipids, glycoproteins, and mucopolysaccharides. Although it is not possible to determine why galactose has been selected through evolutionary pressure to fulfill its preeminent role in these diverse structural elements, it must be acknowledged that glucose will not substitute for galactose under normal circumstances. Galactose has been shown to be an important specific antigenic determinant of bacterial cell wall polysaccharides,

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of blood group substances, and, more recently, has been implicated to malignant transformation.[2] Galactose, as well as other carbohydrates, is incorporated into these larger moieties by uridine diphosphate sugars, the role of which has been elucidated by Leloir and coworkers.[3-6]

Galactose is usually ingested as the disaccharide lactose, which is hydrolyzed by lactase to glucose and galactose. The conversion of ingested galactose is mediated by a series of four enzymes (Fig. 1) located within the soluble fraction of the cell. The interconversion of galactose to glucose and vice versa is carried out at the level of the nucleoside diphosphate and is mediated by a specific epimerase. This particular reaction is likely to be of major importance during the periods when galactose or lactose are in short supply as well as periods characterized by continuing elaboration of glycolipid structures, such as myelin, membranes, and ground substance. Absence of the epimerase in microorganisms leads to disordered cell wall architecture.[7]

This review will be concerned with evaluating the importance of galactose in the economy of the organism as well as considering the manner in which its utilization is controlled. In addition, because clinical disorders of galactose metabolism occupy such a critical position in helping us gain an understanding of the importance of galactose to the organism, these conditions will be explored focusing on the disordered biochemistry encountered. Finally, the search for animal models of these disorders has opened up areas of concern more far reaching than the inability to metabolize galactose, and these models will be evaluated as to their pertinence and ability to answer questions that still remain.

Metabolic regulation in a multicellular organism can be viewed from a number of levels of organization and control, some of which are not directly related to the material in this review but have been surveyed by Stadtman.[8] Here we shall be concerned with three broad avenues of regulation: genetic, developmental, and enzymatic.

Mutational events undoubtedly play a crucial role in cellular regulation by denying to the cell critical enzymatic capabilities. Modulation of enzyme levels due to genetic events may occur over a more protracted course than regulation of enzyme activity on the basis of factors other than genetic, e.g., cofactor and substrate availability. The normal disposition of galactose is severely

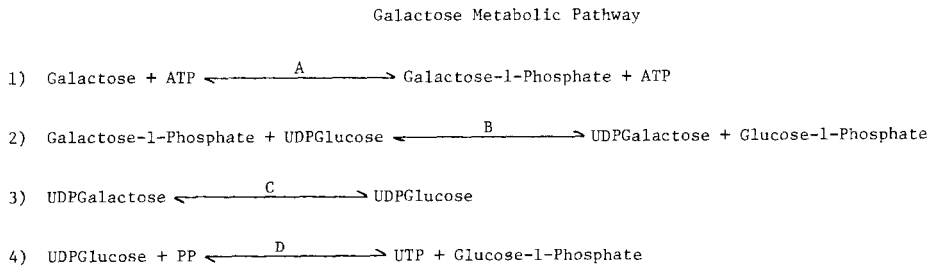

Fig. 1. Reactions responsible for the interconversion of galactose and glucose. A, galactokinase; B, galactose-1-phosphate uridyltransferase; C, UDPgalactose-4-epimerase; and D, UDPglucose pyrophosphorylase.

impaired in a number of disease states in which enzyme activity normally present is either absent or compromised. Study of these individuals has led to some understanding of the importance of these enzymes in the human. In addition, a genetic heterogeneity has been uncovered by studying populations of presumed normals and affected individuals and serves for the moment to complicate any simple interpretation of enzyme activity and the presence of clinical symptoms. Obviously, at the level of the organism a number of interactions determine under what conditions of stress or dietary challenge a particular partial enzymatic defect will become manifest at a clinical level.

Studies on the time course of development of these enzymes provide insight into the susceptibility of the fetus at a galactose load as well as documenting the lack of increased ability to metabolize galactose with advancing age. Another area from which we may expect some clarification in the near future is the relationship between lactase deficiency and the enzymes involved in galactose metabolism.

