# Activities of serine palmitoyltransferase (3-ketosphinganine synthase) in microsomes from different rat tissues

**Alfred H. Merrill, Jr.,[1] Daniel W. Nixon, and Robert D. Williams**

Department of Biochemistry, the Winship Clinic for Neoplastic Diseases, and the Division of Hematology Oncology, Emory University School of Medicine, Atlanta, GA 30322

**Abstract** Serine palmitoyltransferase [EC 2.3.1.50] catalyzes the first unique reaction of sphingolipid biosynthesis. To determine whether or not different rat tissues are capable of initiating this pathway, its activity was determined for microsomes from rat liver, lung, brain, kidney, intestine, spleen, muscle, heart, pancreas, testes, ovary, and stomach. Serine palmitoyltransferase was found in every tissue, and, when compared to the microsomal glycerol 3-phosphate acyltransferase, the activities correlated directly with their sphingomyelin levels as a percentage of total phospholipids. This suggests that the activities were comparable to expected cellular needs for long-chain bases, if the initial enzymes of glycerolipid and sphingolipid biosynthesis influence the phospholipid composition of cells by determining the relative partitioning of fatty acyl-CoA's toward these two lipid classes. Serine palmitoyltransferase activities were also determined using different fatty acyl-CoA's and were consistently greatest with CoA thioesters of saturated fatty acids with 16 ± 1 carbon atoms. This suggests that the predominance of 18-carbon long-chain bases in vivo is due to the higher activity of this enzyme with palmitoyl-CoA. Together, these findings indicate a role for serine palmitoyltransferase in regulating both the type and amount of long-chain bases found in tissues. — **Merrill, A. H., Jr., D. W. Nixon, and R. D. Williams.** Activities of serine palmitoyltransferase (3-ketosphinganine synthase) in microsomes from different rat tissues. *J. Lipid Res.* 1985. **26:** 617–622.

**Supplementary key words** sphingolipids • sphingosine • phospholipid

Sphingolipids are major constituents of biological membranes and lipoproteins. They influence membrane structure, affect the functioning of enzymes and receptors, and contribute to cell-cell communication, cell-surface antigenicity, and some aspects of nerve impulse transmission (1–3). Sphingolipid biosynthesis is initiated by serine palmitoyltransferase, which catalyzes the condensation of L-serine and palmitoyl-CoA to yield 3-ketosphinganine (4–6). This intermediate is converted to sphinganine by an NADPH-dependent reductase (4–7) and a fatty acid is added to the 2-position in amide linkage (8–10). Much of the resulting dihydroceramide is apparently converted to ceramide (which contains a double bond between carbons 4 and 5 of the backbone moiety called sphingosine) (11) and both are incorporated into complex sphingolipids. Phytosphingosine, the 4-hydroxyl analog of sphinganine, is also synthesized by microsomal enzymes (12).

In addition to sphingosine, sphinganine, and phytosphingosine, smaller amounts of homologous compounds with 16 to 22 carbon atoms exist (13) and this entire group of compounds is commonly referred to as long-chain, or sphingoid, bases. The intestine degrades most of the long-chain bases consumed in the diet (14); hence, it would appear that their major source for mammals is de novo biosynthesis. Nonetheless, except for liver and brain (2–6), it is not known whether mammalian tissues are able to initiate long-chain base synthesis or only obtain these molecules by their uptake as part of the sphingomyelin of lipoproteins (15).

To answer this question, serine palmitoyltransferase activities have been measured in microsomes from different rat tissues. As described herein, all contained this enzyme in levels comparable to the tissue's sphingomyelin content. Furthermore, the relative activities with different fatty acyl-CoA's agreed well with the types of long-chain bases occurring in vivo.

---

Abbreviation: NEM, N-ethylmaleimide. Nomenclature: serine palmitoyltransferase is the name recommended by the IUPAC-IUB Commission on Biochemical Nomenclature (1978) for this activity, but it has also been called 3-ketosphinganine synthase and referred to as the condensing enzyme.

[1]To whom reprint requests should be addressed at the Department of Biochemistry, Emory University School of Medicine, Atlanta, GA 30322.

