Enzymology of long-chain base synthesis by aorta: induction of serine palmitoyltransferase activity in rabbit aorta during atherogenesis

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Abstract Serine palmitoyltransferase [EC 2.3.1.50] initiates the biosynthesis of sphingolipids by catalyzing the condensation of a fatty acyl-CoA with serine to yield the committed intermediate 3-ketosphinganine or one of its homologues. The presence of serine palmitoyltransferase in aorta was established under optimal assay conditions using microsomes from New Zealand White rabbits. Its activity was dependent on microsomal protein, L-serine, pyridoxal 5'-phosphate, and palmitoyl-CoA. Although several different saturated and unsaturated fatty acyl-CoA thioesters were utilized as substrates, maximal activity was with palmitoyl-CoA, suggesting that this enzyme contributes to the predominance of 18-carbon long-chain bases in sphingolipids from aorta. Rabbits, fed a Purina lab chow supplemented with 2% cholesterol, were used to study serine palmitoyltransferase activity in aorta during experimental atherogenesis. An increase in activity from intimal-medial preparations was detectable prior to prominent lipid accumulation or cellular proliferation. Activity continued to elevate over the 12-week duration of feeding concurrent with the increase in serum cholesterol and in proportion to the development of plaques resulting in a 3.7-fold increase in activity (20.7 ± 2.6 pmol per min per mg microsomal protein ± SE in the cholesterol-fed group versus 5.6 ± 1.9 in the pair-fed controls also matched for age and sex; $P < 0.005$). Thus, the accumulation of sphingomyelin that occurs in aorta during experimental atherogenesis may be related to increased long-chain base synthesis by serine palmitoyltransferase. — **Williams, R. D., D. S. Sgoutas, and G. S. Zaatari.** Enzymology of long-chain base synthesis by aorta: induction of serine palmitoyltransferase activity in rabbit aorta during atherogenesis. *J. Lipid Res.* 1986. **27:** 763–770.

Supplementary key words arteriosclerosis • atherosclerosis • low density lipoproteins • phospholipids • sphingolipids • sphingomyelin

Sphingomyelin accumulates within atheromas formed in man and animal models, constituting as much as 80% of the total phospholipid (1–6). In a series of investigations by Newman, McCandless, and Zilversmit (7) and McCandless and Zilversmit (8), ^{32}P was found to incorporate at an accelerated rate into sphingomyelin in aorta from rabbits maintained on a cholesterol-rich diet, thus demonstrating that increased synthesis in situ was a major contributor to its accumulation (9). Similar results were obtained using human aorta (10). Although several studies have revealed the synthesis of sphingomyelin by the arterial wall, identification and characterization of the enzymes that synthesize the long-chain base precursors[2] of sphingomyelin and all other sphingolipids have not been reported (11–14).

Serine palmitoyltransferase, whose activity appears to influence cellular sphingomyelin concentrations in several tissues, catalyzes the initial reaction of sphingolipid synthesis, i.e., the condensation of palmitoyl-CoA with serine to yield the long-chain base, 3-ketosphinganine (15–17). Other fatty acyl-CoA thioesters are utilized as substrates to generate the corresponding long-chain bases (17, 18). These long-chain bases may be modified or used directly in the synthesis of the ceramide intermediates of sphingolipids (19–22). Herein, we demonstrate the presence of serine palmitoyltransferase in aorta from the New Zealand White rabbit. Its activity was found to increase in rabbits fed a diet supplemented with 2% cholesterol over the duration of feeding, with elevated serum cholesterol and in proportion to the development of plaques. Thus, induction in long-chain base synthesis may contribute to the accumulation of sphingomyelin during experimental atherogenesis.

MATERIALS AND METHODS

Chemicals

The commonly used chemicals including dithiothreitol (DTT), N-2-hydroxy-ethylpiperazine-N'-2-ethanesulfonic

Abbreviations: HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; DTT, dithiothreitol; PLP, pyridoxal 5'-phosphate; LDL, low density lipoprotein. Serine palmitoyltransferase is the name recommended by the IUPAC-IUB Commission on Biochemical Nomenclature, but it has also been referred to as 3-ketosphinganine synthetase, 3-ketodihydrosphingosine synthetase, and the condensing enzyme.