Enzymatic regulation involves considerations of kinetics as well as substrate and cofactor availability, competing reactions for the same cofactors and compartmentation within the cell. Obviously, modulation of enzyme activity provides the most rapid means by which metabolic pathways can be controlled. Modulation of enzyme activity by modification of the covalent structure, as occurs with phosphorylation and dephosphorylation at the behest of hormones and cylic AMP, is another important form of control, which, however, has not yet been shown to play a role in control of galactose economy within the higher organism. The importance of NAD^+ for the epimerase has been amply documented, and the inhibition of galactose metabolism caused by ethanol ingestion has been shown to be due to accumulation of NADH, a potent inhibitor of the epimerase.[9,10]

Genetic Abnormalities and Clinical Disorders

Most physicians are cognizant of galactose because of the two clinical disorders of galactose metabolism, galactose-1-phosphate uridyl transferase deficiency galactosemia characterized by malnutrition, failure to thrive, vomiting, liver dysfunction, cataracts, and mental retardation; and galactokinase deficiency galactosemia characterized by cataracts. In addition, Gitzelman has reported on deficiency of the epimerase in red cells of a normal infant,[11] while demonstrating epimerase activity in cultured skin fibroblasts. Epimeraseless bacterial mutants have been observed with alterations in cell wall composition, and one would expect such a mutant in higher organisms to have far-reaching consequences involving cell membrane and brain glycolipid elaboration. The fact that no anemia or hemolytic component was discovered in this infant is most intriguing. Since the clinical features and management of transferase deficiency have recently been presented in detail,[12,13] they will be dealt with only briefly here.

In transferase deficiency, laboratory investigation reveals abnormal liver function tests, elevated blood galactose, galactosuria, hyperchloremic acidosis, albuminuria, and aminoaciduria. The diagnosis should be considered on discovering a urinary reducing substance, which is glucose oxidase negative.

One must make the definitive diagnosis by demonstrating the absence of galactose-1-phosphate uridyl transferase, since lactosuria may occur in intestinal lactase deficiency and inability to metabolize galactose may be found in severe liver disease.

Elimination of galactose from the diet is the key to treatment, and a compilation of permitted foods is available.[14] Institution of such a diet in a child with transferase deficiency will result in cessation of gastrointestinal symptoms, regression of cataracts and resumption of normal growth pattern. However, despite early institution of the galactose-free diet, a number of patients with normal IQs manifest learning handicaps involving spatial relationships and mathematics, as well as inadequate drive, shyness, and withdrawal.[15] Individualization of the student–teacher relationship may be salutary in this regard.

Intravenous administration of ^{14}C-galactose to a group of normal and galactosemic individuals has uncovered two populations within the patient group, one composed of Caucasians with much delayed excretion of $^{14}CO_2$ and a second group of Negroes with near normal rates of oxidation.[16] These findings are shown in Fig. 2. The observation of very delayed excretion of $^{14}CO_2$ by Caucasian galactosemics has also been made in tissue culture.[17] Assay of intestinal mucosa[18] and liver[19] from the Negro group has revealed transferase levels of about 10% of normal, while the Caucasians had undetectable activity. Thus, residual transferase activity in liver and intestine appears to account for the ability of Negro patients to oxidize a galactose

