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Substrate-Induced Conjugation of Bilirubin in Genetically Deficient Newborn Rats

Abstract. *Bilirubin appears to induce its own conjugation with glucuronide. Bilibrubin uridine diphosphate–glucuronyltransferase activity is relatively high at birth in heterozygous offspring of permanently jaundiced female rats. The postnatal development of the transferase is accelerated in hyperbilirubinemic heterozygotes.*

Human infants, unlike newborn rats, frequently develop neonatal jaundice which clears within a few days even in relatively immature newborns. "Physiological" hyperbilirubinemia of the newborn is thought to reflect incomplete development of the enzyme-catalyzing bilirubin conjugation—bilirubin uridine diphosphate–glucuronyltransferase (E.C. 2.4.1.17) (BGT) (*1*). The presence of direct-reacting (conjugated) bilirubin in amniotic fluid (*2*) and in serums of severely jaundiced infants (*3*) and the experimental stimulation of bilirubin conjugation in utero with pulse injections of the pigment (*4*) suggest that substrate induction may play a role in the perinatal development of the bilirubin conjugating system. This hypothesis has been substantiated with the use of a naturally occurring animal model.

Postnatal BGT activity was studied in heterozygous offspring of a mutant Wistar strain of rats (Gunn) with an autosomal recessive defect in bilirubin conjugation (*5*). Homozygous animals of this strain exhibit permanent unconjugated hyperbilirubinemia caused by deficiency of BGT activity. Jaundiced Gunn rats were mated with normal Wistar rats to produce two types of heterozygotes capable of conjugating bilirubin: type 1, delivered by jaundiced females bred to normal males; and type 2, produced by anicteric normal females bred to jaundiced males. The two types were identical in all respects except for continuous exposure of type 1 fetuses to high concentrations of bilirubin crossing the placenta from the jaundiced mother (*6*).

Newborn litters of both types of heterozygotes and control litters of Wistar rats were divided in groups of three to five animals. Animals were weighed, and runts were excluded. Animals within each group were killed by decapitation at birth or at specific intervals afterward, and blood was collected for serum bilirubin determinations. The liver was homogenized in nine volumes of ice-cold 0.25*M* sucrose containing 1 m*M* ethylenediaminetetraacetate (disodium salt), *p*H 7.4. The relative BGT activity in homogenates was assayed with a micromodification (*7*) of the incubation procedure of Van Roy and Heirwegh (*8*) which measures bilirubin monoglucuronide formation. Serum bilirubin was determined by the Malloy-Evelyn procedure (*9*).

In normal rats, BGT activity approximated 30 percent of adult amounts at birth (Table 1). The capacity for conjugation began to increase from 3 to 6 hours after birth, reached adult values between days 2 and 3, and exceeded adult rates in 4- to 7-day-old animals. Weanlings conjugated bilirubin less actively than mature rats did.

The BGT activity in type 1 heterozygotes was equivalent to that in normal animals at birth with little further development during the first 24 hours. In contrast, type 2 heterozygotes converted bilirubin at half the normal rates at birth, but BGT activity began to increase within 6 hours. Conjugation in both types of heterozygotes developed more rapidly than in normal rats after day 1. As a result of accelerated development, conjugation rates in partially deficient heterozygotes approached normal rats on days 2 and 3. A decline from these rates began on day 5, reducing BGT activity to 50 percent of normal in weanling and mature heterozygotes.

The serum of normal rats and of mature heterozygous Gunn rats was anicteric. Normal newborns occasionally ex-

Table 1. Bilirubin UDP-glucuronlytransferase activity (expressed as the number of micrograms of bilirubin conjugated per gram of liver per 30 minutes). Homozygous Gunn rats had no detectable activity. The numbers in parentheses are the number of animals used. Values are the mean ± S.D.