## EXPERIMENTAL

### Chemicals

Radioactive [G-$^3$H]L-serine was purchased from ICN Radiochemicals and was rountinely purified by chromatography on a column of Dowex 50W-X8 as described previously (6) and diluted with unlabeled L-serine to a specific activity of approximately 25 mCi/mmol. The [2-$^3$H]glycerol was purchased from ICN and used to synthesize glycerol 3-phosphate enzymatically (16). Fatty acyl-CoA's were purchased from Sigma (St. Louis, MO) and PL Biochemicals (Milwaukee, WI). All biochemicals were purchased from Sigma, or from sources cited in previous reports from this laboratory (6, 17, 18).

### Animals

Female and male buffalo rats were obtained from Harlan Industries (Walkerstown, MD) and fed laboratory chow (Purina) ad libitum until 18 hr before experimentation. The animals were maintained in quarters on a 12-hr cycle of light/dark beginning at 7:00 AM and were killed between 8:00 and 10:00 AM.

### Assays

Microsomes were prepared as described by Williams, Wang, and Merrill (6) and assayed within weeks of freezing at −80°C. No significant change in activities was found over at least several months at this temperature.

Serine palmitoyltransferase was assayed by following the incorporation of radiolabel from [$^3$H]serine into chloroform-soluble products (6). Glycerol 3-phosphate acyltransferase was assayed in the presence and absence of N-ethylmaleimide (NEM) using [$^3$H]glycerol 3-phosphate (16). For examination of serine palmitoyltransferase activities with different fatty acyl-CoA thioesters, the alternative substrates were substituted for palmitoyl-CoA in the usual assay mixture (6).

Protein was measured using the method of Bensadoun and Weinstein (19) with bovine serum albumin as the standard. Thin-layer chromatography was conducted using Silica Gel 60 plates (EM Laboratories) developed with $CHCl_3$–$CH_3OH$–2 N $NH_4OH$ 40:10:1. The spots were visualized with $I_2$ vapor and, after most of the color had faded, the plates were cut into strips and counted in 4 ml of Aquasol-2 (New England Nuclear, Boston, MA).

## RESULTS

### Serine palmitoyltransferase activities of microsomes from rat tissues

Microsomes from every rat tissue examined catalyzed the formation of 3-ketosphinganine from [$^3$H]serine and palmitoyl-CoA (**Fig. 1**; chromatographic data for liver and brain are not shown because they have been reported previously in refs. 6 and 17). In most cases, this product accounted for 85 to 95% of the radiolabel observed by thin-layer chromatography of chloroform extracts of the assay mixtures (Fig. 1). In many samples a small fraction of the total radiolabel migrated near the solvent front (a common artifact with 3-ketosphinganine) (6, 20) or coincident with the sphinganine and sphingosine standards. Microsomes from lung, pancreas, and testes produced a compound that migrated slightly above the origin and was probably phosphatidylserine. This can be formed by a serine base-exchange enzyme that requires $Ca^{2+}$ (21). To routinely correct for counts that were not due to compounds formed by serine palmitoyltransferase (including chloroform-soluble decomposition products of [$^3$H]serine), assays were conducted with and without palmitoyl-CoA and cpm in the latter (typically less than 10%) were subtracted from the former.

The specific activities of serine palmitoyltransferase varied among the tissues examined (**Table 1**). Lung microsomes had the highest activity followed in decreasing order by muscle (although SEM of activities measured with this tissue were very large), kidney, spleen, liver, stomach, intestine, brain, ovary, heart, and pancreas. Measurements of total activities using tissue homogenates were not successful, probably due to greater