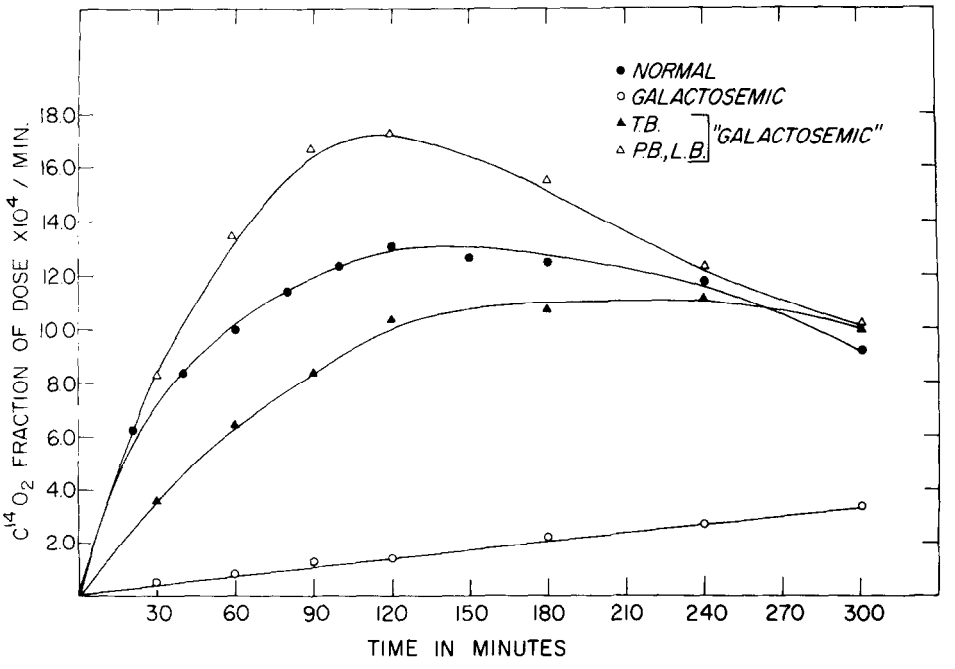

Fig. 2. The excretion of $^{14}CO_2$ in expired air in normal individuals and patients with transferase deficiency after intravenous administration of 1-g quantities of sugar containing galactose-1-^{14}C. (By permission.[16])

load. It is noteworthy that red blood cells of these patients do not have transferase activity, indicating mosaicism of enzyme activity in the tissues of these individuals.[16]

Inheritance of transferase deficiency galactosemia follows an autosomal recessive mode, and obligate heterozygotes have 50% of normal activity. The Duarte variant[20] manifests no clinical symptoms and is characterized by decreased erythrocyte transferase activity, the homozygote manifesting about 50% of normal activity and the mixed heterozygote 75% of normal enzyme activity. The Duarte allele appears to control production of two distinct enzyme bands, which migrate faster on starch gel electrophoresis than the normal single band, suggesting that the enzyme is composed of subunits.[21]

In addition to the Negro variant and Duarte variant, an abnormally slow moving variant has been detected by electrophoresis in two siblings with clinical galactosemia and has been denoted the "Rennes" variant.[22] More recently, the "Indiana" variant[23] has been characterized as an unstable enzyme in a patient with galactosemia. Red cell transferase activity was approximately 35% of control values and was found to be unstable when the patient's cells were stored. On starch gel electrophoresis, the enzyme from the patient's mother and maternal grandmother had slower mobility than transferase from normal control subjects or classic heterozygotes for galactosemia, and enzyme activity was 75% of normal. The enzyme activity from the father, however, was 50% of normal and behaved electrophoretically like a galactosemic heterozygote. No transferase band was detected with the patient's hemolysate, possibly as a consequence of inactivation during electrophoresis. It thus appears that the patient is a double heterozygote with the classic galactosemia gene inherited from the father and the Indiana variant inherited from the mother.

Tedesco and Mellman[24] have shown that galactosemia is a CRM(+) mutation by demonstrating that a protein that reacts with antibody to normal erythrocyte transferase is present in galactosemic cells. Recently, Tedesco[25] purified transferase from postmortem human liver and erythrocytes and showed that red cell extracts from normal, Duarte, and galactosemic individuals are immunologically similar molecules with a molecular weight in the range of 90,000. The enzyme variation encountered is ascribed to small structural gene mutations.

Nadler et al.[26] have presented evidence for the reconstitution of galactose-1-phosphate uridyl transferase activity in hybrid cells formed from the fusion of human diploid fibroblasts obtained from different patients with galactosemia. They presented this work as evidence for interallelic complementation and further suggested that such a phenomenon supports the contention that the mutations at the transferase locus are not identical but rather are point mutations. While the putative hybrid enzyme has Km, pH optimum and electrophoretic mobility on starch gel that is similar to the normal enzyme, it differed from it in respect to V_{max} and thermal stability. The authors speculate that the hybrid enzyme is formed through association of altered subunits. The authors did not dismiss the possibility that somatic recombination rather than interallelic complementation was the basis for their findings.

