and sarcomas. Our results raise the possibility that activation of type C viruses may also play a role in the induction of hepatomas by chemical carcinogens.

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11. Abbreviations are as follows. The center dot indicates that the polynucleotide chains before and after the dot are hybridized; $(dT)_{10}$, a ten-unit oligomer of deoxythymidylate; $(dA)_n$, a polymer (length unspecified) of deoxyadenylate; $(A)_n$, a polymer of adenylate; $(dT)_n$, a polymer of deoxythymidylate; $(dA\text{-}dT)_n$, a polymer of deoxyadenylate and deoxythymidylate units in alternating sequence; dGTP, deoxyguanosine triphosphate; dATP, deoxyadenosine triphosphate; dCTP, deoxycytidine triphosphate; dTTP, deoxythymidine triphosphate; dTMP, deoxythymidine monophosphate.
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## Ceramidase Deficiency in Farber's Disease (Lipogranulomatosis)

Abstract. *Ceramidase activity could not be demonstrated in the kidney and cerebellum from a deceased patient with Farber's disease, whereas the activities of six control acid hydrolase enzymes appeared normal. This enzyme defect presumably accounts for the accumulation that has been described in two patients and may represent the biochemical basis of this disorder.*

Farber's disease is a disorder that affects young children and is characterized by progressive arthropathy, subcutaneous nodules, nutritional failure, and, in most instances, psychomotor retardation. The basic lesion is a histiocytic granuloma which contains variable numbers of foam cells. In the nervous system the pathological changes resemble those seen in the neuronal lipidoses (*1–3*). In one family the illness occurred in a brother and a sister (*1*); all the other eight reported patients were the only affected members of their families (*3*).

A 10- to 60-fold excess of ceramide was demonstrated in the visceral organs of a 9-month-old girl with Farber's disease who died in 1965, and a high concentration of ceramide was found in a subcutaneous nodule (*3*). Comparable ceramide accumulation has been demonstrated in the kidney and subcutaneous nodule, but not in the other tissues, of another patient with Farber's disease who had survived until he was 16 years old, and who appeared to be more mildly affected than other patients (*4*). The observation that ceramide was found to be accumulated in two patients prompted us to examine ceramidase activity in this disease.

We have now examined the stored postmortem tissues of the above-mentioned 9-month-old girl with Farber's disease; other biochemical findings in this patient have been reported (*3*). Immediately after autopsy, which had been performed 6 hours after death, the tissues were placed in sealed plastic bags and stored at −60°C. As controls we used tissues stored under identical conditions for various periods from patients whose ages and causes of death are listed in Table 1.

The substrate for ceramidase, *N*-[1-$^{14}$C]oleoylsphingosine, was synthesized chemically from [1-$^{14}$C]oleoyl chloride and sphingosine (*5*). After treatment with 0.1*N* methanolic NaOH for 30 minutes, the labeled ceramide was purified by silicic acid column chromatography and preparative thin layer chromatography (*3, 6*).

Ceramidase activity was measured in homogenates of tissue in two volumes of a solution of 0.25*M* sucrose containing 1 m*M* EDTA. Incubations were performed for 1 hour at 37°C at the optimum *p*H for each organ with 0.25 m*M* *N*-[1-$^{14}$C]oleoylsphingosine (specific activity, 2678 count min$^{-1}$ nmole$^{-1}$), except for those shown in Fig. 1, where the concentration of the substrate was 0.38 m*M* (specific activity, 1019 count min$^{-1}$ nmole$^{1-}$). Each assay mixture also contained 25 $\mu$l of 1*M* citric acid–phosphate buffer, *p*H 4.5 (for kidney) or *p*H 4.0 (for cerebellum), 0.1 mg of Tween 20, 0.25 mg of Triton X-100, 0.4 mg of sodium cholate, and 0.3 to 4.0 mg of protein in a final volume of 200 $\mu$l. The data in Table 1 were obtained with 0.7 to 1.3 mg of protein. Under these conditions, enzyme activity was linear as a function of the amount of protein added. Control assays were performed with each tissue, and contained homogenate that had been heated at 100°C for 5 minutes. Before addition of buffer and homogenate, the radioactive ceramide and an aqueous solution of the detergents were mixed and subjected to sonication for 10 minutes. After the incubation, carrier oleic acid, 500 $\mu$g in a mixture of $CHCl_3$ and $CH_3OH$ (2 : 1, by volume), was added, and the reaction mixture was partitioned with Dole's solvents (*7*). The fatty acid fraction including [1-$^{14}$C]oleic acid released was further purified by preparative thin layer chromatography (silica gel G), with $CHCl_3$, $CH_3OH$, and $CH_3COOH$ (94 : 1 : 5, by volume) as the solvent system. The fatty acid band was located by means of iodine vapor, with authentic standards for comparison; the band of silica gel containing the fatty acid region was scraped off and the radioactivity was counted in Aquasol (New England Nuclear) in a liquid scintillation counter.