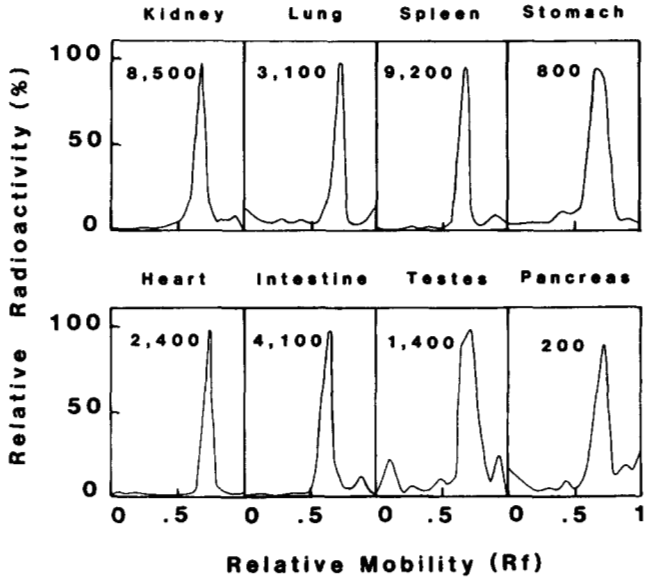


**Fig. 1.** Analysis of the chloroform-soluble products from assays of serine palmitoyltransferase using microsomes from different rat tissues. The samples were prepared and chromatographed on silica gel thin-layer plates as described under Experimental. The cpm in different portions of the chromatograms are expressed relative to the region with the highest counts. The total cpm for each tissue is given inside each panel. For comparison, the $R_f$ of the standards were: ceramide, 0.95; 3-ketosphinganine, 0.70; sphingosine, 0.53; sphinganine, 0.45; and phosphatidylserine, N- and O-palmitoylserine, and ethanolamine, 0.06.

TABLE 1. Activity of serine palmitoyltransferase in microsomes from different rat tissues

| Tissue | Serine Palmitoyltransferase Activity Mean ± SE | N |
|---|---|---|
| | *pmol/min per mg* | |
| Liver | 42.5 ± 3.7 | 14 |
| Lung | 127 ± 14 | 14 |
| Heart | 16.8 ± 2.5 | 13 |
| Brain | 30.1 ± 4.2 | 9 |
| Spleen | 45.6 ± 5.6 | 9 |
| Muscle | 71.7 ± 57.3 | 13 |
| Stomach | 37.5 ± 9.4 | 10 |
| Kidney | 59.3 ± 8.9 | 13 |
| Pancreas | 2.7 ± 0.6 | 3 |
| Intestine | 32.7 ± 17.2 | 9 |
| Testes | 10.0 ± 3.2 | 10 |
| Ovary | 18.0 ± 5.6 | 2 |

Microsomes were isolated from fasted rats and assayed as described in the Experimental section.

TABLE 2. Activity of glycerol 3-phosphate acyltransferase in microsomes from different rat tissues

| Tissue | Glycerol 3-Phosphate Acyltransferase + NEM | – NEM |
|---|---|---|
| | *nmol/min per mg* | |
| Liver | 0.28 ± 0.06 (8) | 1.38 ± 0.26 (9) |
| Lung | 0.09 ± 0.03 (5) | 0.68 ± 0.12 (7) |
| Heart | 0.08 ± 0.04 (7) | 0.74 ± 0.22 (9) |
| Brain | 0.14 ± 0.03 (3) | 1.13 ± 0.29 (6) |
| Spleen | 0.20 ± 0.11 (6) | 0.89 ± 0.23 (9) |
| Stomach | 0.13 ± 0.03 (5) | 0.77 ± 0.13 (6) |
| Kidney | 0.17 ± 0.10 (5) | 1.67 ± 0.44 (9) |
| Pancreas | ND | 0.01 ± 0.01 (3) |
| Muscle | ND | 0.29 ± 0.29 (7) |

Microsomes were isolated from fasted rats and glycerol 3-phosphate acyltransferase was assayed with and without N-ethylmaleimide as described in the Experimental section.

hydrolysis of the fatty acyl-CoA substrate, as has been observed previously with rat liver (6) and brain (17). Therefore, to compare this variation with another microsomal enzyme of lipid biosynthesis, glycerol 3-phosphate acyltransferase was also assayed for many of the tissues.

### Glycerol 3-phosphate acyltransferase activities of microsomes from rat tissues

This enzyme is found in microsomes, mitochondria, and peroxisomes; however, since the microsomal activity it selectively inhibited by NEM (22), contamination of microsomes by these other forms can be estimated by assays with and without this reagent. Nearly all of the activity (78 to 90%) in microsomes from different tissues was NEM-sensitive (**Table 2**); hence, the total activities were used in subsequent computations.

