lator in the primary culture. Unrelated persons were arbitrarily considered highly cross-reactive for a given LD haplotype if they restimulated at least 80 percent as much as the average for family members known to have the specific haplotype.

Table 2 shows the average response, in a standard 5-day MLC, of each family member to those unrelated persons cross-reacting highly in PLT tests with both, one, or neither of the LD haplotypes of that family member. For each family member the trend of MLC response magnitudes is in the predicted direction: Those persons that are highly cross-reactive by PLT for both LD haplotypes of a particular responder stimulate that responder the least. Similarly those that are highly cross-reactive with one haplotype stimulate less than those which do not cross-react highly with either haplotype. The probability of finding this consistent trend by chance alone is less than .0025. It is important to note that the observed low stimulation in the standard primary MLC by certain unrelated individuals "highly cross-reactive" for one or two LD haplotypes was predicted from the high stimulation by those same individuals in specific secondary MLC combinations (PLT tests).

As a further test of the ability to do LD typing by our method, two groups of three unrelated persons were chosen by PLT criteria. One group consisted of three persons that were all "highly cross-reactive" for one LD haplotype, and the other three persons were "highly cross-reactive" for a different LD haplotype. Standard MLC tests were done between all possible pairs from these six persons. The average MLC (in counts per minute) when responder and stimulator were from opposite groups was 7550; the average when responder and stimulator were from the same group was only 5154, about two-thirds as great. This difference was highly significant ($P < .001$), demonstrating that, on the average, individuals shared more LD antigens with persons in their own group than with persons in the other group. These results, and those in Table 2, show that our method does identify cell components that cause MLC stimulation—that is, that it does type for LD antigens.

Because of the rapidity of the method, and the ease of obtaining PLT cells, the PLT test can easily be applied in an international LD typing program. PLT cells for any LD antigen can be obtained, with the use of cells from appropriate members of any family in which that antigen is found. Since we have found that fresh PLT cells and those preserved by freezing give quantitatively similar results, the freezing methods of Netzel *et al.* (*11*) could be used to prepare typing trays that contain frozen PLT cells defining many different LD antigens. The LD type of any person could be rapidly determined by stimulation of the different PLT cells in this panel.

The ability to identify LD antigens may be important in other ways besides its obvious application to transplant matching. LD antigens are controlled by the same region of the MHC (*12*) that controls the magnitude of immune responses to certain specific antigens (*13*). Moreover, the LD region has been associated with susceptibility to oncogenic viral infections in the mouse (*14*), and certain LD antigens have been associated with disease of immune etiology in man (*15*). Thus PLT may be an important criterion for diagnosing human disease, as well as a specific probe allowing greater understanding of the function and genetic fine structure of the MHC in man and in other species.

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Lactosyl Ceramidosis: Normal Activity for Two Lactosyl Ceramide β-Galactosidases

Abstract. *Lactosyl ceramide β-galactosidase activities in the fibroblasts from the previously described patient with so-called "lactosyl ceramidosis" were reexamined with the two recently developed assay methods which appear to measure two genetically distinct enzymes that can degrade this substrate. No deficiency of either of the lactosyl ceramide–cleaving enzymes was observed. In addition, sphingomyelinase activity was only one-sixth of normal, while all other enzymes examined were within the normal ranges.*

In 1970 Dawson and Stein (*1*) reported on a child with a severe neurovisceral disease which has come to be called "lactosyl ceramidosis." This child had symptoms which included mental regression, spasticity, cerebellar ataxia, increased deep tendon reflex, and increasing redness of the maculae. Biopsy of liver demonstrated storage of glycolipids, especially glucosyl ceramide, lactosyl ceramide (lac-cer), and ganglioside GM_{M3}. Lactosyl ceramide was also increased in red cells, plasma, bone marrow, urine sediment, and brain (*1*). The concentration of sphingomyelin in these tissues was within normal limits according to the initial and subsequent report (*1, 2*).

