The differences in the distribution of LADH enzyme activity in adult, newborn, and fetal samples are statistically significant ($\chi^2 = 17.378$, $.001 < P < .01$) (Table 1). The significance is due to differences in the distribution of adult and newborn samples versus the fetal samples ($\chi^2 = 10.827$, $P < .001$). There is no significant difference in the distribution of enzyme levels between adult and newborn samples.

In another investigation, gradient elution from a carboxymethyl cellulose column demonstrated three peaks with LADH activity in some individuals and two LADH activity peaks in others (*8*). This variation was similar to the variation in number of cathodal adult isoenzymes found in our study.

Our results indicate at least three transitional periods in the ontogeny of LADH in man; the first occurs during late fetal life, the second at from 4 to 6 years of age, and the third between 11 and 14 years of age. Although the developmental variation in creatine phosphokinase (*4*), esterase (*3*), lactate dehydrogenase (*2*), and hemoglobins (*1*) seems to be almost complete or complete by the newborn period, this study indicates that developmental change probably continues for a longer period in human LADH than in other developmental markers thus far described in man. Since the primary metabolic function of LADH in man is unknown, no reason can be given for this variation.

Because LADH from human fetuses has not yet been purified, any differences in primary structure between the fetal and adult enzyme are still unknown. However, the finding that, compared with the adult enzyme, fetal LADH has a different K_m and optimum *p*H value as well as a characteristic electrophoretic pattern (*6*) suggests that fetal and adult LADH are structurally different proteins.

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Krabbe's Globoid Cell Leukodystrophy: Deficiency of Galactocerebrosidase in Serum, Leukocytes, and Fibroblasts

Abstract. *The activity of galactocerebroside β-galactosidase was extremely low in serum, leukocytes, and cultured fibroblasts of patients with Krabbe's disease. Antemortem diagnosis is possible without organ biopsies. The parents of patients showed enzyme activities generally lower than that of normal controls. This finding provides supportive evidence that the deficient activity of galactocerebroside β-galactosidase is the genetically determined enzymatic defect underlying the disease. Demonstration of this deficiency requires the use of the specific substrate, galactocerebroside. Assays carried out with synthetic, unnatural substrates, such as 4-methylumbelliferyl β-galactoside, do not distinguish patients or heterozygous carriers from normal individuals.*

Krabbe's globoid cell leukodystrophy is a genetically determined and rapidly fatal neurological disorder of infants, characterized by almost total loss of myelin, severe gliosis, and massive infiltration of the unique multinucleated globoid cells in white matter. Deficient activity of galactocerebroside β-galactosidase has been demonstrated in this disease (*1*). We have extended our study to readily available materials such as peripheral blood and cultured fibroblasts in an attempt to establish a means of antemortem diagnosis of the disease. We also assayed samples from parents of patients with Krabbe's disease, because a partial deficiency of the enzyme would be expected in heterozygous carriers, if the lack of galactocerebroside β-galactosidase activity is indeed the genetically determined defect.

Serum was separated from blood clot by centrifugation, and the enzyme assays were performed immediately, because both galactocerebroside β-galactosidase and 4-methylumbelliferyl β-galactosidase were unstable in serum and rapidly lost activities even if serum samples were stored frozen or refrigerated (Fig. 1). A similar instability of enzyme in serum was reported for another specific galactosidase, galactosylgalactosylglucosylceramide:galactosyl hydrolase (*2*). Leukocyte suspensions were prepared essentially by the method of Snyder and Brady (*3*), with minor modifications (*4*). Fibroblasts were cultured from skin (*5*) and collected by trypsinization. Both leukocytes and fibroblasts were frozen and thawed several times before enzyme assays, and fibroblasts were disrupted with high-frequency sound. β-Galactosidases in leukocytes were stable when kept frozen, with only a slight loss of galactocerebroside β-galactosidase activity over an 8-week period (Fig. 1). The protein concentrations of leukocyte and fibroblast preparations were determined

Table 1. Activity of β-galactosidases in serum.