The specific activities were greatest in kidney and liver, and lower in the order: brain, spleen, stomach, heart, lung, muscle, and pancreas (Table 2). The activities were generally similar to other reports using microsomes from some of these tissues (22–24).

### Comparison of serine palmitoyltransferase and glycerol 3-phosphate acyltransferase activities

The ratio of the activities of serine palmitoyltransferase and glycerol 3-phosphate acyltransferase and the percentage of sphingomyelin in total phospholipids were compared for tissues where data for all three were found (the percentages of sphingomyelin were taken from refs. 16, 22, 25, and 26), including results from a previous study of fetal liver and Morris hepatoma 7777 (27). There was a weak correlation between these variables when all tissues were analyzed by linear regression ($r = 0.44$); however, a much more significant relationship ($r = 0.80$, $P < 0.005$) was obtained if only the ratio for muscle was omitted. This omission may be justified by the uncertainty in the mean for the serine palmitoyltransferase activity of muscle (the mean ± SE was 71.7 ± 57.3 for n = 13). The results omitting muscle are shown in **Fig. 2.**

### Activities of serine palmitoyltransferase with different fatty acyl-CoA thioesters

Assays of microsomes from most rat tissues were conducted using saturated fatty acyl-CoA's with alkyl chain lengths from 14 to 18 carbons (**Fig. 3**). For every tissue, the activities were much greater with substrates

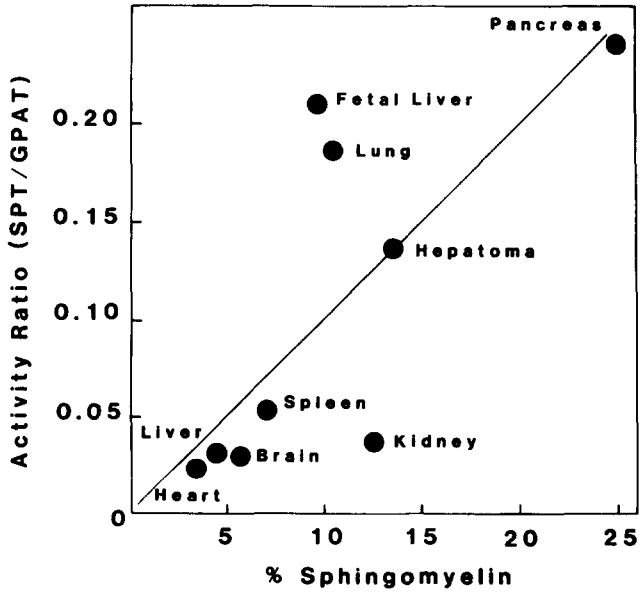


**Fig. 2.** Relationship between the ratio of activities of serine palmitoyltransferase versus glycerol 3-phosphate acyltransferase and the % sphingomyelin for different rat tissues. Activities were obtained by assays conducted as described under Experimental or, for fetal liver and intestine, from references 16 and 22, respectively. The % sphingomyelin was obtained from references 25 and 26.

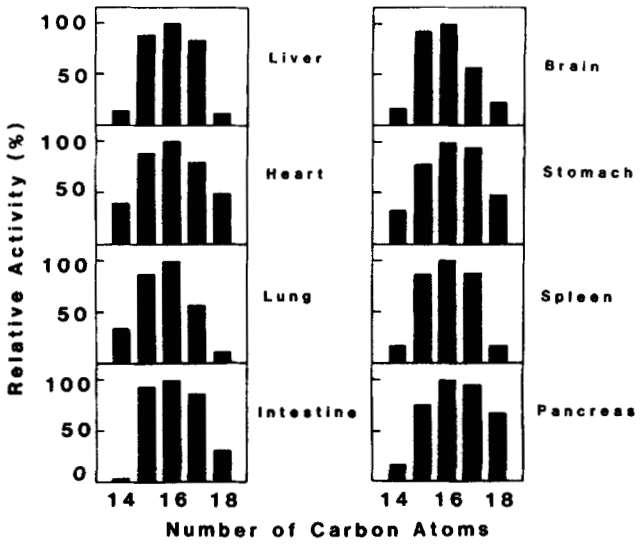


**Fig. 3.** Serine palmitoyltransferase activities with different fatty acyl-CoA thioesters. Assays were conducted as described under Experimental with the CoA thioesters of saturated fatty acids with 14 to 18 carbon atoms. The activities with microsomes from the tissues are expressed as the % relative to palmitoyl-CoA.