Physiology

Man's capacity to dispose of large quantities of orally or intravenously administered galactose through the sugar nucleotide pathway is very efficient as evidenced by the rapid elimination of galactose from blood and oxidation of ^{14}C-galactose to $^{14}CO_2$.[16,27] The liver appears to be the most important organ involved in the disposition of galactose, and the enzymes involved in the metabolism of galactose have all been studied in livers of a number of different animals. Plasma glucose rises after a galactose load, and intravenous administration of tracer amounts of radioactive galactose leads to 50% of the radioactivity in body glucose pools within 30 min. Assessment of the quantitative ability of patients with galactosemia to metabolize galactose is based on estimation of conversion of intravenous administered ^{14}C-galactose to $^{14}CO_2$.[16,27] With this test, normals excrete 30%–35% of the radioactivity in 5 hr. Caucasian galactosemics effected slow conversion, excreting 0%–8% of administered ^{14}C, while the Negro galactosemic oxidized the sugar at nearly normal rates. Clearance of blood galactose by liver appears to be saturated at plasma levels of 50 mg/100ml.[28] Liver damage and ethanol administration are associated with a decreased rate of galactose removal from blood, the latter causing tissue elevation of NADH, a potent inhibitor of UDPGal-4-epimerase.[9,10] Tygstrup et al.[29] have recently examined the effect of changes in the lactate/pyruvate ratio on galactose utilization in liver slices from man and rat. They found that while puruvate increased the utilization rate, lactate had no effect and ethanol decreased it by about 20%. They were thus able to show a dissociation between cytoplasmic NADH/NAD ratio and lactate/pyruvate concentration in the medium.

Cellular Regulation of Galactose Metabolism

The enzymes of the sugar nucleotide pathway have been studied in a number of tissues and control characteristics have been explored in order to understand the regulation of this pathway within the cell and the role of galactose in the economy of different tissues within the body.

While it is not possible to state with certainty the relative quantitative contribution of different modes of galactose disposition by different tissues, especially because of its role in synthetic reactions, the main route of metabolism of administered galactose in liver, erythrocytes, and kidney is the uridine nucleotide pathway seen in Fig. 1. It is noteworthy that evidence in cat[30] and chick[31] suggests that the kidney plays a significant role in galactose utilization. Recently, McNamara and Segal[32] examined metabolism of galactose in rat kidney cortex and found that on a weight basis, kidney oxidized approximately twice the amount of galactose as did liver. A fuller understanding of galactose metabolism requires a closer examination of the enzymes comprising the Leloir pathway.

Galactokinase

The enzyme catalyzes a phosphotransferase reaction involving $MgATP^{-2}$ and galactose that results in the production of galactose-1-phosphate. It has been studied in rat,[33] pig liver,[34] human red cells[35] and placenta.[36] The enzyme

requires thiol groups to express full activity, and both substrate and product inhibition have been found, such regulation tending to decrease formation of galactose-1-phosphate, a possible toxic metabolite. Cuatrecasas and Segal[33] found different Km and V_{max} values for the newborn and adult rat liver, but this was not found by Walker.[37] However, Mathai and Beutler[38] found fetal and adult type enzymes in red blood cells from cord blood and adults. Recently, erythrocyte galactokinase[39] was purified over 3000-fold and placental enzyme was purified 350-fold.[36] A comparison of their kinetic properties revealed significant differences in Km and V_{max} and cochromatography of the two enzyme preparations permitted separation of two peaks of enzyme activity, with distinctly different kinetic characteristics corresponding to the fetal and adult tissue, which served as the enzyme source. It is interesting that Vigneron et al.[40] reported a child with transient galactokinase deficiency immediately after birth, who manifested increase in enzyme activity subsequently, suggesting that there are multiple forms of the enzyme presumably under separate genetic controls.

Tedesco et al.[41] have presented evidence for racial polymorphism for galactokinase within the black population, noting three levels of enzyme activity: high, comparable to the white population surveyed; low, approximately 30% of the "high" activity; and an intermediate value. The authors suggest that this allele is distinct from the one which in the homozygous state is responsible for galactokinase deficiency and speculate that there may be a relationship between this polymorphism and the high incidence of lactase deficiency in blacks so that their galactokinase is not challenged by high levels of galactose.

Recently, Zacchello et al.[42] have demonstrated enhancement of galactokinase activity in cultured human fibroblasts by addition of galactose to the medium as well as a lack of response in cells derived from a patient with galactokinase deficiency.

