Studies of the pathogenesis of Gaucher's disease: tissue distribution and biliary excretion of [^{14}C]L-glucosylceramide in rats

Toshiharu Tokoro,* Andrew E. Gal,* Linda L. Gallo,† and Roscoe O. Brady[1,*]

Developmental and Metabolic Neurology Branch,* National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892, and Department of Biochemistry,† George Washington University Medical Center, Washington, DC 20037

Abstract The time course of the clearance from the blood and the tissue localization of [^{14}C]L-glucosylceramide, a nonmetabolizable enantiomorph of D-glucosylceramide that accumulates in Gaucher's disease, has been determined. ^{14}C-labeled L-glucosylceramide injected intravenously in the form of micelles or liposomes is rapidly removed from the circulation. Most of this lipid is taken up by the liver where it is found in both hepatocytes and nonparenchymal cells. This sphingolipid analog is promptly cleared from hepatocytes and a significant portion is recovered in the bile. The clearance of [^{14}C]L-glucosylceramide from Kupffer cells is greatly prolonged in comparison with its brief residence in hepatocytes. These findings have significant implications regarding the pathogenesis and treatment of Gaucher's disease.—**Tokoro, T., A. E. Gal, L. L. Gallo, and R. O. Brady.** Studies of the pathogenesis of Gaucher's disease: tissue distribution and biliary excretion of [^{14}C]L-glucosylceramide in rats. *J. Lipid Res.* 1987. **28:** 968–972.

Supplementary key words glucocerebroside • liposomes • Kupffer cells • hepatocytes

In patients with Gaucher's disease, glucocerebroside-storing cells (Gaucher cells) in organs and tissues outside of the central nervous system are derived from elements of the monocyte/macrophage system (reticuloendothelial cells) such as Kupffer cells in the liver (1). It is well established that there is little, if any, accumulation of glucocerebroside in liver parenchymal cells (hepatocytes). Evidence suggesting the biliary excretion of glucocerebroside was obtained several years ago (2). In order to document this pathway of glucocerebroside excretion and to try to determine whether this lipid was transferred from Kupffer cells to hepatocytes and eventually excreted via the bile, we examined the organ and cellular disposition and turnover in rats of [^{14}C]L-glucosylceramide, a nonmetabolizable enantiomorph of natural D-glucosylceramide (glucocerebroside).

MATERIALS AND METHODS

Lipids

[^{14}C]L-glucosylceramide with a specific activity of 1.17 mCi/mmol was synthesized as described by Gal et al. (3). Egg-Yolk PtdCho, DCP, PtdSer and cholesterol were purchased from Sigma.

Preparation of micelles

Ten parts of PtdSer were mixed with one part of [^{14}C]L-glucosylceramide. Aliquots of 2 mg of the mixtures were suspended in 3 ml of phosphate-buffered saline solution (pH 7.4) and sonicated for 10 min at room temperature with a Heat System Cell Sonifier at a power setting of 4. The suspensions were heated at 50°C for 10 min and sonicated for a second 10-min period.

Preparation of liposomes

Two species of MLV were prepared essentially by the method of Zborowski, Roerdink, and Scherphof (4). The first contained PtdCho, cholesterol, DCP, and [^{14}C]L-glucosylceramide in a molar ratio of 2:2:1:0.15, and the second contained PtdCho, cholesterol, DCP, PtdSer, and [^{14}C]L-glucosylceramide in a molar ratio of 2:2:1:0.6:0.15, respectively. The lipids were dissolved in a solution of chloroform–methanol 2:1 (v/v) and the solvent was evaporated in a rotary evaporator under vacuum to form a

Abbreviations: PtdCho, phosphatidylcholine; PtdSer, phosphatidylserine; DCP, dicetylphosphate; MLV, multilamellar vesicles; HEPES, 4-(2-hydroxyethyl-l-piperazineethanesulfonic acid; HBSS, Hanks' balanced salt solution; PC, parenchymal cells; NPC, nonparenchymal cells.

[1]To whom correspondence should be addressed at: Building 10, Room 3D04, National Institutes of Health, Bethesda, MD 20892.

film which was subsequently dried overnight over anhydrous $CaSO_4$ in a desiccator. MLV were prepared for intravenous injection by adding 0.5 ml of a solution of 0.15 M NaCl, 5 mM Tris-HCl, pH 7.4 (NaCl-Tris buffer solution) per mg of lipid with vigorous mixing for 15 min at room temperature under N_2. The suspension was centrifuged at 100,000 *g* for 10 min. The pelleted material (40 mg of lipid) was resuspended in 7 ml of the same buffer solution and kept at 4°C until use.