having 16 ± 1 carbon atoms than with shorter or longer homologs. Small differences among the tissues (e.g., proportionately greater activities with stearoyl-CoA for pancreatic microsomes) may be artifacts of the low activities in these tissues, the micellar nature of these substrates (28), or the presence of other microsomal enzymes that compete for them as substrates (e.g., thioesterases) (6, 17). Nonetheless, these results demonstrate that, given these substrates in equal amounts, serine palmitoyltransferase of all rat tissues most rapidly produced 18-carbon long-chain bases.

## DISCUSSION

This study established that the major rat tissues contain the enzyme necessary to initiate long-chain base formation. For all, serine palmitoyltransferase activities were lower than those reported (7) for the next step of sphinganine synthesis. Its activities as a percentage of 3-ketosphinganine reductase were: liver (6%), spleen (17%), muscle (14%), lung (29%), brain (7%), and kidney (50%). In addition, when liver (6), brain (29), and yeast (4, 30) microsomes were assayed under conditions where both enzymes function (i.e., by including NADPH), 3-ketosphinganine did not accumulate. We have also found that addition of [$^3$H]- or [$^{14}$C]serine to cells in culture results in rapid appearance of radiolabel in sphinganine without detectable levels of 3-ketosphinganine (data not shown). These observations suggest that serine palmitoyltransferase catalyzes the rate-limiting step of long-chain base biosynthesis.

To estimate whether or not these activities were commensurate with the long-chain base needs of cells, the specific activities and sphingomyelin levels were compared. These were poorly correlated ($r < 0.3$); however, there was a significant relationship between the ratio of serine palmitoyltransferase versus glycerol 3-phosphate acyltransferase activities and the sphingomyelin percentage of most tissues (Fig. 2). A similar correlation between the relative activity of serine palmitoyltransferase and the sphingomyelin percentages of lungs from rats maintained in elevated versus normal levels of oxygen (31) has been reported recently. This correlation may exist because these enzymes share a common pool of fatty acyl-CoA's and cellular concentrations of glycerol 3-phosphate and serine are near the apparent $K_m$ for these substrates

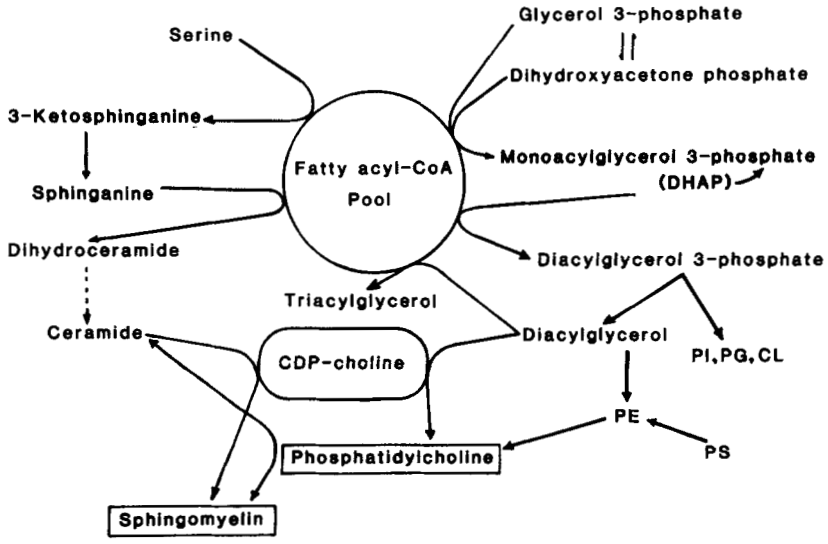


**Fig. 4.** Interrelationships between sphingolipid and glycerolipid biosyntheses.

(6, 22); therefore, they could determine the rates of overall flux through these pathways and the partitioning of fatty acyl-CoA's toward glycerolipid or sphingolipid synthesis (**Fig. 4**). It is noteworthy that sphingolipid and glycerolipid metabolism also overlaps at later steps where ceramides compete with diacylglycerols for the phosphocholine group of CDP-choline (32–34) or phosphatidylcholine (35–37).