Because of the storage of lac-cer and the indication that lipid storage diseases, as a rule, are caused by a deficiency of a catabolic enzyme, the activity of lactosyl ceramide β-galactosidase was measured in liver from this patient (*1*). The patient was found to have decreased ability to degrade this natural glycosphingolipid. However, the patient still had 16 to 20 percent of normal activity for this enzyme. Further studies by Dawson *et al.* using cultured skin fibroblasts from their patient and her parents appeared to confirm their earlier report (*3*).

New findings on the degradation of lac-cer via β-galactosidase have been reported. Wenger *et al.* (*4*) reported that tissues from patients with Krabbe's disease (globoid cell leukodystrophy) were almost completely deficient in lac-cer β-galactosidase activity. Furthermore, the use of lac-cer β-galactosidase activity to identify patients and carriers of Krabbe's disease in leukocytes and fibroblast cultures has been reported (*5*). The use of pure sodium taurocholate to stimulate this reaction is essential. Studies in human tissues, especially brain, have indicated that the same β-galactosidase can degrade galactosyl ceramide and lac-cer (*6*). Conflicting reports by Suzuki and co-workers on the catabolism of lac-cer by human liver have appeared (*7*). They reported normal ability to degrade lac-cer in Krabbe disease patients and decreased ability in patients with various types of G_{M1} gangliosidosis. Recently Tanaka and Suzuki determined the reason for the apparent discrepancy between the two laboratories (*8*). While the assay method of Wenger *et al.* (*6*) measures exclusively the lac-cer–cleaving activity due to galactosyl ceramide β-galactosidase, the assay of Tanaka and Suzuki (*8*) appeared to determine preferentially the lac-cer–cleaving activity of another enzyme, which is genetically distinct from galactosyl ceramide β-galactosidase. This second enzyme appears to be identical to the β-galactosidase that can degrade synthetic β-galactosidase substrates and G_{M1} ganglioside. In light of these findings, which demonstrate the presence of two enzymes in humans that can degrade lac-cer, we have reevaluated the lac-cer β-galactosidase activity in the patient with so-called lactosyl ceramidosis.

Fibroblast cultures were grown both in Chicago and in Denver. The cells from this one patient (S.H.), from patients with other lysosomal storage diseases, and from controls were grown in Eagle's minimum essential medium (MEM) plus 15 percent fetal calf serum; they were in at least their third passage when they were harvested and assayed. The confluent cells were harvested with a rubber policeman and homogenized (*5*). The protein concentration of the total homogenate was determined by the method of Lowry *et al.* (*9*). Assays for the synthetic glycosides were carried out as follows: 0.1 ml of each 4-methylumbelliferyl (4MU) substrate was added to the fibroblast homogenate and distilled water was added to a total incubation volume of 0.2 ml. All synthetic substrates (Koch-Light) were prepared in the following concentrations: 0.5 m*M* 4MU-β-galactoside in 0.1*M* acetate (*p*H 4.0)–0.1*M* NaCl; 10 m*M* 4MU-α-galactoside in 0.1*M* citrate–0.2*M* phosphate (*p*H 4.4); 1 m*M* 4MU-β-*N*-acetylglucosaminide in 0.1*M* citrate–0.2*M* phosphate (*p*H 4.4); 1 m*M* 4MU-α-fucoside in 0.1*M* citrate–0.2*M* phosphate (*p*H 6.0). After incubation at 37°C for a specific time, 3.8 ml of glycine-carbonate buffer was added and fluorescence was read on a fluorometer (Perkin-Elmer). Glucosyl ceramide (labeled with ^{14}C in the glucose moiety) β-glucosidase was assayed as follows. [^{14}C]Glucosyl ceramide (50 nmole) plus 500 μg of pure sodium taurocholate (Calbiochem) and 50 μg of oleic acid (Schwarz/Mann) were added in a mixture of chloroform and methanol (2 : 1), and the solvent was then evaporated; 0.1 ml of 1*M* acetate buffer (*p*H 5.0) and fibroblast homogenate were added plus enough distilled water to make a total incubation volume of 0.2 ml. After incubation for 1 hour at 37°C the released glucose was measured in the upper phase as described for galactosyl ceramide β-galactosidase (*10*). Sphingomyelinase was measured with the use of [^{14}C-methyl]sphingomyelin prepared as described (*11*). Sphingomyelin (20 nmole) plus 200 μg of Triton X-100 were added in a mixture of chloroform and methanol (2 : 1), and the solvent was evaporated under a stream of nitrogen. To the residue 0.1 ml of 1*M* acetate buffer (*p*H 5.0) and fibroblast homogenate were added plus enough distilled water to make a total incubation volume of 0.2 ml. After 1 hour at 37°C, the mixture was partitioned in a mixture of chloroform and methanol (2 : 1) as described for galactosyl ceramide β-galactosidase (*10*). [^{3}H]Galactosyl ceramide was prepared and assayed (*5, 10*). Lactosyl ceramide was also labeled with tritium in the C-6 position (*12*). Lactosyl ceramide β-galactosidase was assayed in fibroblasts either by the method of Wenger *et al.* (*5*) or under the conditions described for liver by Tanaka and Suzuki (*8*). Crude sodium taurocholate was purchased from Sigma.