Subject	Galactocerebroside β-galactosidase (nmole/hr/100 ml)	4-Methylumbelliferyl β-galactosidase (nmole/hr/ml)
Patient	0	13.8
Father	6.5	4.8
Mother	3.2	5.6
Brother 1	5.8	4.8
Brother 2	27.7	6.0
Child control	15.5	6.0
Adult control 1	19.2	8.6
Adult control 2	23.6	5.0
Adult control 3	15.1	7.8

Table 2. Activity of β-galactosidases in leukocytes and fibroblasts.

Subject	Galactocerebroside β-galactosidase (nmole/hr/mg protein)	4-Methylumbelliferyl β-galactosidase (nmole/hr/mg protein)
Leukocytes—Family 1		
Patient	0.03	67.2
Father	0.37	67.5
Mother	1.61	68.7
Control 1	1.39	59.0
Control 2	1.58	64.6
Leukocytes—Family 2		
Father	0.48	18.1
Mother	0.34	30.9
Control 1	1.14	21.2
Control 2	1.19	23.8
Leukocytes—Family 3		
Patient	0.04	90.4
Father	2.25	123
Mother	1.10	133
Brother 1	0.31	81.6
Brother 2	1.69	86.2
Control*	3.21	115
Fibroblasts		
Patient	0.53	312
Control 1	4.26	197
Control 2	4.93	888
Control 3	2.78	799
Control 4	6.61	406

* This was the only control sample prepared simultaneously with the experimental samples, but this was prepared in our own laboratory and the average control activities of leukocyte preparations ($n = 11$) prepared in our laboratory are 3.23 ± 1.46 for galactocerebroside β-galactosidase and 98.0 ± 20.2 for 4-methylumbelliferyl β-galactosidase.

by the Lowry method (*6*). The assay system for galactocerebroside β-galactosidase has been described previously (*1*). 4-Methylumbelliferyl β-galactosidase was assayed at *p*H 5.0 (leukocytes and fibroblasts) or 4.0 (serum) by spectrofluorometry (*7*). The values given in Tables 1 and 2 are the results of single determinations, but, when several samples were assayed repeatedly for both galactocerebroside β-galactosidase and 4-methylumbelliferyl β-galactosidase, the results were generally reproducible within the range of 10 percent.

Fresh serum samples were obtained from a patient with Krabbe's disease and from members of her family (Table 1). Galactocerebroside β-galactosidase activity was not detectable in the patient's serum, and the activities in the serums of both parents and one of the two siblings were lower than that of normal controls. The activity of 4-methylumbelliferyl β-galactosidase was similar in all samples tested. The moderately high activity of 4-methylumbelliferyl β-galactosidase in the patient's serum, despite the undetectable galactocerebroside β-galactosidase, is not surprising, because the assay gives the total activity of all β-galactosidases which can use the artificial substrate nonspecifically.

Leukocyte samples were collected from members of three families (Table 2). Leukocytes of the members of families 1 and 2 were prepared in another laboratory with the same technique and sent to us. Family 3 is the same family in which we assayed fresh serums for the enzyme activities, and leukocytes were prepared in our laboratory. The results are tabulated separately for each family because there were considerable variations in normal control values from one series to another, probably due to the inevitable slight differences in the conditions of leukocyte preparation in different laboratories. This also points out the importance of including specimens from normal individuals whenever pathological samples are prepared. Consistent results were obtained within each experimental series. The activity of galactocerebroside β-galactosidase was extremely low in the two patients. Four of the six parents showed low activity of this enzyme, but two of them appeared to have normal activity. The mother of family 1 showed normal activity not only in her leukocytes but possibly also in serum, although the result on serum may not be valid because of the storage for 3 weeks. The father of family 3, whose serum showed definitely low galactocerebroside β-galactosidase activity, appeared to have normal activity in leukocytes. A separate blood sample obtained several days later gave the same results, for both serum and leukocytes. Galactocerebroside β-galactosidase was also deficient in cultured skin fibroblasts of a patient, but there was a higher residual activity (10 to 20 percent normal) than that in serum or leukocytes (Table 2).