We think these findings suggest that all tissues are responsible for synthesizing a major portion of their long-chain bases. It is not known whether the similarities in the relative activities and the sphingomyelin percentages merely reflect a close match between the rate of long-chain base synthesis and cellular needs or constitute biosynthetic control of this pathway. Degradatory enzymes have been thought to be most important to the regulation of sphingolipid metabolism; however, when long-chain base formation is inhibited in vivo by vitamin $B_6$ deficiency (38) or the inhibitors cycloserine (39) or $\alpha$-fluoropalmitic acid (40), the levels of various sphingolipids are affected. The involvement of serine palmitoyltransferase in regulating long-chain base synthesis would be more efficient than regulation solely through degradation, and might protect cells from the toxic effects of these amphipathic molecules (19).

This enzyme probably also helps determine the type of long-chain bases made by cells, since activities are consistently greatest with fatty acyl-CoA's of 16 ± 1 carbon atoms (studies with liver and brain have also shown that unsaturated substrates are used poorly) (6, 17). Since tissues contain primarily palmitoyl- and stearoyl-CoA's, which are present in comparable amounts (41), this apparently explains the predominance of long-chain bases of 18 carbon atoms and the lower amounts of other homologs in most sphingolipids (13, 42), as was earlier suggested by Snell, Di Mari, and Brady from studies of the yeast enzyme. Because other fatty acyl-CoA's can be used, although less well, large differences in the fatty acyl-CoA pool could affect the long-chain base composition of mammalian sphingolipids, as has been shown with yeast (4, 43).

This work was supported by grants from the Georgia Affiliate of the American Heart Association (A.H.M.), The Emory University Research Committee (A.H.M.), and a State of Georgia Cancer-Nutrition Contract Grant (D.W.N.). The authors thank Mrs. Marion Little for help in preparing the manuscript, and Drs. Dean Jones, Dave Lambeth, Wendy Innis, and Vicky Stevens for helpful suggestions.

