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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References and Notes

1. G. H. Lathe and M. Walker, *Biochem. J.* **70**, 705 (1958).
2. F. H. Jansen, K. P. M. Heirwegh, A. Devriendt, *Lancet* **1969-I**, 702 (1969).
3. L. E. Harris, F. J. Farrell, R. G. Shorter, E. A. Banner, D. R. Mathieson, *Proc. Staff Meet. Mayo Clin.* **37**, 574 (1962).
4. A. F. Bakken, *Pediat. Res.* **205**, 3 (1969); *Acta Paediat. Scand.* **59**, 148 (1970).
5. R. Schmid, J. Axelrod, L. Hammaker, R. L. Swarm, *J. Clin. Invest.* **37**, 1123 (1958).
6. S. Schenker, N. H. Dawber, R. Schmid, *ibid.* **40**, 1442 (1961); S. Schenker, R. A. Bashore, F. Smith, in *Bilirubin Metabolism*, J. A. Bouchier and B. H. Billing, Eds. (Blackwell, Oxford, 1967), p. 199.
7. M. Black and B. H. Billing, *N. Engl. J. Med.* **280**, 1266 (1969).
8. F. P. Van Roy and K. P. M. Heirwegh, *Biochem. J.* **107**, 507 (1968).
9. H. T. Malloy and K. A. Evelyn, *J. Biol. Chem.* **119**, 481 (1937).
10. I thank Dr. R. Schmid for making a colony of Gunn rats available. Supported by PHS grant HD-03148.

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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

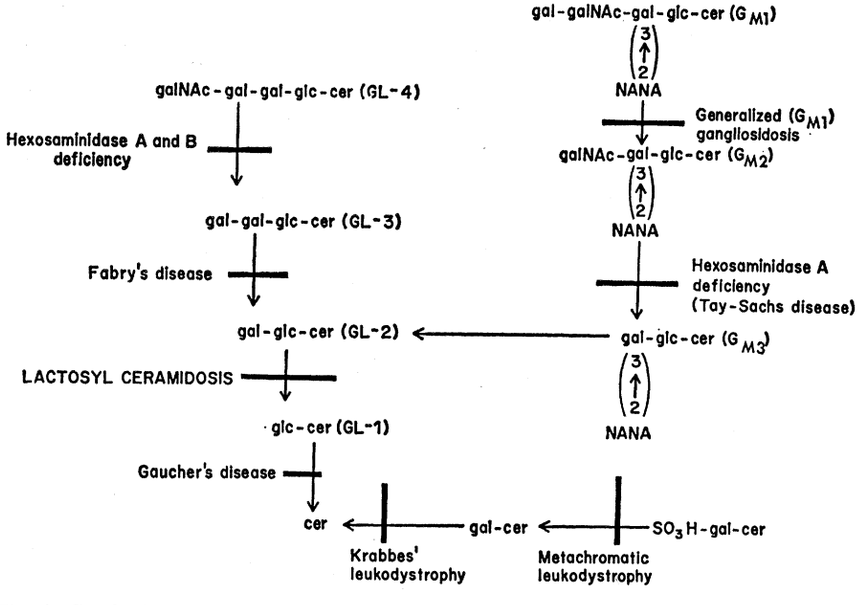

Fig. 1. Catabolism of the major human glycosphingolipids showing the sites of known inborn errors of metabolism.

opisthotonos. The liver was enlarged 5 cm and the spleen 2 cm below the costal margins, suggestive of a storage disease. Examination of the bone marrow showed mononuclear storage cells —similar to those seen in Niemann-Pick disease—about 40 μm in diameter and filled with clear foamy cytoplasm. Initial clinical laboratory data revealed an elevation of serum acid phosphatase, which is a nonspecific characteristic of lipid storage diseases. Subsequent analyses indicate that the disease is characterized by the presence of abnormally high concentrations of lactosyl ceramide in all the blood and biopsy samples studied.