Galactocerebrosidase and glucocerebrosidase were assayed in the supernatant remaining after the cell nuclei were removed, according to the method of Hajra *et al.* (*8*) and Radin and Arora (*9*) with 0.1 m*M* $^3$H-labeled galactocerebroside (specific activity, 2473 count min$^{-1}$ nmole$^{-1}$) and 0.1 to 1.4 mg of protein per assay in 0.1*M* citric acid–phosphate buffer, *p*H 4.0, or 0.1 m*M* $^{14}$C-labeled glucocerebroside (spe-

Table 1. Enzyme activities in kidney and cerebellum.

| Diagnosis | Sex | Age at death | Time stored | Activity per milligram of protein per hour: Ceramidase (nmole) | Neuraminidase (nmole) | β-Hexosaminidase (μmole) | β-Galactosidase (μmole) | Acid phosphatase (μmole) |
|---|---|---|---|---|---|---|---|---|
| | | | *Kidney* | | | | | |
| Farber's disease | F | 9 months | 7 years | < 0.01 | 1.01 | 8.00 | 0.54 | 1.34 |
| Biliary cirrhosis | M | 2 years | 4 years | 2.48 | 0.77 | 3.66 | 0.96 | 1.51 |
| Reye's syndrome | M | 2 years | 4 years | 1.73 | 1.28 | 4.17 | 0.65 | 0.95 |
| Familial microcephaly | F | 4 years | 4 years | 2.28 | 0.70 | 3.02 | 0.67 | 1.03 |
| Coarctation of aorta | F | 2 months | 5 years | 2.26 | 1.52 | 7.99 | 0.96 | 1.69 |
| Congenital heart disease | F | 3 months | 1 month | 3.21 | 0.90 | 3.96 | 0.61 | 0.98 |
| Burkitt lymphoma | M | 11 years | 1 month | 2.85 | 1.76 | 3.80 | 0.47 | 0.86 |
| Metachromatic leukodystrophy | M | 2 years | 1 year | 3.03 | | 4.45 | 0.67 | 1.10 |
| Infantile Gaucher | M | 2 years | 11 years | 0.66 | | 4.20 | 0.72 | 1.61 |
| | | | *Cerebellum* | | | | | |
| Farber's disease | F | 9 months | 7 years | < 0.001 | | 0.77 | 0.09 | 0.45 |
| Congenital heart disease | F | 1 month | 1 day | 1.34 | | 0.68 | 0.16 | 0.64 |
| Metachromatic leukodystrophy | M | 2 years | 1 year | 0.68 | | 0.89 | 0.11 | 0.62 |
| B-12 defect and homocystinuria (*16*) | M | 2 months | 4 years | 0.74 | | 0.92 | 0.20 | 0.76 |
| $G_{M2}$ gangliosidosis, type AB (*17*) | M | 4 years | 6 years | 0.33 | | 1.27 | 0.14 | 0.61 |
| Hallervorden-Spatz | F | 24 years | 5 years | 0.44 | | 0.33 | 0.14 | 0.49 |
| Infantile Gaucher | M | 2 years | 11 years | 0.18 | | 0.65 | 0.13 | 0.53 |

cific activity, 236 count $min^{-1}$ $nmole^{-1}$) and 0.3 to 0.6 mg of protein per assay in 0.1*M* citric acid–phosphate buffer, *p*H 5.0.