*Manuscript received 24 October 1984.*

## REFERENCES

1. Barenholtz, Y., and T. E. Thompson. 1980. Sphingomyelins in bilayers and biological membranes. *Biochim. Biophys. Acta.* **604:** 129–158.
2. Barenholtz, Y., and S. Gatt. 1982. Sphingomyelin: metabolism, chemical synthesis, chemical and physical properties. *In* Phospholipids. J. N. Hawthorne, and G. B. Ansel, editors. Elsevier/North Holland Biomedical Press, Amsterdam. Chapter 4.
3. Hakomori, S. 1981. Glycosphingolipids in cellular interaction, differentiation, and oncogenesis. *Annu. Rev. Biochem.* **50:** 733–784.
4. Snell, E. E., S. J. Di Mari, and R. N. Brady. 1970. Biosynthesis of sphingosine and dihydrosphingosine by cell-free systems from *Hansenula ciferri*. *Chem. Phys. Lipids.* **5:** 116–138.
5. Stoffel, W. 1970. Studies on the biosynthesis and degradation of sphingosine bases. *Chem. Phys. Lipids.* **5:** 139–158.
6. Williams, R. D., E. Wang, and A. H. Merrill, Jr. 1984. Enzymology of long-chain base synthesis by liver: characterization of serine palmitoyltransferase in rat liver microsomes. *Arch. Biochem. Biophys.* **228:** 282–291.
7. Stoffel, W., D. Le Kim, and G. Sticht. 1968. Stereospecificity of the NADPH-dependent reduction reaction of 3-oxodihydrosphingosine (2-amino-1-hydroxyoctadecane-3-one. *Hoppe-Seyler's Z. Physiol. Chem.* **349:** 1637–1644.
8. Sribney, M. 1966. Enzymatic synthesis of ceramide. *Biochim. Biophys. Acta.* **125:** 542–547.
9. Ullman, M. D., and N. S. Radin. 1972. Enzymatic formation of hydroxyceramides with enzymes forming non-hydroxy-ceramides. *Arch. Biochem. Biophys.* **152:** 767–777.
10. Singh, I. 1983. Ceramide synthesis from free fatty acids in rat brain: function of NADPH and substrate specificity. *J. Neurochem.* **40:** 1565–1570.
11. Ong, D. E., and R. N. Brady. 1973. *In vivo* studies on the introduction of the 4-*t*-double bond of the sphingenine moiety of rat brain ceramides. *J. Biol. Chem.* **248:** 3884–3888.
12. Crossman, M. W., and C. B. Hirschberg. 1977. Biosynthesis of phytosphingosine by the rat. *J. Biol. Chem.* **252:**5815–5819.
13. Karlsson, K-A. 1970. On the chemistry and occurrence of sphingolipid long-chain bases. *Chem. Phys. Lipids.* **5:** 6–43.
14. Nilsson, A. 1968. Metabolism of sphingomyelin in the intestinal tract of the rat. *Biochim. Biophys. Acta.* **164:** 575–584.
15. Verdery, R. B., and R. Theolis. 1982. Regulation of sphingomyelin long-chain base synthesis in human fibroblasts. *J. Biol. Chem.* **257:** 1412–1417.
16. Coleman, R. A., and E. B. Haynes. 1983. Selective changes in microsomal enzymes of triacylglycerol and phosphatidylcholine synthesis in fetal and postnatal rat liver. *J. Biol. Chem.* **258:** 450–456.
17. Merrill, A. H., Jr., and R. D. Williams. 1984. Utilization of different fatty acyl-CoA thioesters by serine palmitoyltransferase from rat brain. *J. Lipid Res.* **25:** 185–187.
18. Merrill, A. H., Jr. 1983. Characterization of serine palmitoyltransferase activity in Chinese hamster ovary cells. *Biochim. Biophys. Acta.* **754:** 284–291.
19. Bensadoun, A., and D. Weinstein. 1976. Assay of proteins in the presence of interfering materials. *Anal. Biochem.* **70:** 241–250.
20. Braun, P. E., and E. E. Snell. 1968. Biosynthesis of sphingolipid bases. II. Keto intermediates in synthesis of sphingosine and dihydrosphingosine by cell-free extracts of *Hansenula ciferri*. *J. Biol. Chem.* **243:** 3775–3783.
21. Kanfer, J. N. 1980. The base exchange enzymes and phospholipase D of mammalian tissue. *Can. J. Biochem.* **58:** 1370–1380.
22. Schlossman, D. M., and R. M. Bell. 1977. Microsomal *sn*-glycerol 3-phosphate and dihydroxyacetone phosphate acyl-