Total lipids were extracted by the Folch (*8*) procedure from samples of erythrocytes (2 to 4 ml), plasma (5 to 10 ml), bone marrow cells (2 to 4 ml), urine sediment (24-hour collection centrifuged and lyophilized), liver biopsy (1 g), and brain biopsy (0.25 g). Liver and brain biopsy specimens were homogenized in 0.15*M* NaCl (nine volumes) prior to extraction, whereas the other material was extracted directly. A portion of the total lipid extract was retained for a two-dimensional thin-layer chromatographic peptide pattern (*9*), which allowed a qualitative appraisal of any lipid abnormalities; the remainder was fractionated into neutral lipids, glycolipids, and phospholipids (*10*). Individual glycosphingolipids were isolated and characterized by the modification of the procedure of Vance and Sweeley (*10*), as described (*11*), quantitative analyses being carried out by gas-liquid chromatography. Gangliosides were purified by a combination of silicic acid chromatography (*12*) after dialysis of the Folch upper phase, and thin-layer chromatography (two sequential separations on coated silica gel plates, obtained from Quantum Industries) in a mixture of chloroform, methanol, and 2.5*N* NH_4OH (60 : 40 : 9). Individual gangliosides were visualized by iodine, removed from the plate by scraping, eluted with a mixture of chloroform, methanol, and water (100 : 42 : 6), and estimated by gas-liquid chromatography. Hematoside G_{M3}, the major glycosphingolipid of normal liver, was found to partition almost equally into the two phases of the Folch extract on the basis of fatty acid chain lengths; the partition of the other glycosphingolipids appeared to be complete.

In all the samples studied GL-2 was elevated (Table 1), suggesting that this glycolipid was involved in the primary metabolic defect. Cholesterol, cholesterol esters, triglycerides, free fatty acids, sphingomyelin, other phospholipids, GL-3, GL-4, and gangliosides were present in normal concentrations except for hematoside (G_{M3}), which showed a twofold elevation in some tissues, such as liver, where it is normally the major glycosphingolipid (*13*). The concentrations of galactosyl ceramide and sulfatide in brain were significantly reduced, suggestive of demyelination. Glucosyl ceramide (GL-1) was slightly elevated in erythrocytes and plasma, while a sixfold excess was found in liver. However, brain, bone marrow cells, and urine sediment contained normal amounts of GL-1. This is in contrast to the finding (*14*) of large amounts of GL-1 in urine sediment from a patient with Gaucher's disease. The analysis of urine sediment, together with the distinctly different clinical picture (delayed onset, no opisthotonos, hepatomegaly greater than splenomegaly, cerebromacular degeneration) enables the disease to be distinguished from Gaucher's disease. Subsequent studies on skin fibroblasts (*15*) revealed further differences.

Table 1. Lactosyl ceramide (GL-2) concentrations in S.H. and normal patients of approximately the same age and sex. With the exception of brain and liver biopsies these results are based upon at least two separate determinations. Values for urine sediment are based on dry weight. Values for brain and liver are based on the fresh weight of the sample.

Subject	Red cells (μmole/ml)	Plasma (μmole/ml)	Bone marrow cells (μmole/ml)	Urine sediment (μmole/g)*	Brain† (μmole/g)	Liver† (μmole/g)
Normal	1.34	0.39	3.40	0.18	0.05	0.06
S.H.	5.33	1.81	6.85	0.54	0.39	0.45

* Dry weight. † Fresh weight.

No lipid storage disease has previously been characterized by the storage of GL-2 as the primary abnormality. Increased amounts of GL-2, together with GL-1 and GL-3, have been found in the brain of patients with Krabbe's leukodystrophy (*16*), generalized gangliosidosis, and Tay-Sachs disease (*17*). However, the primary metabolic defect in these diseases is characterized by the inability to catabolize glycosphingolipids other than GL-2 (Fig. 1). Similarly, elevated levels of GL-2 have been reported in the spleen of some patients with Gaucher's disease (*18*), where the primary metabolic defect is a glucosyl hydrolase deficiency leading to the accumulation of GL-1.

A generalized elevation of GL-2 could result from either a catabolic or anabolic defect but, by analogy with other glycosphingolipidoses, we suspected a catabolic enzyme deficiency. The lactosyl ceramidase activity of liver biopsy of our patient (S.H.) was compared to that of liver obtained at autopsy from females of comparable age. As the substrate we used GL-2 labeled with ^3H at carbon-6 of the terminal galactose residue (*19*). Liver samples (50 to 100 mg, wet weight) were sonicated in 0.1*M* sodium acetate, *p*H 5.0, and centrifuged at 600*g* for 30 minutes to remove cell debris. Portions of the supernatant (100 to 400 μl) were incubated with the [^3H]GL-2 (20,000 count/min) in sodium taurocholate (2 mg/ml), total volume 0.5 ml at 37°C for 2 hours. The reaction was stopped by the addition of chloroform and methanol (2 : 1) (5 ml) followed by water (0.5 ml). Liberated [^3H]galactose was found exclusively in the upper phase of the resulting Folch partition. The activity of lactosyl ceramidase in S.H. liver was only 16 to 20 percent of normal (Table 2), providing good evidence for a catabolic enzyme deficiency. This result has been subsequently confirmed in fibroblasts cultured from a skin biopsy (*15*). The accumulation of GL-2 in the patient's tissues was not responsible for the depressed galactosyl hydrolase activity since addition of excess GL-2 did not affect activity in controls. The activities of other lysosomal enzymes such as β-glucosidase, β-*N*-acetylhexosaminidase, and β-glucuronidase were determined