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[2]Several different long-chain bases exist, but the most common are sphinganine, sphingosine, and phytosphingosine (ref. 11).

acid (HEPES), L-serine, pyridoxal 5'-phosphate (PLP), ethylenediaminetetraacetic acid (EDTA), fatty acid-free bovine serum albumin (fraction V, BSA), sphinganine, sphingosine, ceramides, sucrose, sodium carbonate, copper sulfate, sodium tartrate, Folin–Ciocalteau reagent, calf-thymus DNA, 4',6-diamidino-2-phenylindole, and fatty acyl-CoA thioesters were from the Sigma Chemical Company (St. Louis, MO). Palmitoyl-, stearoyl-, and oleoyl-CoA thioesters were purchased from P-L Biochemicals (Milwaukee, WI) and Sigma. The methyl esters were analyzed by gas–liquid chromatography to determine purity (23). The [G-^{3}H] and [3-^{3}H]L-serine, purchased from Amersham (Arlington Heights, IL) and ICN Radiochemicals (Cleveland, OH), respectively, were purified by column chromatography over Dowex 50W-X8, then diluted with unlabeled L-serine to specific activities ranging from 20,000 to 50,000 cpm/nmol as previously described (15). Stock solutions were prepared monthly and refrigerated at 4°C until used. 3-Ketosphinganine was prepared according to Mendershausen and Sweeley (24). Silica Gel 60 thin-layer chromatography plates were from E. Merck (Germany). Diethylether for anesthesia and the scintillation cocktail, Maxifluor, were from J. T. Baker (Phillipsburg, NJ). Organic solvents were HPLC grade and reagents were analytical grade.

Animals

Young, male New Zealand White rabbits (1.5 to 2.5 kg at arrival from Myrtle or H&D Labs, Georgia) were housed in individual wire-bottomed cages at 22°C. After several days, the animals were divided into groups of three to six animals and placed on either a standard Purina laboratory chow containing approximately 61 ppm cholesterol or the same formula supplemented with 2% cholesterol (w/w). Each chow was pelleted. The cholesterol-supplemented diet was prepared by Purina within weeks of its delivery to us, then stored at 4°C. Animals on this diet were allowed to feed ad libitum. Those on the standard chow were pair-fed.

Isolation of microsomes

Rabbits were killed under ether anesthesia 1, 2, 3, 6, 9, and 12 weeks after starting on the diets. Each aorta, from the semilunar valve to the bifurcation, was quickly removed, rinsed with cold phosphate-buffered saline, and blotted dry. The adventitia was stripped away and the remaining tissue (intima and media) was weighed, evaluated for gross morphological characteristics, and sectioned for histological analysis or isolation of microsomes.

Tissue allocated for the isolation of microsomes was placed in a cold 20% (w/v) solution containing 0.25 M sucrose, 50 mM HEPES, pH 7.4, at 4°C, 5 mM EDTA, and 5 mM DTT and minced fine with scissors, then homogenized at 4°C in three 15-sec treatments with a Brinkman Polytron PT 10. The homogenates were centrifuged for 10 min at 22,000 *g* in a Beckman L5-75B ultracentrifuge (50Ti rotor) yielding supernatant fractions that were centrifuged for 40 min at 150,000 g. The resulting microsomal pellets were suspended in 20% glycerol, 5 mM HEPES, pH 7.4, at 25°C, 5 mM EDTA, and 5 mM DTT on a 500 μl/g original tissue basis using a ground-glass hand-held homogenizer and immediately frozen in aliquots at −60°C.