Administration of [^{14}C]L-glucosylceramide to experimental animals

Micelles and MLV suspended in the NaCl-Tris buffer solution were injected intravenously into the tail veins of 200-to 300-g male Sprague-Dawley rats that had been fasted for 24 hr. Each rat received 1.2 nmol of [^{14}C]L-glucosylceramide per g of body weight. Rats were killed at various time intervals by the intraperitoneal injection of 60 mg of sodium pentobarbital per kg of body weight. Aliquots of 0.1 g of liver, spleen, kidney, and lung tissue, 1×10^7 PC (primarily hepatocytes), and $2\text{-}10 \times 10^6$ NPC were extracted with 8 ml of a solution of chloroform-methanol 2:1 (v/v). Two ml of water was added to the mixtures and the glycolipids and radioactivity in the lower phase were analyzed as described previously (5).

Isolation of PC and NPC from the liver

PC and NPC were prepared by minor modifications of the method of Berry and Friend (6). Livers were perfused with HBSS (pH 7.4) without Ca^{2+} that had been gassed with 95% O_2/5% CO_2 for 10 min at 37°C. After clearing the blood from the organ, the same buffered solution containing 90 mg of collagenase (Type II, Worthington Enzymes) was perfused through the liver for 30 min at a flow rate of 15 ml per min under oxygenation. Following perfusion, the liver was minced in 100 ml of oxygenated HBSS with Ca^{2+} at pH 7.4. The cell suspension was filtered several times through 0.5×0.3 mm mesh nylon cloth and then quickly centrifuged at 50 *g* for 2 min. The sediment which was enriched in PC was washed three times with oxygenated HBSS with Ca^{2+} and the cells were counted with a hemacytometer. NPC in the supernatant suspension were further purified by layering 25 ml of the suspension over 12 ml of a solution containing 16% (w/v) Metrizamide, 10 mM HEPES buffer, 6.7 mM KCl, and 1.2 mM $CaCl_2$ at pH 7.6. Following centrifugation at 4°C for 45 min at 3000 *g*, the NPC were recovered at the Metrizamide-buffer interface. The isolated cells were washed three times with 50 ml of Krebs-Henseleit buffer, resuspended in this buffer solution, and counted. Kupffer cells and endothelial cells were distinguished by their difference in peroxidase content as described by Wisse et al (7). Aliquots of 0.1 g of liver tissue, 1×10^7 PC and $2\text{-}10 \times 10^6$ NPC were used to determine the distribution of accumulated [^{14}C]L-glucosylceramide. Corrections for losses during the isolation of various types of cells were made on the basis of published data regarding the number of cells in liver tissue; (100×10^6 PC, 54×10^6 NPC, 42×10^6 endothelial cells, 12×10^6 KC)per gram of tissue (8).

Collection of bile

Rats were fasted overnight and anesthetized with pentobarbital. The common bile duct was cannulated with 0.28-mm diameter polyethylene tubing essentially as described by Borja, Vahouny, and Treadwell (9) and a 1.4-mm polyethylene intragastric tube was inserted through the abdominal wall. Rats were placed in a modified Bollman cage and infused continuously at a rate of 50 ml per day with a solution of 5% glucose and 0.9% NaCl. Rats were also provided a continuous supply of this solution ad libitum. Bile was collected continuously on ice at 24-hr intervals over a period of 8 days.

Tissue culture of isolated liver cells

NPC obtained from rat liver were seeded at a density of 5×10^5 cells per cm^2 in a T25 flask that had been precoated with fibronectin (10). The cells were cultured in RPMI 1640 medium (Flow Laboratories) supplemented with 20% heat-inactivated fetal calf serum for 4 days (11). The amount of [^{14}C]L-glucosylceramide in NPC and in the medium was determined following extraction and partitioning with chloroform-methanol-water (12).

RESULTS

Micelles containing [^{14}C]L-glucosylceramide are rapidly cleared from the blood and are chiefly taken up by PC in the liver **(Table 1).** Negatively charged MLV containing [^{14}C]L-glucosylceramide are also cleared rapidly from the blood and enrichment of MLV with PtdSer accelerated this process **(Fig. 1).** PtdSer has been shown to increase the uptake of MLV by NPC (13, 14). These cells are the principal elements involved in the catabolism of sphingolipid components of senescent erythrocytes and leukocytes from which much of the accumulating glucocerebroside in Gaucher's disease is derived (1, 15). Therefore, except as noted, PtdSer-enriched MLV were used in the present experiments in order to mimic the situation in the human condition as closely as possible.