transferase activities from liver and other tissues. *Arch. Biochem. Biophys.* **182:** 732–742.
23. Yamada, K., and H. Okuyama, 1979. Possible involvement of acyltransferase systems in the formation of pulmonary surfactant lipid in rat. *Arch. Biochem. Biophys.* **196:** 209–219.
24. Daae, L. N. W. 1973. The acylation of glycerol 3-phosphate in different rat organs and in liver of different species (including man). *Biochim. Biophys. Acta.* **306:** 186–193.
25. Rouser, G., G. J. Nelson, S. Fleischer, and G. Simon. 1968. Lipid composition of animal cell membranes, organelles and organs. *In* Biological Membranes. D. Chapman, editor. Academic Press, New York. Chapter 2.
26. White, D. A. 1973. The phospholipid composition of mammalian tissues. *In* Form and Function of Phospholipids. G. B. Ansell, J. N. Hawthorne, and M. C. Dawson, editors. Elsevier/North Holland Biomedical Press, Amsterdam. Chapter 16.
27. Williams, R. D., D. W. Nixon, and A. H. Merrill, Jr. 1984. Comparison of serine palmitoyltransferase in Morris hepatoma 7777 and rat liver. *Cancer Res.* **44:** 1918–1923.
28. Powell, G. L., J. R. Grothusen, J. K. Zimmerman, C. A. Evans, and W. W. Fish. 1981. A re-examination of some properties of fatty acyl-CoA micelles. *J. Biol. Chem.* **256:** 12740–12747.
29. Braun, P. E., P. Morell, and N. S. Radin. 1970. Synthesis of C18- and C20-dihydrosphingosines, ketosphingosines, and ceramides from microsomal preparations from mouse brain. *J. Biol. Chem.* **245:** 335–341.
30. Stoffel, W., D. Le Kim, and G. Sticht. 1968. Biosynthesis of dihydrosphingosines *in vitro*. *Hoppe-Seyler's Z. Physiol. Chem.* **349:** 664–670.
31. Merrill, A. H., Jr., E. Wang, J. Stevens, and G. W. Brumley. 1984. Activities of the initial enzymes of glycerolipid and sphingolipid synthesis in lung microsomes from rats exposed to air or 85% oxygen. *Biochem. Biophys. Res. Commun.* **119:** 995–1000.
32. Kennedy, E. P., and S. B. Weiss. 1956. The function of cytidine coenzymes in the biosynthesis of phospholipids. *J. Biol. Chem.* **222:** 193–214.
33. Lyman, E. M., C. L. Knowles, and M. Sribney. 1976. Biosynthesis of sphingomyelin in rat liver endoplasmic reticulum. *Can. J. Biochem.* **54:** 358–364.
34. Stoffel, W., and I. Melzner. 1980. Studies *in vitro* on the biosynthesis of ceramide and sphingomyelin. A reevaluation of proposed pathways. *Hoppe-Seyler's Z. Physiol. Chem.* **361:** 755–771.
35. Bernert, J. T., Jr., and M. D. Ullman. 1981. Biosynthesis of sphingomyelin from *erythro*-ceramides and phosphatidylcholine by a microsomal cholinephosphotransferase. *Biochim. Biophys. Acta.* **666:** 99–109.
36. Marggraf, W. D., R. Zertani, F. A. Anderer, and J. N. Kanfer. 1982. The role of endogenous phosphatidylcholine and ceramide in the biosynthesis of sphingomyelin in mouse fibroblasts. *Biochim. Biophys. Acta.* **710:** 314–323.
37. Voelker, D. R., and E. P. Kennedy. 1982. Cellular and enzymic synthesis of sphingomyelin. *Biochemistry.* **21:** 2753–2759.
38. Kurtz, D. J., and J. N. Kanfer. 1973. Composition of myelin lipids and synthesis of 3-keto dihydrosphingosine in the vitamin $B_6$-deficient developing rat. *J. Neurochem.* **20:** 963–968.
39. Sundaram, K. S., and M. Lev. 1984. Inhibition of sphingolipid synthesis by cycloserine *in vitro* and *in vivo*. *J. Neurochem.* **42:** 577–581.
40. Soltysiak, R. M., F. Matsura, D. Bloomer, and C. C. Sweeley. 1984. D,L-α-fluoropalmitic acid inhibits sphingosine base formation and accumulates in membrane lipids of cultured cells. *Biochim. Biophys. Acta.* **792:** 214–226.
41. Olbrich, A., B. Dietl, and F. Lynen. 1981. Determination and characterization of long-chain fatty acyl-CoA thioesters from yeast and mammalian liver. *Anal. Biochem.* **113:** 386–397.
42. Naoi, M., and E. Klenk. 1972. The sphingosine bases of the gangliosides from developing human brain and from brains of amaurotic idiots. *Hoppe-Seyler's Z. Physiol. Chem.* **353:** 1677–1683.
43. Kulmacz, R. J., and G. J. Schroepfer, Jr. 1978. Dramatic alteration of sphingolipid bases of *Hansenula ciferri* by exogenous fatty acid. *Biochem. Biophys. Res. Commun.* **82:** 371–377.