Assay of serine palmitoyltransferase

A selective-partition assay was used to quantitate serine palmitoyltransferase activity by measuring the incorporation of aqueous-soluble [^{3}H]serine into the chloroform-soluble long-chain base, 3-ketosphinganine. Each tube (100 μl final volume) contained 0.1 M HEPES (pH 8.3, at 25°C), 5 mM DTT, 2.5 mM EDTA, 1 mM [^{3}H]L-serine, 50 μM PLP, and various amounts of microsomal protein. The reaction was initiated with the addition of palmitoyl-CoA to an assay concentration of 0.2 mM. Control tubes contained all of the assay constituents except for palmitoyl-CoA which was substituted with water. The tubes were quickly placed in a water-bath maintained at 37°C and allowed to incubate with gentle agitation. The reactions were performed in dim light to protect the PLP. Assays were terminated after 10 min of incubation with

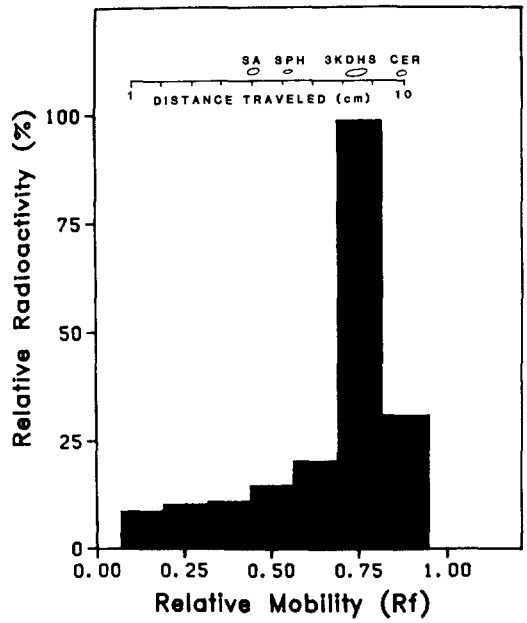

Fig. 1. Thin-layer chromatography of chloroform-soluble products from an assay of serine palmitoyltransferase using microsomes from rabbit aorta. The chloroform extracts were dried under nitrogen, redissolved in chloroform–methanol 1:2 (v/v) and spotted along with standards on a Silica Gel 60 plate. The plate was air-dried, then developed in chloroform–methanol–2 N ammonium hydroxide 40:10:1 (v/v/v). The spots were visualized with brief exposure to iodine. After the iodine evaporated, the plate was divided and cut into sections, then extracted. The radioactivity was determined by scintillation counting. The abbreviations represent relative mobilities: SA, sphinganine (dihydrosphingosine); SPH, sphingosine; 3KDHS, 3-ketosphinganine; CER, ceramide.

the addition of 1.5 ml of chloroform–methanol 1:2 (v/v). Carrier (20 μg of sphinganine) was added to facilitate recovery. One ml of chloroform and 2 ml of water were added, and products were then separated from reactants by extraction using the method of Bligh and Dyer (25). The chloroform phase was washed a total of three times with water to remove unreacted [^{3}H]serine.

Other analyses

Protein was determined by Bensadoun and Weinstein's modification of the Lowry method (26). DNA was measured in cellular homogenates using the fluorescent enhancement of 4',6-diamidine-2-phenylindole (27). Microsomal recoveries were determined using the NADPH cytochrome c marker (28). Total serum cholesterol was measured with an Abbott Bichromatic Analyzer 100 according to the manufacturer's instructions. Calcium was determined by Moorehead and Biggs' method (29). Tissue sections were prepared and stained with hematoxylin and eosin or Sudan IV according to Lillie and Fuller (30).

RESULTS

Validation of the assay using rabbit aorta microsomes

Serine palmitoyltransferase activity was demonstrated in microsomes from aorta using intimal–medial preparations from New Zealand White rabbits. The majority of the chloroform-soluble radioactive products (more than 60%) comigrated with authentic 3-ketosphinganine by thin-layer chromatography (**Fig. 1**). Only minor amounts of radioactivity were found at the origin where phosphatidylethanolamine or phosphatidylserine may have occurred. EDTA was present in the assay buffer to chelate divalent cations such as calcium, thus preventing incorporation of [^{3}H]serine into phosphatidylserine by the microsomal serine-base exchange enzyme (15). Total calcium, measured in microsomes from the aorta of normal and atherosclerotic rabbits, did not exceed 1.0 mM.