β-Galactosidase was assayed by incubating homogenate for 15 minutes in 0.05*M* citric acid–phosphate buffer, *p*H 4.0, containing 5 m*M* *p*-nitrophenyl-β-D-galactopyranoside and 0.25*M* sodium chloride, with 0.2 to 0.3 mg of protein. β-Hexosaminidase was assayed by incubating as described above for 30 minutes in 0.05*M* sodium acetate buffer, *p*H 5.0, containing 7.5 m*M* *p*-nitrophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside, with 0.02 to 0.03 mg of protein. Except for the assay of β-galactosidase in the cerebellum, both reactions were terminated with 1.8 ml of 0.2*M* glycine–0.3*M* sodium carbonate, *p*H 9.9, and liberated *p*-nitrophenol was determined by its absorbancy at 400 nm. In the assay for cerebellum β-galactosidase, the reaction was terminated with 0.8 ml of cold 5 percent trichloroacetic acid; the mixture was then centrifuged (2500*g* for 5 minutes), an 0.5-ml portion of the supernatant was added to 0.5 ml of 0.2*M* glycine–0.3*M* sodium carbonate, and the *p*-nitrophenol was determined as above. Acid phosphatase was assayed for 30 minutes in 0.25*M* sodium acetate buffer, *p*H 5.0, containing 0.05*M* β-glycerophosphate, with 0.5 to 0.75 mg of protein, and the reaction was terminated with 1.3 ml of 10 percent trichloroacetic acid. The mixture was chilled and centrifuged (2500*g* for 5 minutes) and the liberated inorganic phosphate was determined on 1.0-ml portions of the supernatant (*10*). These three assays were performed at 37°C in a final volume of 200 μl; the homogenate used was treated with Triton X-100 to give a final concentration of 0.2 percent. Neuraminidase was assayed according to Kolodny *et al.* (*11*) and Cumar *et al.* (*12*), with the use of a disialosyl ganglioside ($G_{D1b}$) specifically labeled (*13*) with tritium in the *N*-acetylneuraminyl moiety (specific activity, 302 count $min^{-1}$ per nanomole of sialic acid) and 0.6 to 1.2 mg of protein per assay. Assays were performed with a precision greater than ± 6 percent and are reported as the average of two or more determinations.

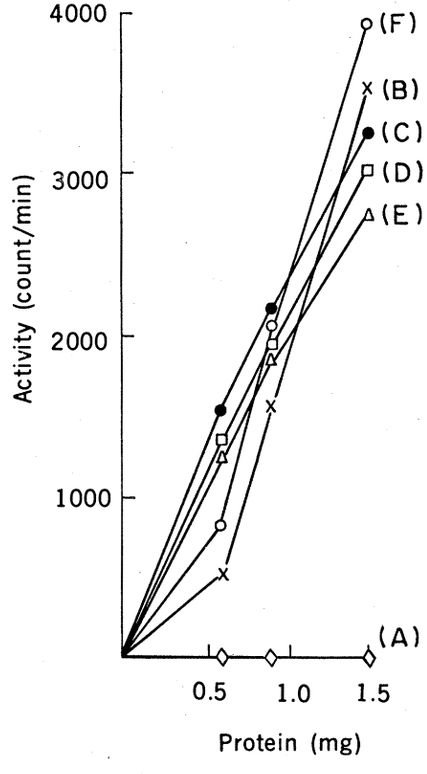


Fig. 1. Ceramidase activity in homogenates of kidney from the patient with Farber's disease and from a control patient (biliary cirrhosis), and in mixtures of homogenates. Where two types of homogenate were present in the same assay, protein values of the varying amounts of untreated control homogenate are designated by the abscissa, while constant quantities of the other types of homogenate were added as specified: (A) Farber's disease homogenate; (B) control homogenate; (C) control homogenate plus 1.5 mg of untreated homogenate from Farber's disease; (D) control homogenate plus 1.5 mg of boiled Farber's disease homogenate; (E) control homogenate plus 1.5 mg of boiled control homogenate; (F) control homogenate plus 1.5 mg of bovine serum albumin (Schwartz-Mann) in 0.25*M* sucrose–1 m*M* ethylenediaminetetraacetic acid.