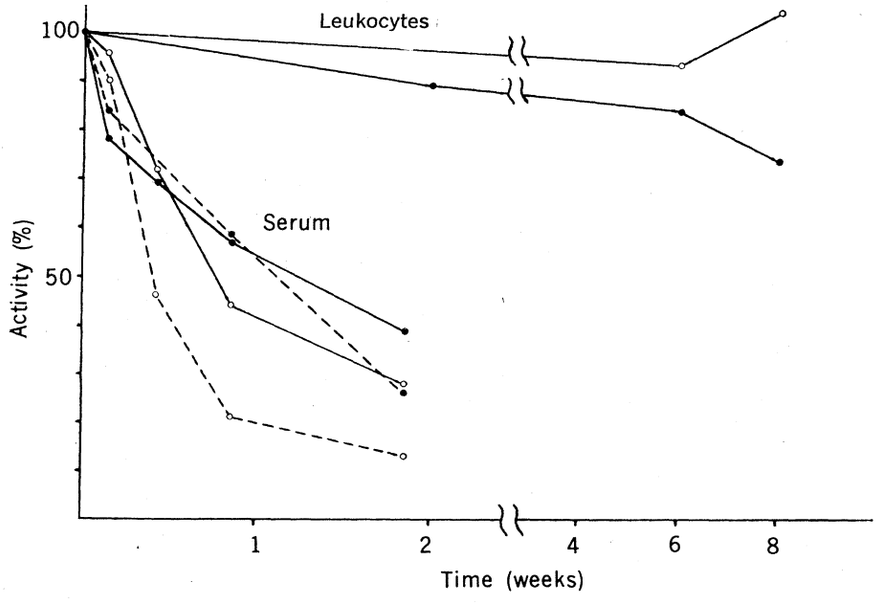

Fig. 1. Effect of storage on the activities of β-galactosidases. Serum and leukocytes were prepared and stored either frozen or refrigerated for designated periods of time before enzyme assays. Closed circles, galactocerebroside β-galactosidase; open circles, 4-methylumbelliferyl β-galactosidase. Solid lines represent samples stored frozen at −20°C, and dashed lines for samples stored refrigerated at 4°C.

Galactocerebroside β-galactosidase activity is deficient in serum, leukocytes, and cultured fibroblasts of patients with globoid cell leukodystrophy. This deficiency can be demonstrated only with the use of the specific natural substrate, galactocerebroside, and not by artificial chromogenic substrates. The disease can be diagnosed utilizing these readily available materials, without resorting to organ biopsies. The disease was diagnosed in the patient of family 3 first on the basis of the blood enzyme assay, prior to morphological confirmation by a brain biopsy. If enzyme assays can be performed immediately, serum provides the most convenient and reliable source for the assay, but if separated samples must be stored for more than a few days, leukocytes are more reliable, because the enzyme is much more stable in leukocytes.

Interpretation of the enzyme activities in parents is not as simple as in those of patients. Krabbe's disease is

believed to be transmitted as an autosomal recessive trait. Parents of patients can generally be considered to be obligate heterozygous carriers of the disease. If lack of galactocerebroside β-galactosidase activity is the genetically determined defect, parents are expected to have low enzyme activity in all somatic cells and probably also in serum, because the deficiency of this enzyme in homozygous patients is systemic and not limited to the brain (*1*). Four of the six parents we examined gave, as expected, low enzyme activity. However, the two exceptions require careful evaluation.

The mother of family 1 is exceptional, because she showed normal activity of galactocerebroside β-galactosidase not only in leukocytes but possibly also in serum. She was pregnant when the blood sample was drawn. We plan to follow up the enzyme activity of this individual in order to rule out the possible effect of pregnancy or any other extraneous factors which might have affected the enzyme activity. We cannot exclude another rare possibility, germinal mosaicism, in which only the gonad carries the mutation (*8*).