Age (hours)	Bilirubin UDP-glucuronyltransferase activity		
	Normal rats	Heterozygous rats	
		Type 1	Type 2
0	355 ± 15 (25)	385 ± 18 (20)	145 ± 12 (20)
3	342 ± 18 (15)	367 ± 17 (20)	152 ± 14 (20)
6	344 ± 22 (20)	344 ± 15 (20)	200 ± 13 (25)
12	470 ± 33 (25)	352 ± 12 (25)	240 ± 16 (20)
24	664 ± 47 (20)	421 ± 24 (25)	413 ± 28 (30)
48	943 ± 54 (25)	825 ± 38 (25)	800 ± 47 (25)
72	1120 ± 109 (25)	1030 ± 89 (25)	1070 ± 98 (25)
96	1280 ± 113 (20)	1054 ± 95 (25)	1090 ± 102 (25)
120	1360 ± 120 (20)	1000 ± 103 (20)	980 ± 68 (25)
21 days	1035 ± 60 (15)	488 ± 54 (20)	500 ± 60 (20)
1 year	1147 ± 135 (10)	630 ± 70 (7)	580 ± 65 (10)

Table 2. Serum bilirubin concentration in rats (expressed as the number of milligrams per 100 ml). Values are mean ± S.D. Not detectable, n.d.

Age (hours)	Normal rats	Heterozygous rats		Homozygous Gunn rats
		Type 1	Type 2	
0	0.1 ± 0.1	1.4 ± 0.3	0.1 ± 0.1	1.2 ± 0.8
3	0.1 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	1.4 ± 0.9
6	n.d.	0.1 ± 0.1	0.8 ± 0.2	3.0 ± 1.0
12	n.d.	n.d.	1.0 ± 0.2	4.0 ± 0.5
24	n.d.	0.3 ± 0.2	1.3 ± 0.3	5.0 ± 0.8
48	0.1 ± 0.1	0.8 ± 0.1	1.2 ± 0.1	6.2 ± 1.2
72	n.d.	0.8 ± 0.1	0.8 ± 0.2	6.9 ± 1.5
96	n.d.	0.6 ± 0.2	0.6 ± 0.2	7.8 ± 1.8
120	n.d.	0.4 ± 0.1	0.3 ± 0.1	8.2 ± 1.8
21 days	n.d.	n.d.	n.d.	11.5 ± 2.0
1 year	n.d.	n.d.	n.d.	12.8 ± 2.8

hibited serum bilirubin concentrations just above the limits of detection immediately after birth (Table 2). Type 1 heterozygotes cleared the bilirubin transmitted from their jaundiced mothers during the first 24 hours, whereas type 2 heterozygotes began postnatal existence with trace amounts of serum bilirubin that, 24 hours after birth, increased to an average concentration of 1.3 mg/100 ml. Mild hyperbilirubinemia persisted to day 5 in both types.

These findings indicate that embryonic differentiation results in elaboration of sufficient BGT activity to prevent jaundice in normal but not in partially deficient newborns. Newborn heterozygous animals exposed to large amounts of bilirubin throughout gestation conjugate the pigment twice as efficiently as those in similar, but unexposed, litters. Newborns of the first type initially clear the bilirubin transmitted from their jaundiced mothers, whereas newborns of the second type develop a hyperbilirubinemia shortly after birth. Hyperbilirubinemic animals achieve maximum BGT activities more rapidly than normal animals in the course of postnatal development, but relative rates of conjugation in genetically deficient rats decline to approximately half the normal rates as the jaundice clears. Thus, postnatal changes in BGT activity follow the serum bilirubin pattern.

These results show that the presence of bilirubin is associated with accelerated induction of BGT before and after birth. Substrate accumulated by maternal animals with an inherited error of metabolism has, in this instance, the paradoxical effect of stimulating the same function in heterozygous fetuses. The competence of this hepatic mechanism is most vigorously tested in the presence of a partial deficiency due to genetic factors or immaturity at birth, or both. Transient hyperbilirubinemia in premature infants appears, therefore, to reflect a perinatal adaptive process necessary for eventual survival.