No ceramidase activity was detected in the kidney or cerebellum of our patient with Farber's disease (Table 1). Storage of tissues at −60°C for periods of up to 11 years has no marked effect on the activities of the control enzymes; the reason for lowered ceramidase activity in the case of infantile Gaucher tissue is not clear. In addition, the levels of glucocerebrosidase and galactocerebrosidase were determined in two control kidneys and were found to be, respectively, 10.2 and 0.81 nmole per milligram of protein per hour in biliary cirrhosis and 14.8 and 1.25 nmole per milligram of protein per

hour in the case of coarctation of aorta; in kidney from Farber's tissue these two enzyme activities were similar to control levels, 13.6 and 0.93 nmole per milligram of protein per hour. In preliminary studies, which we reported previously, the activity of ceramidase in the liver of this same patient had appeared to be normal (*3*). However, these assays were performed at *p*H 7.0, which was later shown to be far from the optimum *p*H, and the assay included a technique that failed to separate the fatty acids completely from the remaining uncleaved substrate.

A specific inhibitor of ceramidase is absent in Farber's tissue (Fig. 1). In addition, it can be calculated from the data in (*3*) that dilution of the substrate or inhibition of enzyme activity by tissue ceramide could not be responsible for the observed low ceramidase activity in the case of Farber's disease, since the assay samples of Farber's tissue contained, at most, 6 percent of the amount of ceramide added as substrate. Also, there is a stimulation of activity caused by factors present in the added homogenate, whether it had been boiled or not, and by added bovine serum albumin itself, at lower protein concentrations.

The demonstrated accumulation of ceramide in two patients with Farber's disease, coupled with the deficiency of ceramidase in our patient, suggests that a genetically determined defect in ceramide degradation forms the biochemical basis of this disorder. Ceramide levels, however, have been reported in only two patients with this disease (*3, 4*), and to our knowledge assays of ceramidase activity have been carried out only in the patient reported here. A block in ceramide degradation could, secondarily, lead to the ganglioside and glycolipid accumulation which has been reported in this patient and in others (*1, 3, 14*). There is still no explanation for the glycosaminoglycan accumulation reported in two patients with Farber's disease (*15*). Yavin and Gatt in their studies with rat brain concluded that the same enzyme catalyzed the synthesis and the degradation of ceramide (*7*).

We have observed that ceramide synthetase of human kidney—assayed by the method of Yavin and Gatt (*7*) at *p*H 4.5, with 1.03 m*M* sphingosine and 4.13 m*M* [1-$^{14}$C]oleic acid as substrates—has a specific activity of 1.44 to 2.58 nmole of ceramide synthesized per milligram of protein per hour in six patients without Farber's disease, whereas in the kidney of the patient with Farber's disease the specific activity was found to be less than 0.02 nmole per milligram of protein per hour. This finding raises anew the question of what pathways are utilized for biosynthesis of ceramide (*18*).

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## Adrenergic and Cholinergic Innervation of the Hamster Harderian Gland

Abstract. *Examination of Harderian glands of adult male and female golden hamsters by appropriate histochemical techniques reveals that adrenergic nerves are associated only with the blood vessels. Acetylcholinesterase-positive fibers are present in the connective tissue surrounding the gland, along the ducts, and among the acini.*

The gland of Harder, present in the orbits of many vertebrates that possess a nictitating membrane, is a compound tubuloalveolar gland that is separate from, but drains into, the bulbar surface of the nictitating membrane (*1*). In addition to numerous lipid droplets, this gland in several species of mammals is rich in porphyrins (*2*). The function of this gland is uncertain, but several investigations suggest that in rats and hamsters it may be a component of a system involving light, the pineal gland, and the reproductive organs. Wetterberg and his colleagues reported a regulatory effect of the Harderian gland on pineal serotonin and hydroxyindole-*O*-methyltransferase (HIOMT) in blinded suckling rats (*3*). Data from our studies of pineal–Harderian gland interaction in the hamster indicate that the pineal, which has a pronounced antigonadotropic action in the light-deprived hamster (*4*), has a regulatory effect on cell type and porphyrin metabolism in the Harderian glands of blinded male and female animals (*5, 6*). However, in an earlier study similar to ours, Hoffman (*7*) concluded that blinding alters Harderian gland metabolism but does so by a pathway exclusive of the pineal gland. Reiter and Klein (*8*) found that Harderian glands of adult female rats regress after exposure to constant light for 9½ weeks and that removal of the Harderian glands from rats on a cyclic lighting regimen leads to slight uterine enlargement; however, they observed no relation between the Harderian gland and pineal HIOMT or *N*-acetyltransferase activity. Since much is still unknown about the Harderian gland, knowledge of its innervation should be useful in determining how these various interactions may be mediated.

Adult golden hamsters of both sexes (100 to 120 g) were decapitated after