Table 2. Lactosyl ceramide galactosyl hydrolase activity. Activity is expressed as [^3H]galactose released (counts per minute) per milligram of protein per 60 minutes. Each sample contained GL-2 (20,000 count/min) in sodium taurocholate (2 mg/ml) added to the lysosomal fraction of sonicated liver (0.1 to 0.4 ml) in acetate (*p*H 5.0) buffer, the total volume being 0.5 ml. Boiled tissue was used as the blank, and analyses were performed in triplicate. The amount of [^3H]galactose liberated was proportional to the protein concentration in the range of 0.1 to 0.4 ml of supernatant of the sonicated material.

Specimen	Enzyme activity
Control liver (autopsy)	231 ± 30
S.H. liver (biopsy)	39

by Dr. R. Matalon (*20*) and found to be normal in the livers of S.H. and control subjects.

With the possible exception of Fabry's disease and the adult form of Gaucher's disease (*4*), which both spare the nervous system, all the glycosphingolipidoses so far described are characterized by an almost total specific enzyme deficiency and rapid neurological deterioration after the first few months of life. In contrast to this, S.H. appeared to develop normally, although somewhat slowly, for 2½ years before the start of progressive neurologic degeneration. Because of the central role of GL-2 in both major glycosphingolipid catabolic pathways, a total enzyme deficiency should be at least as severe as infantile Gaucher's disease; the finding of partial lactosyl ceramidase activity in our patient can be interpreted as an explanation for the more prolonged course of the disease. The discovery of this disorder means that an enzyme defect is known for each step in the catabolism of red cell globoside to ceramide. Further, four clinically distinct glycosphingolipidoses, namely, generalized gangliosidosis, Fabry's disease, Krabbe's leukodystrophy (*21*), and lactosyl ceramidosis have now been attributed to specific galactosyl hydrolase deficiencies. Even though these four enzymes have not been characterized, nevertheless the use of specific substrates enables us to detect the diseases and to offer genetic counseling.

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References and Notes

1. K. Sandhoff, *Fed. Eur. Biochem. Soc. Lett.* **4**, 351 (1969).
2. K. Sandhoff, U. Andreae, H. Jatzkewitz, *Life Sci.* **7**, 283 (1968).
3. C. C. Sweeley and B. Klionsky, in *The Metabolic Basis of Inherited Disease*, J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, Eds. (McGraw-Hill, New York, 1966), p. 618; R. O. Brady, A. E. Gal, R. M. Bradley, E. Martenson, A. L. Warshaw, L. Laster, *N. Engl. J. Med.* **276**, 1163 (1967); C. A. Mapes, R. L. Anderson, C. C. Sweeley, *Fed. Eur. Biochem. Soc. Lett.* **7**, 180 (1970).
4. R. O. Brady, J. N. Kanfer, D. Shapiro, *Biochem. Biophys. Res. Commun.* **18**, 221 (1965).
5. J. S. O'Brien, M. B. Stern, B. H. Landing, J. K. O'Brien, A. Donnel, *Amer. J. Dis. Child.* **109**, 338 (1965); S. Okada and J. S. O'Brien, *Science* **160**, 1002 (1968).
6. L. Svennerholm, *Biochem. Biophys. Res. Commun.* **9**, 436 (1962); S. Okada and J. S. O'Brien, *Science* **165**, 698 (1969); E. H. Kolodny, R. O. Brady, B. W. Volk, *Biochem. Biophys. Res. Commun.* **37**, 526 (1969); B. Hultberg, *Lancet* **1969-II**, 1195 (1969).
7. Abbreviations: cer, ceramide or 2-*N*-acylsphingosine; galNAc, *N*-acetylgalactosamine; gal, galactose; glc, glucose; NANA, *N*-acetylneuraminic acid; SO_3H, sulfate. GL-1, GL 2, GL-3, and GL-4 are explained in Fig. 1.
8. J. Folch, M. Lees, G. H. Sloane-Stanley, *J. Biol. Chem.* **226**, 497 (1957).
9. G. Rouser, G. Kritchevsky, C. Galli, A. Yamamoto, A. G. Knudson, Jr., in *Inborn Disorders of Sphingolipid Metabolism*, S. M. Aronson and B. W. Volk, Eds. (Pergamon Press, London, 1967), p. 303.
10. D. E. Vance and C. C. Sweeley, *J. Lipid Res.* **8**, 621 (1967).
11. R. Matalon, A. Dorfman, G. Dawson, C. C. Sweeley, *Science* **164**, 1522 (1969).
12. J. R. Wherrett, *Neurology* **19**, 489 (1969).
13. Normal liver contains 0.17 μmole per gram of G_{M3} (fresh weight), representing approximately 50 percent of the total glycosphingolipid content.
14. R. J. Desnick, personal communication. In the urine sediments of patients there is a tenfold excess of GL-1 in Gaucher's, GL-3 in Fabry's, and GL-4 in Sandhoff's (visceral Tay-Sachs) diseases.
15. G. Dawson, R. Matalon, A. O. Stein, in preparation.
16. J. Menkes, C. Duncan, J. Moossy, *Neurology* **16**, 581 (1966); J. E. Evans and R. H. McCluer, *J. Neurochem.* **16**, 1393 (1969).
17. K. Suzuki and G. C. Chen, *J. Lipid Res.* **8**, 105 (1967); H. Pilz, K. Sandhoff, H. Jatzkewitz, *J. Neurochem.* **13**, 1273 (1966).
18. M. Philippart, B. Rosenstein, J. H. Menkes, *J. Neuropathol. Exp. Neurol.* **24**, 290 (1965).
19. This compound was a gift from Dr. N. Radin, Mental Health Research Institute, University of Michigan, Ann Arbor. See N. S. Radin, L. Hof, R. M. Bradley, R. O. Brady, *Brain Res.* **14**, 497 (1969).
20. We thank Dr. R. Matalon, Department of Pediatrics, University of Chicago, for help and advice with the enzyme studies.
21. M. J. Malone, *Trans. Amer. Soc. Neurochem.* **1**, 56 (1970); K. Suzuki and Y. Suzuki, *Proc. Nat. Acad. Sci. U.S.* **66**, 302 (1970).
22. Supported by PHS Mental Retardation Research Center grant HD 04583-01 and USPHS grant RR 00305. We thank J. Oh for technical assistance. G.D. and A.O.S. are Joseph P. Kennedy, Jr., scholars.