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Lactosyl Ceramidosis: Catabolic Enzyme Defect of Glycosphingolipid Metabolism

Abstract. *A 3-year-old Negro female showed clinical evidence of a neurovisceral storage disorder that has been characterized by the specific elevation of lactosyl ceramide in erythrocytes, plasma, bone marrow, urine sediment, liver biopsy, and brain biopsy. A galactosyl hydrolase deficiency was demonstrated by the inability to cleave lactosyl ceramide labeled with tritium in the terminal galactose. The enzyme deficiency may be the primary cause of this previously unreported sphingolipidosis.*

A number of clinically distinct lipid storage diseases have been characterized as inborn errors of glycosphingolipid catabolism. Visceral Tay-Sachs disease (*1, 2*), Fabry's disease (*3*), and Gaucher's disease (*4*) are characterized by the absence of specific glycosidases in the stepwise catabolism of red cell globoside (Fig. 1) and the accumulation of glycosphingolipid, mainly in the visceral organs. Similarly, the absence of specific glycosidases has been demonstrated in G_{M1} gangliosidosis (*5*) and Tay-Sachs disease (*1, 6*), leading to the accumulation of glycosphingolipid material primarily in the brain. Lactosyl ceramide [gal(1→4)glc ceramide] (*7*) is a common catabolite of both globoside and ganglioside catabolism (Fig. 1), and any defect in its catabolism might be expected to have neurovisceral implications. No such disease has previously been described, but we have recently studied a patient who clinically and biochemically manifests such a disorder.

A 3-year-old Negro female (S.H.) was born prematurely, and early development was slow but unremarkable. Examination at 2½ years revealed mild hypotonia. Shortly thereafter neurologic development slowed, and she began a course of accelerating mental regression. The neurologic deterioration has been marked by cerebellar ataxia together with loss of mental function, spasticity, increased deep tendon reflexes, bilateral Babinski reflexes, and increasing redness of the maculae. There has been no

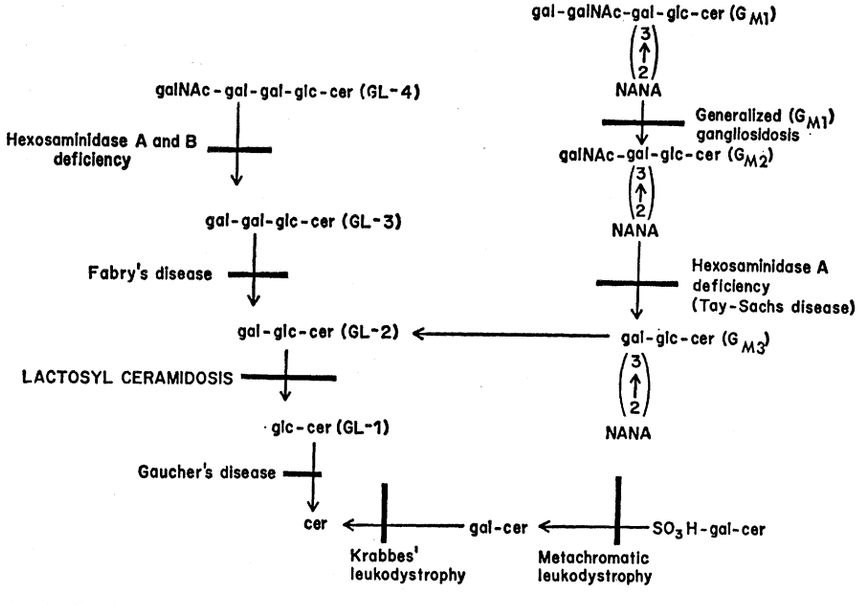

Fig. 1. Catabolism of the major human glycosphingolipids showing the sites of known inborn errors of metabolism.