Most of the [^{14}C]L-glucosylceramide injected in the form of MLV becomes localized in the liver **(Table 2).** This tissue distribution did not change appreciably over the next 8 hr. However, during the 8 days postinfusion, considerable [^{14}C]L-glucosylceramide appeared in the bile **(Table 3).** The amount of radioactivity excreted in the bile can only partially account for the decrease in PC (Table 3 and **Table 4).** We speculate that some of the unaccounted [^{14}C]L-glucosylceramide may reside in

TABLE 1. Hepatocellular distribution of [^{14}C]L-glucosylceramide 1 hr following injection

Localization	Form Injected: Micelles	Form Injected: MLV with PtdSer
	percent of ^{14}C injected	
Whole liver	64 (65.1, 62.8)[a]	63 (60.2; 65.7)
PC	54 (50.4, 57.8)	24 (20.0; 27.4)
NPC	5.6 (3.2, 8.1)	40 (42.2; 36.8)
Blood	1.5 (1.0, 2.0)	0.9 (0.72; 1.12)

[a]The values indicate the percentage distribution of [^{14}C]L-glucosylceramide 1 hr after injection. The first number is the mean of the two experimental data points which appear in parentheses. Abbreviations: MLV, multilamellar vesicles; PtdSer, phosphatidylserine; PC, parenchymal cells; NPC, nonparenchymal cells.

TABLE 2. Distribution of [^{14}C]L-glucosylceramide following intravenous injection of MLV enriched with PtdSer

Organ	Time (hr): 1	2	4	8
	percent of ^{14}C injected			
Whole liver[a]	55 (54, 56)	56 (55, 57)	46 (43, 49)	53 (46, 59)
Spleen[b]	8.9	9.7	8.3	8.9
Lung[b]	1.2	1.8	0.74	0.62
Kidney[b]	0.09	0.06	0.04	0.09
Blood[b]	0.9	0.32	0.38	0.31

[a]The values in the table indicate the percent of [^{14}C]L-glucosylceramide incorporated at various time intervals. The first number is the mean of the two experimental data points in parentheses.
[b]These values were determined in a single experiment. Abbreviations: MLV, multilamellar vesicles; PtdSer, phosphatidylserine.

capillary bile ducts. Much of the biliary radioactivity appears to have arisen from [^{14}C]L-glucosylceramide in PC since the quantity of this lipid in these cells in the liver decreased much more rapidly than that in NPC (Table 4). Although we searched in more than a dozen experiments at different time points, we found no evidence of a significant transfer of [^{14}C]L-glucosylceramide from NPC (sinusoidal endothelial cells and Kupffer cells) to PC (hepatocytes). This deduction is supported by the data presented in Table 4 that indicated little or no reduction of [^{14}C]L-glucosylceramide in NPC over a period of 9 days, while there was a marked decrease in the amount of radioactivity in PC. An additional experiment along this line was performed by loading NPC with [^{14}C]L-glucosylceramide in vivo and examining the release of labeled lipid from these cells after their being transferred to tissue culture. No evidence was obtained indicating release of radioactivity from these cells into the culture medium **(Table 5).** Such observations are completely consistent with the accumulation of glucocerebroside, primarily in Kupffer cells, in patients with Gaucher's disease.

Several additional observations of potential importance were made in the course of these investigations. We looked for evidence of [^{14}C]L-glucosylceramide absorption from the intestines into the blood after oral administration of [^{14}C]L-glucosylceramide. The amount of radioactivity

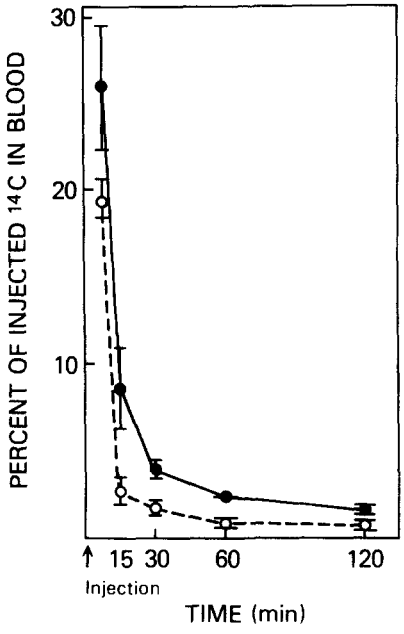

Fig. 1. Kinetics of the clearance of [^{14}C]L-glucosylceramide from the circulation of rats. [^{14}C]L-glucosylceramide was injected intravenously in the form of MLV without (closed circles) and with PtdSer (open circles). Each experimental point was determined in two rats. The open and closed circles represent the mean of the two determinations. The upper and lower crosshatches represent the data points. Abbreviations used: PtdSer, phosphatidylserine; MLV, multilamellar vesicles.