Galactose-1-Phosphate Uridyl Transferase

A widely distributed enzyme, it has been studied in a number of mammalian tissues including rat liver,[43] human red cells,[44] leukocytes,[45] cultured fibroblasts,[45] liver,[19] and intestinal mucosa.[18] The mammalian transferase has a pH optimum of 8.5 and a partial requirement for sulfhydryl stabilization. The Km of the liver enzyme for UDPG is 0.1–0.2m*M*, a value approximating that found within the cell, making regulation by substrate availability likely. At values exceeding the Km, UDPG causes substrate inhibition of the enzyme. The product glucose-1-phosphate is also an inhibitor of the reaction and UTP, UDP, and UMP are potent inhibitors of UDPG binding to the enzyme at concentrations normally found in liver tissue.[46] Figure 3 shows the effects of UTP on uridyltransferase velocities.

Uridyl Diphosphate Galactose-4-Epimerase

This enzyme mediates the inversion of the hydroxyl group at C-4 of the hexose ring to form UDPG from UDPGal as well as the reverse reaction. As noted earlier, while the enzyme functions in liver to effect disposition of a

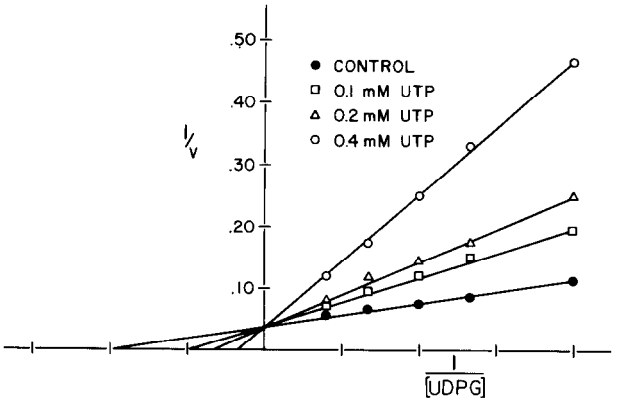

Fig. 3. Effect of UTP on rat liver galactose-1-phosphate uridyltransferase velocity with respect to UDPglucose as varied substrate and galactose - 1 - phosphate at 0.35m*M*. Initial velocities (v) are nmoles of UDP-galactose formed per min per mg soluble protein. (By permission.[46])

galactose load, in brain[47,48] and intestine[49] its function may be concerned with myelin formation and glycocalyx elaboration, respectively.

In microorganism, 1 mole of NAD^+ is bound to the enzyme, while in mammals exogenous NAD^+ must be supplied. NADH is a potent inhibitor, and ethanol ingestion by increasing NADH levels can interfere with liver metabolism of galactose.[10,50] Studies with various nucleotides and nucleotide sugars indicate that intracellular concentration of UDPG as well as other uridine nucleotides may also regulate enzyme activity in liver,[51,52] brain,[53] mammary gland,[54] and erythrocytes.[55] Studies with mouse brain demonstrate a peak of enzyme activity at 8–16 days postpartum, a time coincident with rapid myelination.

The enzyme catalyzes a fascinating reaction, the mechanism of which one might expect to be relatively easy to define and prove. However, such has not been the case. Recent investigations have postulated and presented evidence to substantiate 3-keto,[56] 2-keto,[57] and 4-keto[58] intermediates. The difficulties so far have been insuperable, and at present it appears that the structure of the presumed intermediate cannot be assigned with certainty.

Alternate Pathways

Four additional enzymatic routes of galactose metabolism have been described. The first involves UDPgalactose pryophosphorylase, which catalyzes the reaction of galactose-1-phosphate and UTP to form UDPgalactose and pyrophosphate in a reaction totally analogous to that catalyzed by UDPglucose pyrophosphorylase (Fig. 1). By this reaction, the block caused by deficiency of the transferase could be circumvented. However, studies with human liver UDPgalactose pyrophosphorylase[59] have demonstrated that the activity is highest in the neonatal period, that it decreases with increasing age, and, further, that the ratio of transferase to UDPgalactose pyrophosphorylase activity is 100:1. Moreover, studies by Knop and Hansen[85] with purified UDPglucose pyrophosphorylase indicate that it possesses a small amount of activity towards UDPgalactose and that this accounts for the reaction. Recently, Chacko et al.[60] have reported on studies with human skin fibroblasts from normal and galactosemic individuals that UDPglucose

pyrophosphorylase and UDPgalactose pyrophosphorylase are distinct enzymes on the basis of thermal inactivation studies only, while disregarding the evidence that they are the same enzyme furnished by evidence of similar electrophoretic mobility, pH optima and Km values. The thermal stability studies which showed greater retention of activity by the UDPglucose pyrophosphorylase could also be interpreted to indicate relatively greater persistence of the enzymatic activity toward the physiologic substrate as against activity for an alternate substrate.