TABLE 1. The effect of each assay component on the activity of serine palmitoyltransferase in microsomes from aorta

Assay Component Omitted	Specific Activity	
	pmol/min per mg	%
None	5.8	100
Palmitoyl-CoA	0.4	7
HEPES	5.8	100
EDTA[a]	12.1	208
Dithiothreitol	4.1	71
Pyridoxal 5'-phosphate	4.5	78
Sphinganine carrier[b]	4.8	84

[a] The increase in apparent activity was due to increased serine-base exchange activity.
[b] The carrier was added after the reaction was terminated during the extraction procedure.

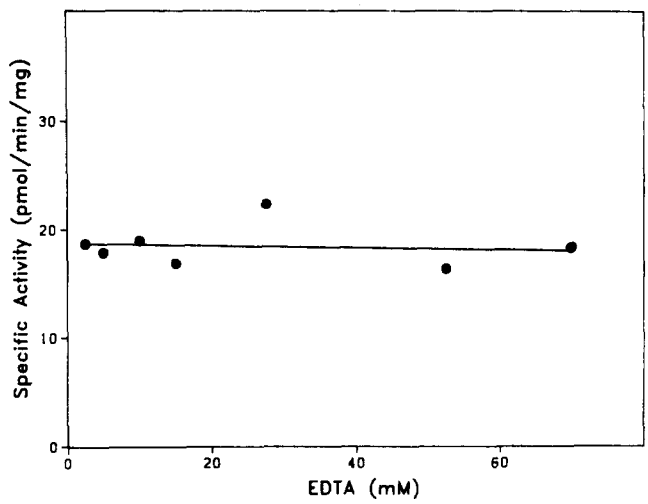

Fig. 2. The effect of increasing concentrations of EDTA on activity measurements using 56 μg of protein from a rabbit fed the cholesterol-supplemented diet for 12 weeks.

Although, in the absence of EDTA from the assay, an increase in apparent activity was observed (**Table 1**), assay concentrations of EDTA from 2.5 mM to 70 mM (**Fig. 2**) had no effect on activity when microsomes from calcified, atherogenic aorta were used. Thus, the accumulation of calcium frequently accompanying atherosclerosis did not affect the activity of serine palmitoyltransferase under the assay conditions routinely utilized, i.e., 2.5 mM EDTA.

Activity was found to be linear over time to 20 min and proportional to the concentration of microsomal protein from 29 to 290 μg, suggesting negligible interference by nonenzymatic reactions or other enzymes presumed to be present, such as the fatty acyl-CoA hydrolase (15). The effect of each assay component on activity was determined by omitting them individually from the assay with other parameters remaining unchanged (Table 1). It was found that activity was dependent on the presence of palmitoyl-CoA. HEPES buffer did not affect activity. The omission of pyridoxal 5'-phosphate resulted in a 22% loss of activity, thus the majority of the enzyme remained holo after a several hundred-fold dilution of the protein. As mentioned, EDTA was effective in reducing base-exchange activity at the routine concentration of 2.5 mM. When EDTA was omitted from the assay and products were separated by TLC, an increase in radioactivity near the origin where phosphatidylserine migrates was observed with no concurrent increase in radioactivity comigrating with 3-ketosphinganine. DTT was found to preserve activity, presumably by maintaining reduced sulfhydryl groups (31), and sphinganine improved the yield when added during the extraction procedure.

One-third (33.3% ± 2.0) of the total serine palmitoyltransferase activity was measured in the microsomes relative to homogenates. NADPH cytochrome c reductase

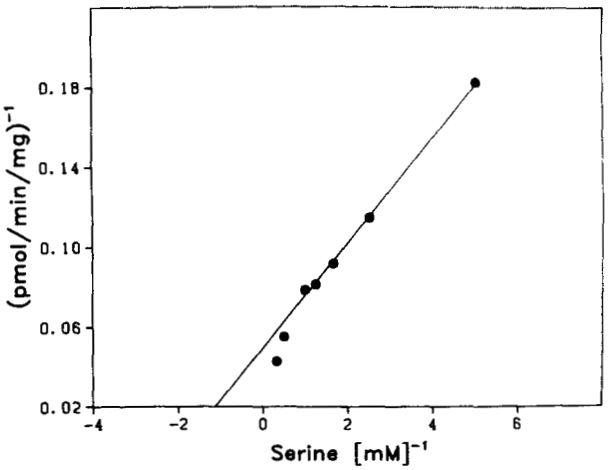

Fig. 3. The dependence of serine palmitoyltransferase activity on the concentration of serine is presented as a double reciprocal plot. The assay was conducted using microsomes from the aorta of a rabbit fed cholesterol for 12 weeks.