TABLE 3. Excretion of [^{14}C]L-glucosylceramide in the bile following intravenous injection

Time after Injection	Form Administered: Micelles	MLV with PtdSer	
days	*percent of injected dose*		
1	1.9 (1.4, 2.3)[a,b]	0.50[c]	
2	1.7 (1.3, 1.8)	0.36	1.3[d]
3	1.4 (0.92, 1.8)	0.39	
4	1.5 (1.1, 1.8)	0.79	1.2
5	1.1 (0.87, 1.4)	0.73	
6	0.80 (0.64, 0.96)	0.63	1.8
7	0.69 (0.38, 0.99)	0.54	
8	0.54 (0.34, 0.74)	0.50	1.6
Total	9.63	4.44	5.9

[a]The values indicate the percent of [^{14}C]L-glucosylceramide excreted into the bile.
[b]Two rats received injections in the form of micelles and bile samples were collected each day.
[c]One rat was given MLV with PtdSer and samples were collected each day.
[d]One rat was given MLV with PtdSer and samples were collected every 2 days. These values represent the amount excreted into the bile over 2-day periods. Abbreviations used: MLV, multilamellar vesicles; PtdSer, phosphatidylserine.

TABLE 4. Hepatic content and cellular distribution of [^{14}C]L-glucosylceramide after injection of MLV enriched with PtdSer

	Time after Injection						
	1 hr	2 hr	3 hr	4 hr	2 days	9 days	40 days
	nmol per g of liver						
Whole liver	16.2[a]	15.6	14.7	12.9	10.5	9.3	1.1
PC	6.0	6.3	3.5	2.3	1.8	1.0	0.10
NPC	11.4	10.9	11.2	12.1	12.5	9.2	0.66

[a]Data for each time point were obtained from individual experimental animals. Abbreviations used: MLV, multilamellar vesicles; PtdSer, phosphatidylserine; PC, parenchymal cells; NPC, nonparenchymal cells.

recovered in the feces approximated 97% of that given and there was no detectable [^{14}C]L-glucosylceramide in the liver, spleen, or on repeated sampling of the blood. This observation is not surprising since such lipids may have to be at least partially hydrolyzed before their products can reach the circulation (16). In this regard, it has been repeatedly shown that the small intestine is rich in sphingoglycolipid hydrolases (17, 18) and that unhydrolyzed sphingolipid accumulates in this tissue when a specific catabolic enzyme is lacking (19).

DISCUSSION

The present experiments provide considerable insight into the disposition of glucocerebroside and have important implications concerning the pathogenesis and therapy of Gaucher's disease. One of the major pathogenetic aspects of this disorder is the fact that glucocerebroside accumulates in the liver exclusively in Kupffer cells; hepatocytes appear to be spared from this burden (15). One of the reasons for this cellular specialization was suspected to be due to excretion of glucocerebroside via the bile (2). This excretory route was confirmed in the present experiments. The validity of experiments concerning the disposition of [^{14}C]L-glucosylceramide in comparison with the natural D-enantiomorph rests on identical physical properties of the two compounds (20) and the fact that hepatic [^{14}C]L-glucosylceramide was recovered in inclusion bodies with a density of 1.08 g/ml (2) which is the density reported for D-glucosylceramide-rich deposits found in Gaucher's disease (21).

TABLE 5. Retention of [^{14}C]L-glucosylceramide within NPC in tissue culture

Days in Culture	dpm per mg Cell Protein
1	9550 (9380, 9720)[a]
2	9040 (8730, 9350)
3	8430 (7930, 8930)
4	10380 (9560, 11200)

Rats were injected with PtdSer-enriched MLV containing [^{14}C]L-glucosylceramide and the animals were killed 1 hr later. The liver was perfused and NPC were isolated and placed in tissue culture as described in the Methods. Data represent values from two separate experiments.
[a]The first number is the mean of the two experimental data points in parentheses. Abbreviations used: NPC, nonparenchymal cells; PtdSer, phosphatidylserine; MLV, multilamellar vesicles.