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Sodium Cyclamate and Bladder Carcinoma

It was informative to read that Bryan and Ertürk (*1*) in their first paragraph summarily discarded previous evidence of the carcinogenic effect of sodium cyclamate, notwithstanding all the pros and cons on this subject. While their data are quantitatively impressive in showing that surgically implanted pellets of cholesterol plus cyclamate result in bladder carcinomas in laboratory mice, the control category in their experiments seems to be incomplete. The central technical question at stake is which of the large variety of molecular species ingested via normal diets, when pelletized with cholesterol and implanted in this way, would give the same low incidence of carcinomas as cholesterol pellets alone.

If we ignore, as the authors did, the entire problem of which molecules would normally end up in urinary bladders in amounts similar to the cyclamate concentrations introduced by their technique, then it is interesting to speculate that sucrose, for example, might also have a carcinogenic effect under the same experimental conditions. If such a finding were documented to the same degree as in Bryan and Ertürk's report, could we legitimately conclude that sugar causes bladder carcinoma?

It seems also worth noting that the authors did not rule out the possibility of synergistic effects in their experiment.

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Reference

1. G. T. Bryan and E. Ertürk, *Science* **167**, 996 (1970).

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LSD: Teratogenicity in Mice

Roux, Dupuis, and Aubry (*1*) report that LSD failed to cause abnormalities in rats, mice, and hamsters. I wish to comment specifically about the work performed with mouse embryos.

The teratogenic effect of injected LSD was originally demonstrated in studies on a large series of animals from several inbred mouse lines (*2*). These studies were confirmed and extended in other laboratories (*3, 4*). Dipaolo *et al.* (*4*), moreover, emphasized the fact that in their study teratogenicity was observed in inbred animals but not in outcrossed, general purpose mice. In those studies, as well as in our own, the injection of LSD appears to lead to an increased incidence of those developmental defects which also occur "spontaneously" in these lines.

There is thus no inconsistency in the reported observations. Teratogens always act against a background of genetically influenced susceptibility; LSD is no exception. But since, as the authors point out, extrapolation to man is difficult and extensive clinical observations are needed, one must in the interim at least recognize that LSD can be teratogenic in mice.

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References

1. C. Roux, R. Dupuis, M. Aubry, *Science* **169**, 588 (1970).
2. R. Auerbach and J. A. Rugowski, *ibid.* **157**, 1325 (1967).
3. J. K. Hanaway, *ibid.* **164**, 574 (1969).
4. J. A. Dipaolo, H. M. Givelber, H. Erwin, *Nature* **220**, 490 (1968).

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