The patient (S.H.) was found to have normal activity for the synthetic substrates tested, including α- and β-galactosidase, α-fucosidase, and β-glucosaminidase (including a normal percentage of hexosaminidase A) (Table 1). Another series of enzyme assays in one of our laboratories also indicated normal activities of G_{M1}-ganglioside and asialo G_{M1}-ganglioside β-galactosidase. Previously, synthetic β-mannosidase and β-glucuronidase activities were found to be normal in this

Table 1. Lysosomal hydrolase activity in cultured skin fibroblasts from patient (S.H.) and from controls. Activity is expressed as nanomoles of substrate hydrolyzed per milligram of protein per hour (± standard deviation).

Substrate	Activity		
	Control	Patient S.H.	Percentage of control
4MU-β-galactoside	348.8 ± 48.2	371	106
4MU-α-galactoside	65.4 ± 11.6	74	113
4MU-α-fucoside	54.4 ± 18.9	47	87
4MU-β-*N*-acetylglucosaminide	2893 ± 757	3067	106
(percentage of hexosaminidase A)	(70)	(76)	
Glucosyl ceramide	161.0 ± 48.9	126	78
Galactosyl ceramide	4.5 ± 1.6	13.8	307
Sphingomyelin	89.0 ± 20.4	15.1	16

Table 2. Lactosyl ceramide β-galactosidase activity in cultured skin fibroblasts from patient (S.H.) and from controls. Activity is expressed as nanomoles of substrate hydrolyzed per milligram of protein per hour (± standard deviation).

Assay method	Activity		
	Control	Patient S.H.	Percent of control
Wenger, Sattler, Clark, and McKelvey (*5*)	16.2 ± 5.6	44.6	278
Tanaka and Suzuki (*8*)	108.0 ± 38.4	106.3	98
Dawson, Matalon, and Stein (*3*)	1.2 ± 0.2*	0.09*	7.5

*These data are recalculated from Dawson *et al.* (*3*).

patient's fibroblasts (*3*). Aryl β-glucosidase activity was shown to be 55 percent of controls (*3*); whereas glucosyl ceramide β-glucosidase activity overlapped with controls (Table 1). However, sphingomyelinase activity was found to be only 15 to 17 percent of normal on repeated testing at all stages of cell growth (Table 1). Galactosyl ceramide β-galactosidase activity was about three times higher than normal in this patient (Table 1). In Table 2 is shown the lac-cer β-galactosidase activity assayed according to the methods of Wenger *et al.* (*5*) and Tanaka and Suzuki (*8*), and compared with that reported by Dawson *et al.* (*3*). The lac-cer β-galactosidase activity in this patient was about three times normal when assayed according to the method of Wenger *et al.* (*5*). This is almost the same as the increase in the activity of galactosyl ceramide β-galactosidase found in this patient's fibroblasts (Table 1). When the same culture was assayed according to the method of Tanaka and Suzuki (*8*) the activity of lac-cer β-galactosidase was not different from the normal control. The deficiency in the patient is shown only with the original data of Dawson *et al.* (*3*). In addition to fibroblasts, the liver and brain of the patient were examined for lac-cer–cleaving activity. No deficiency was found with either of the two assay methods.