Glucocerebroside does not appear to be mobilized for biliary excretion by a readily detectable transfer of this lipid from NPC to PC. This situation appears to be in contrast with evidence indicating that certain phospholipids such as PtdCho may be transferred from Kupffer cells to hepatocytes (22, 23).

The amount of glucocerebroside that is excreted by the bile may be a quantitatively minor portion of the total daily body burden of this lipid and may arise in part from turnover of endogenous sphingoglycolipids in PC. The comparatively high level of glucocerebrosidase activity in these cells (24) suggests that they are involved in glucocerebroside metabolism to some extent under normal conditions. It is interesting to note that [^{14}C]L-glucosylceramide eventually appears to be cleared from NPC (Table 4). The precise pathway involved in the slow removal of this lipid from NPC is not apparent from these investigations.

A number of practical implications can be derived from this investigation. The lack of rapid mobilization of glucocerebroside from NPC is of fundamental importance concerning the pathogenesis of Gaucher's disease (25). Targeting exogenous glucocerebrosidase to NPC in patients with Gaucher's disease (26–28) assumes an even larger potential importance for enzyme replacement therapy since glucocerebroside is not readily released from these cells and its clearance from these sites seems likely to be required for clinical benefit.

The authors wish to express their gratitude to Dr. Peter G. Pentchev for his helpful suggestions.

Manuscript received 9 October 1986 and in revised form 24 February 1987.

REFERENCES

1. Kattlove, H. W., J. C. Williams, E. Gaynor, M. Spivack, R. M. Bradley, and R. O. Brady. 1969. Gaucher cells in chronic myelogenous leukemia: an acquired abnormality. *Blood.* **33:** 279–390.
2. Pentchev, P. G., A. E. Gal, R. Wong, S. Morrone, B. Neumeyer, J. Massey, R. Kanter, A. Sawitsky, and R. O. Brady. 1981. Biliary excretion of glycolipid in induced or inherited glucosylceramide lipidosis. *Biochim. Biophys. Acta.* **665:** 615–618.
3. Gal, A. E., P. G. Pentchev, J. M. Massey, and R. O. Brady. 1979. L-glucosylceramide: synthesis, properties and resistance to catabolism by glucocerebrosidase in vitro. *Proc. Natl. Acad. Sci. USA.* **76:** 3083–3086.
4. Zborowski, J., F. Roerdink, and G. Scherphof. 1977. Leakage of sucrose from phosphatidylcholine liposomes in-