The father of family 3 had low enzyme activity in serum, but his leukocytes showed normal galactocerebroside β-galactosidase activity. This may be the result of technical problems associated with leukocyte preparations. Leukocytes prepared from normal individuals in different laboratories showed significantly different enzyme activities, despite the fact that presumably the same isolation procedure was used. Unlike serum, there are a number of factors which can affect the final calculated enzyme activities of leukocytes, such as a partial blood clotting due to incomplete anticoagulation, contamination by plasma protein due to incomplete washing, incomplete elimination of red cells, errors in cell count or protein determination, or the variable proportions of granulocytes and lymphocytes.

Our study on heterozygous carriers of Tay-Sachs disease also demonstrated better reproducibility in hexosaminidase assays in serum than in leukocytes (*4*). Unlike galactosidases, hexosaminidase was stable in serum, and heterozygous carriers of Tay-Sachs disease could be detected by assaying hexosaminidase A in serum, without overlap of values with normal controls. When hexosaminidase A was assayed in leukocytes, however, there was an overlap between heterozygous carriers and normal controls, although the difference between the two groups was statistically significant.

We conclude tentatively, therefore, that, despite exceptions, the activity of galactocerebroside β-galactosidase is generally lower than normal in parents of patients with Krabbe's disease, as predicted from the assumption that the deficient activity of this enzyme is the genetically determined defect underlying globoid cell leukodystrophy. One of the two siblings of the patient in family 3 is asymptomatic but had partially deficient activity of galactocerebroside β-galactosidase both in serum and leukocytes, and we believe that this individual is heterozygous. For the detection of heterozygous carriers of the disease, serum, if available fresh, appears to give more consistent data.

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Hybrid Cell Line from a Cloned Immunoglobulin-Producing Mouse Myeloma and a Nonproducing Mouse Lymphoma

Abstract. *A hybrid cell line was established by cell fusion between a cloned Balb/c myeloma that is resistant to 8-azaguanine and produces immunoglobulin (γG and free kappa chain) and C57BL/6N lymphoma that is resistant to bromodeoxyuridine and does not produce immunoglobulins. The hybrid cells contained the membrane antigens of both parents; they synthesized free kappa chain; no synthesis of γG (γ_{2a}) heavy chain was detected.*

Recent experiments support the notion that the nucleus of the differentiated cell contains large numbers of unexpressed genes (*1, 2*). Discovery of repressors that control gene expression in bacteria has stimulated the search for evidence of similar nature, which may explain the selective gene expression observed in the differentiated cells of the higher organisms. One approach to this problem has been hybridization of two cells which differ in their expression of specific genes. Experiments along this line have provided evidence both in favor of and against the "repressor mechanism" (*3, 4*). We have examined this question with respect to immunoglobulin synthesis by hybridizing a cloned immunoglobulin G (γG; also called γ_{2a}) producing mouse myeloma and a nonproducing mouse lymphoma cell line. The hybrid cells were capable of continuous growth in culture. They produced kappa light chain but not the γG heavy chain of the immunoglobulin synthesized by the parental myeloma clone.

An RPC-5 myeloma tumor of Balb/c origin (*5*) was adapted to tissue culture and grown in Eagle's minimal essential medium supplemented with glutamine, sodium pyruvate, nonessential amino acids, penicillin, and 10 percent fetal calf serum (MEM+) (*6*). Ten clonal lines were isolated (*7*). Clone number 4 (CL4), one of the two parents in this hybridization study, produced γG and free kappa chain with no qualitative change in immunoglobulin synthesis after 2½ years of continuous culturing. The other parent, E.L.4, was a suspension culture of a lymphoma that was induced in a C57BL/6N mouse by 9,10-dimethyl-1,2-benzanthracene (*8*). The E.L.4 was maintained in MEM+ with 10 percent fetal calf serum, and did not produce any immunoglobulins. 8-Azaguanine-resistant CL4 (CL4-