Recently, Gitzelman and Hansen (personal communication) have demonstrated loss of UDPgalactose pyrophosphorylase activity in hemolysates of galactosemic red cells on addition of antihuman liver UDPglucose pyrophosphorylase. They also found a purification ratio of UDPglucose pyrophosphorylase/UDPgalactose pyrophorylase of 10:1, which persisted during immunotitration studies, both activities precipitating at the same rate, suggesting identity of the enzyme activities. Gitzelmann has suggested, however, that continued activity of the UDPgalactose pyrophosphorylase activity may lead to self-intoxication by continuing to produce galactose-1-phopsphate in the face of a galactose-free diet.

The second alternate path involves reduction of galactose to its polyol, galactitol by aldose reductase an NADPH requiring enzyme possessing broad specificity for aldehydes.[61] While this path does not represent a major route in the normal individual, it probably achieves significance in the transferase- and galactokinase-deficient patients where accumulation of galactitol has been demonstrated.[62] The Km values for purified lens[63] and brain[64] enzyme are 12 and 20 mM, respectively, underscoring the fact that these enzymes will not be quantitatively significant until galactose accumulation occurs. This path and the following two are shown in Fig. 4.

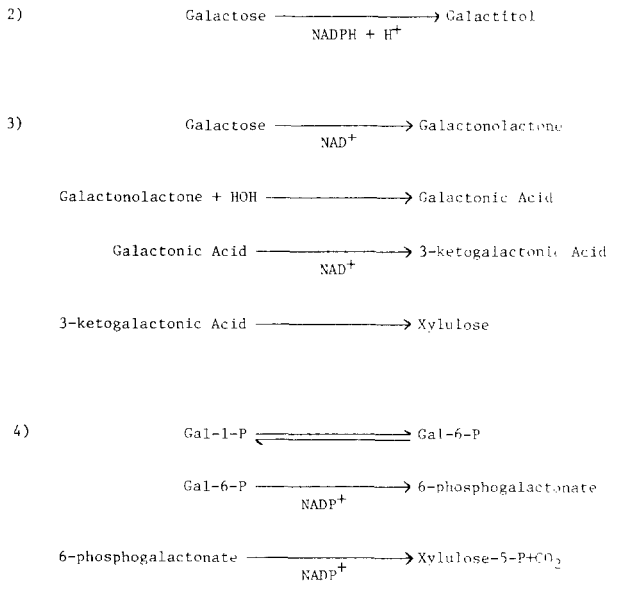

Fig. 4. Alternate routes of galactose metabolism. Route 2 involves the aldose reductase, route 3 shows the galactose dehydrogenase pathway, and route 4 shows the generation of 6-phosphogalactonate from galactose-6-phosphate.

The third route involves oxidation of galactose to galactonate by an NAD^+ requiring enzyme whose Km for galactose is 26 m*M* and has been described in rat liver.[65] The galactonic acid is then oxidized to 3-ketoglalactonic acid, which is converted to xylulose, which may then be further metabolized. While existence of this route has been questioned,[66] Cuatrecasas and Segal[27] have shown that in Caucasian galactosemics there is about twice as much $^{14}CO_2$ liberated from galactose-1-^{14}C than from galactose-2-^{14}C, further substantiating the existence of the oxldative route for galactose. Additional support for the operation of such a pathway was furnished by the study of Bergren et al.[67] in which it was reported that administration of large loads of galactose to normal individuals or to galactosemics resulted in excretion of large amounts of galactonic acid in the urine.

Finally, the conversion of galactose-1-phosphate to galactose-6-phosphate by phosphoglucomutase has been demonstrated in red cells of patients with transferase deficiency, and galactose-6-phosphate has been found in erythrocytes of such patients. This compound can be oxidized in vitro by glucose-6-phosphate dehydrogenase and NADP to 6-phosphogalactonic acid.[68]

Control of Galactose Metabolism

Much of the investigation regarding galactose metabolism has been directed toward elucidation of the characteristics and control mechanisms for the enzymes involved in the sugar nucleotide pathway which permits galactose to enter the glycolytic pathway and other paths. It should be pointed out that in vitro assay of an enzyme under "optimal" conditions of substrate and activator concentration, pH, etc. are not necessarily valid as regards in vivo conditions under which the enzyme is likely to operate, and if approximately valid they define the upper limit of tissue capability to carry out the metabolic pathway. Moreover, as noted by Siebert et al.[69] a low V_{max} is not a universal feature of rate limiting enzymes and in order to estimate actual enzyme rates in vivo, one needs to know concentrations and intracellular distributions of substrates as well as kinetic parameters of the enzyme in the tissues and species under study.