duced by interaction with serum albumin. *Biochim. Biophys. Acta.* **497:** 182–191.
5. Pentchev, P. G., A. E. Gal, A. D. Boothe, F. Omodeo-Sale, J. Fouks, B. A. Neumeyer, J. M. Quirk, G. Dawson, and R. O. Brady. 1980. A lysosomal storage disorder in mice characterized by a dual deficiency of sphingomyelinase and glucocerebrosidase. *Biochim. Biophys. Acta.* **619:** 669–679.
6. Berry, M. N., and D. S. Friend. 1969. High-yield preparation of isolated rat liver parenchymal cells. J. Cell Biol. **43:** 506–520.
7. Wisse, E., F. Roels, D. dePrest, J. Van der Meuler, J. Emeis, and W. Th. Daems. 1973. Peroxidatic reaction of Kupffer and parenchymal cells. *In* Electronmicroscopy and Cytochemistry. E. Wisse, W. Th. Daems, I. Molenaar and P. van Duijin, editors. North-Holland Publishing Co., Amsterdam. 119–126.
8. Knook, D. L., and E. C. Sleyster. 1977. Preparation and characterization of Kupffer cells from rat and mouse liver. *In* Kupffer Cells and Other Liver Cells. E. Wisse and D. L. Knook, editors. North-Holland Publishing Co., Amsterdam. 273–288.
9. Borja, C. R., G. V. Vahouny, and C. R. Treadwell. 1964. Role of bile and pancreatic juice in cholesterol absorption and esterification. *Am. J. Physiol.* **206:** 223–228.
10. Johansson, S., and M. J. Hook. 1984. Substrate adhesion of rat hepatocytes: on the mechanism of attachment to fibronectin. *J. Cell Biol.* **98:** 810–817.
11. Smedsrod, B., and H. Pertoft. 1985. Preparation of pure hepatocytes and reticuloendothelial cells in high yield from a single rat liver by means of Percoll centrifugation and selective adherence. *J. Leukocyte Biol.* **38:** 213–230.
12. Folch, J. M., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
13. Fidler, I. J., A. Raz, W. E. Fogler, R. Kirsh, P. Bugelski, and G. Poste. 1980. Design of liposomes to improve delivery of macrophage-augmenting agents to alveolar macrophages. *Cancer Res.* **40:** 4460–4466.
14. Schroit, A. J., J. W. Madsen, and Y. Tanaka. 1985. In vivo recognition and clearance of red blood cells containing phosphatidylserine in their plasma membranes. *J. Biol. Chem.* **260:** 5131–5138.
15. Brady, R. O., and J. A. Barranger. 1983. Glucosylceramide lipidosis: Gaucher's disease. *In* The Metabolic Basis of Inherited Disease. 5th Edition. J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, J. L. Goldstein, and M. S. Brown, editors. McGraw-Hill, New York. 842–856.
16. Gallo, L., and C. R. Treadwell. 1970. Localization of the monoglyceride pathway in subcellular fractions of rat intestinal mucosa. *Arch. Biochem. Biophys.* **141:** 514–621.
17. Brady, R. O., A. E. Gal, J. N. Kanfer, and R. M. Bradley. 1965. The metabolism of cerebrosides. III. Purification and properties of a glucosyl- and galactosylceramide-cleaving enzyme from rat intestinal tissue. *J. Biol. Chem.* **240:** 3766–3770.
18. Brady, R. O., A. E. Gal, R. M. Bradley, and E. Martensson. 1967. The metabolism of ceramidetrihexosides. I. Purification and properties of an enzyme that cleaves the terminal galactose molecule of galactosylgalactosylglucosylceramide. *J. Biol. Chem.* **242:** 1021–1026.
19. Brady, R. O., A. E. Gal, R. M. Bradley, E. Martensson, A. L. Warshaw, and L. Laster. 1967. Enzymatic defect in Fabry's disease, ceramidetrihexosidase deficiency. *N. Engl. J. Med.* **276:** 1163–1167.
20. Gal, A. E., A. L. Weis, J. M. Quirk, and R. O. Brady. 1986. Animal and cellular models of sphingolipid storage disorders of humans. *Chem. Phys. Lipids.* **42:** 199–207.
21. Lee, R. E., S. P. Peters, and R. H. Glew. 1977. Gaucher's disease: clinical, morphologic and pathogenetic considerations. *Pathol. Annu.* **12:** 309–339.
22. Freise, J., W. H. Müller, C. H. Brolsch, and F. W. Schmidt. 1980. In vivo distribution of liposomes between parenchymal and nonparenchymal cells in rat liver. *Biomedicine.* **32:** 118–123.
23. Roerdink, F., J. Dijkstra, G. Hartman, B. Bolscher, and G. Scherphof. 1981. The involvement of parenchymal, Kupffer and endothelial liver cells in the hepatic uptake of injected liposomes. *Biochim. Biophys. Acta.* **677:** 79–89.
24. Barranger, J. A., P. G. Pentchev, F. S. Furbish, C. J. Steer, E. A. Jones, and R. O. Brady. 1978. Studies of lysosomal function. I. Metabolism of some complex lipids by isolated hepatocytes and Kupffer cells. *Biochem. Biophys. Res. Commun.* **83:** 1055–1060.
25. Gery, I., J. S. Zigler, Jr., R. O. Brady, and J. A. Barranger. 1981. Selective effects of glucocerebroside (Gaucher's storage material) on macrophage cultures. *J. Clin. Invest.* **68:** 1182–1189.
26. Steer, C. J., F. S. Furbish, J. A. Barranger, R. O. Brady, and E. A. Jones. 1978. The uptake of agalactoglucocerebrosidase by rat hepatocytes and Kupffer cells. *FEBS Lett.* **91:** 202–205.
27. Furbish, F. S., C. J. Steer, N. L. Krett, and J. A. Barranger. 1981. Uptake and distribution of placental glucocerebrosidase in rat hepatic cells and effects of sequential deglycosylation. *Biochim. Biophys. Acta.* **673:** 425–434.
28. Brady, R. O. 1984. Enzyme replacement in the sphingolipidoses. *In* Molecular Basis of Lysosomal Storage Disorders. J. A. Barranger and R. O. Brady, editors. Academic Press, Orlando. 461–478.