Therefore the patient with so-called lactosyl ceramidosis was found to have normal or above normal activity for either of the two lac-cer β-galactosidases when assayed by methods recently described (*5, 8*). Dawson *et al.* (*3*) performed their original assays by a method which did not contain either pure or crude sodium taurocholate. They found deficient activity in this patient when they used Triton X-100 as the only detergent. The activity for lac-cer β-galactosidase assayed with Triton X-100 in control fibroblasts was found to be about 1 nmole per milligram of protein per hour, which is about 1 percent of the activity found for controls by the method of Tanaka and Suzuki (*8*) and 5 percent of the activity for controls by the method of Wenger *et al.* (*5*). The present evidence is for two genetically distinct lac-cer β-galactosidase activities. There still is no evidence to support the idea that there may be a third β-galactosidase in humans that degrades lac-cer exclusively. While we cannot conclusively exclude the possibility that the lac-cer–cleaving activity measured in the presence of Triton X-100 might represent the third enzyme, the level of the activity appears too low to be physiologically significant.

Lactosyl ceramide has been reported to be stored to some extent in certain organs in children with specific lysosomal storage diseases such as Tay-Sachs, Niemann-Pick, G_{M1} gangliosidosis, Krabbe's disease, and in the Hurler, Hunter, and Sanfilippo syndromes (*13*). Patients with minimal storage of sphingomyelin, but excess glycolipids including lac-cer, and a partial deficiency of sphingomyelinase activity have been reported (*14*). The reasons for the storage of lac-cer in patients in which the primary lesion has been shown to be the deficiency of a lysosomal enzyme apparently unrelated to lac-cer catabolism is at present unexplained. A partial deficiency (15 to 17 percent of normal) of sphingomyelinase activity found in the fibroblasts may be the primary lesion in this patient. If so, this one patient with so-called lactosyl ceramidosis may be another of the cases of an unusual type of Niemann-Pick disease.

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Visual Membranes: Specificity of Fatty Acid Precursors for the Electrical Response to Illumination

Abstract. *Rat electroretinograms were measured as a function of dietary supplements of purified ethyl esters of linolenic acid, linoleic acid, and oleic acid. Polyunsaturated fatty acids derived from precursors of linolenic and linoleic acids appear to be important functional components of photoreceptor cell membranes, although in equal dietary concentrations, linolenic acid precursors affect electroretinogram amplitudes to a greater extent than linoleic acid precursors. The electrical response of photoreceptor cell membranes appears to be a function of the position of the double bonds as well as a function of the total number of double bonds in fatty acid supplements.*

Vertebrate photoreceptor cells consist of an inner and an outer segment (*1*). The inner segment of the rod cell contains a variety of membrane systems, such as mitochondrial, ribosomal, plasma, and nuclear membranes; the rod outer segments (ROS) are characterized by a stacked array of many hundreds of disk membranes, which are enclosed by a plasma membrane. The visual pigment rhodopsin accounts for 80 to 90 percent of ROS membrane-bound protein (*2*), and phospholipids account for 80 to 85 percent of the total ROS membrane lipid (*3*). Phospholipids are required for both the stability and regenerability of rhodopsin (*4*); and, on the average, each rhodopsin molecule of rat ROS is associated with 60 to 65 phospholipid molecules (*5*).

Docosahexenoic acid, 22 : 6ω3, is the dominant polyunsaturated fatty acid of the phospholipids of vertebrate ROS membranes (*6*). Rats cannot synthesize either ω3 or ω6 fatty acids, and ω3 and ω6 precursors (essential fatty acids) must be obtained from dietary sources (*7*). This dietary requirement, together with the observation that normal rat ROS membranes turn over and are renewed every 9 to 10 days (*8*), suggested that dietary manipulations could be exploited to alter the lipid composition of rat ROS membranes. However, only 8 percent of the polyunsaturated lipids were replaced in ROS mem-