Puck and Hill[55,70,71] in a series of papers have sought to evaluate control of the entire pathway in red cell lysates and have reported that the pseudo first order rate constant for the galactokinase reaction is about a tenth of the transferase constant and a fourth that for the epimerase. Their studies confirm those discussed above with brain and liver that point to the importance of uridine nucleotides as regulatory agents for the activity of the transferase and the epimerase. They further suggest that epimerase activity in the red cell is maintained at a submaximal level by constraints on NAD^+ availability and by the presence of uridine substrates, which are inhibitory. Indeed, K_i values for several of these compounds are well within the range of intracellular levels reported for them,[72] but possible compartmentation of substrates makes a definite regulatory role for any particular nucleotide tentative. These observations furnish some insight regarding the stereospecificity of the enzyme as well as structural requirements of nucleotides, which may interfere with enzyme activity. Of the various nucleoside diphosphate sugars and nucleo-

Table 1. Relationship of In Vivo Concentration of Uridine Nucleotide to K_i Values Determined In Vitro*

Enzyme	Inhibitor	Liver Nucleotide Concentration In Vivo (μmole/g)	K_i (m*M*) In Vitro
Galactose-1-P uridyl transferase	UMP	0.04	2.3
	UDP	0.34	0.35
	UTP		0.13
UDPgalactose 4-epimerase	UMP	0.04	0.4
	UDP	0.34	0.1

*Data for K_i values were obtained for galactose-1-phosphate uridyl transferase and UDP-galactose 4-epimerase from the literature.[46,51] The in vivo data are those Keppler et al.[72] UDP and UTP levels were not measured individually. The value given represents their sum.

tides examined, only those with a pyrimidine base having a C = O group at positions 2 and 4 appear to interact with the enzyme. Table 1 presents values for intracellular concentrations for certain uridine nucleotides and their K_i values for the uridyl transferase and the epimerase.

Recently, Watson[73] has examined the role played by coenzyme derivatives of end-product of pathways, which he terms "rate effectors," and has concluded that regulation of enzyme reactions by these substances provides for control based on the rate of utilization of cofactors, which allows regulations of a pathway branching into a number of divergent paths. The role of UDPglucose and UDPgalactose in complex lipid synthesis is one such focal point, which is likely to be under stringent control by the cell lest there be wasteful production of such key intermediates.

Developmental Aspects

There has been a clinical impression that patients with transferase deficiency galactosemia develop an increased ability to handle galactose with increasing age. However, studies on change of activity of the enzymes of the sugar nucleotide pathway reveal that in liver of the specific activity increases after birth and is highest at about 5 days postpartum in the rat.[13] It is noteworthy that total enzyme activity calculated on the basis of total extractable liver protein increases several-fold from birth to around 25 days when a plateau is achieved.[13]

Recent evaluation of developmental changes of the epimerase[49] showed that in rat intestine specific activity did not rise until 17 days postpartum, reached a peak at 29 days, thereafter becoming constant at adult values (20 ± 1.3 pmole/min/μg protein—twice that in the newborn). Liver epimerase specific activity was highest at birth and 30% of that value in the adult. Particularly striking was the finding that while total epimerase activity in newborn rat liver was 2–5 times higher than corresponding activity in the gut, total epimerase activity in adult intestine was 3–4 times higher than total liver activity. This finding appears somewhat anomalous, since liver is considered

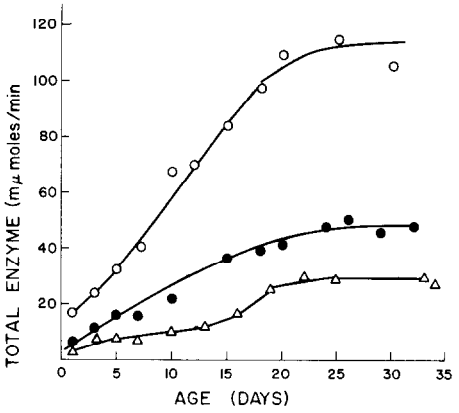

Fig. 5. This is the time course of development of total enzyme activity of the Leloir pathway in rat liver based on the data from the literature[33,43,49] and calculated by a method described previously.[49] Triangles represent epimerase activity; solid circles, galactokinase activity; and open circles, transferase activity.

to be the principal organ involved in galactose disposition, but may be explained by the requirement of the intestine to elaborate its mucopolysaccharide glycocalyx, which must require substantial amounts of UDPgalactose. Figure 5 show the time course of development of total enzyme activity of the enzymes of the Leloir pathway.

Models of Galactosemia

A number of investigators have employed an experimental model of transferase deficiency galactosemia based on administration of high levels of galactose to fetal and young rats and chicks. This model has demonstrated cataract formation[74] and a protective effect of progesterone on galactose-induced cataracts.[75] Aminoaciduria has been induced as well.

As mental retardation is the most significant clinical finding in galactosemia, a number of studies have examined effects of this dietary regimen on brain. Wells and Wells[76] have demonstrated interference with brain myoinositol metabolism as a result of feeding high amounts of galactose to the developing rat. Haworth et al.[77] observed increased galactose-1-phosphate and decreased DNA in brain, indicating reduction in cell number. Wild et al.[78] found considerable decrease in serotonin receptor activity in the brain of galactose fed rats, with the activity localized in the ganglioside fraction. Brunngraber et al.[79] have studied the chemical composition of white and gray matter of a transferase-deficient galactosemic who died at 25 yr of age of bronchopneumonia. They found low values for cerebrosides in white matter, low values for glycosaminoglycans and protein-bound hexosamines and an abnormal distribution of glycoproteins with a 50% decrease in dialyzable glycopeptides in white and gray matter. Recently, they have reexamined the ganglioside pattern in this patient and found an adult type pattern with changes in the carbohydrate content of minor gangliosides but no significant differeces in major gangliosides.[80]

Induction of galactose toxicity in the chick results in a syndrome characterized by shivering, generalized motor seizures, and eventual death.[81] Studies in our laboratory[82] and that of Wells[83] have demonstrated interference with entry of glucose into the brain in these chicks and some slight decrease in

ATP levels after 9 hr of galactose ingestion in 1–2--day-old chicks.[83] In this laboratory, a striking finding in galactose toxic chicks has been the discovery of significant hyperosmolality in conjunction with reversibility of the neurotoxicity within 24 hr of cessation of galactose ingestion.[84] Moreover, Granett et al.[83] note that while on the galactose regimen many of the characteristics of this condition can be reversed by intraperitoneal administration of glucose, underscoring the difference between the acute syndrome in the chick and the subtle persistent mental impairment in the human.

Hence, it is imperative to inquire into the validity of this particular model of transferase deficiency galactosemia. In the first place, the human condition is not characterized by the same high levels of blood galactose that are associated with the chick toxicity, nor is convulsive neurotoxicity found in the inherited disorder. In the second place, patients with galactokinase deficiency galactosemia have no mental retardation despite galactose ingestion and elevated blood galactose concentration, two characteristics they share with the transferase-deficient state. And finally, while hypoglycemia occasionally occurs in transferase deficiency, the postulate of chronic lack of brain glucose as the cause of mental retardation must be serious questioned since other disorders—e.g., glycogen storage disease, having a diminished brain glucose—are not consistently associated with mental retardation. Thus, the etiology of the mental deficiency in galactosemia as well as the aminoaciduria and cirrhosis must be related to biochemical disturbances unrelated to competition of galactose for glucose entry into vital organs.

In view of the clinical distinctions between galactokinase deficiency and transferase deficiency, the role of galactose-1-phosphate, which accumulates in this condition but not in the galactokinase deficiency, must be considered. Implicit in the observation of the differing severity of the two syndromes is the fact that phosphorylation of the sugar must be related to the toxicity. However, there has been no evidence to implicate galactose-1-phosphate as being the toxic factor. The parallelism between the two galactosemic syndromes to that of benign fructosemia and hereditary fructose intolerance is striking, with the disease resulting from the enzymatic defect after phosphorylation being the more severe in each instance.

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