was used to measure microsomal recoveries, and the majority of its activity was associated with the microsomal pellets (62.8% ± 0.8 of the total activity) of which 70.1% ± 22.2 was rotenone-insensitive (28).

Characterization of serine palmitoyltransferase

An apparent K_m of 0.90 mM was obtained from a Lineweaver-Burk plot of the activities with varying serine concentrations (**Fig. 3**). This value is similar to that reported for rat liver (15). Since cellular serine concentrations may vary, they could exert an influence on the activity of serine palmitoyltransferase in aorta, e.g., during hyperalimentation, if adequate amounts of fatty acyl-CoA and pyridoxal 5'-phosphate are present (32).

By varying the assay concentration of palmitoyl-CoA, an activity curve with a plateau at 150 to 200 μM was obtained (**Fig. 4**). Lower activities were found at higher substrate concentrations, therefore assays were routinely conducted at 200 μM.

Activity was maximal with palmitoyl-CoA (C16:0), although pentadecanoyl (C15:0) and heptadecanoyl-CoA (C17:0) were nearly as active (**Fig. 5**). The saturated lower and higher chain-length homologues tridecanoyl-, myristoyl-, stearoyl-, and nonadecanoyl-CoA thioesters were poor substrates. Activities were also low with the unsaturated palmitoleoyl-, oleoyl-, and linoleoyl-CoAs (**Table 2**), presumably due to their nonlinear configurations resulting from *cis* double bonds, whereas palmitelaidoyl-CoA, with a *trans* double bond was utilized similarly to palmitoyl-CoA perhaps due to the linear configuration it can assume. The relatively high activities with pentadecanoyl- and heptadecanoyl-CoAs were not due to the contamination of these substrates with palmitoyl-CoA. Their fatty acid methyl esters were found to be about 90% pure with only minor amounts of contamination from palmitoyl-CoA (23). The low activities with myristoyl- and stearoyl-CoAs were not solubility-effects since similar activities relative to palmitoyl-CoA were found in the presence of 0.2% Triton X-100. Since sphingosine is the predominant long-chain base in aorta with lesser amounts of other homologues (11), the relative in vitro activities of serine palmitoyltransferase with different fatty acyl-CoA thioesters are similar to the relative in vivo amounts of the corresponding long-chain bases.

Serine palmitoyltransferase activity was maximum between pH 8 and 9. The enzyme was found to be stable at the routine assay temperature of 37°C, which was removed sufficiently from the denaturation point measured at 55°C. Pyridoxal 5'-phosphate was determined to be a cofactor for the enzyme in aorta through formation of apoenzyme and reconstitution with PLP (15). The K_m for PLP was found to be 2.7 μM, which is similar to the values in other tissues and species (15–18).

Determination of activity during atherogenesis

The activity of serine palmitoyltransferase was determined in microsomes from the aortas of young male rabbits maintained on a Purina chow supplemented with 2% cholesterol and pair-fed controls at 1, 2, 3, 6, 9, and 12 weeks. Serum cholesterol concentrations for both groups are shown in **Table 3.** Activity in the control animals did not vary appreciably over the feeding period. Activity in the experimental group, which was augmented by 2 weeks ($P < 0.10$), increased significantly with the duration of feeding and the elevation of serum cholesterol levels to a 3.7-fold change on a microsomal protein basis by 12 weeks ($P < 0.005$) (**Fig. 6**). The change was not due to decreased recoveries of microsomal protein, e.g., values at 9 weeks in the cholesterol-fed group (2.48 ± 0.9 mg of microsomal protein per gram original wet weight tissue) were similar to 1-week controls (2.51 ± 0.2). DNA was then measured (27), and the specific activities were

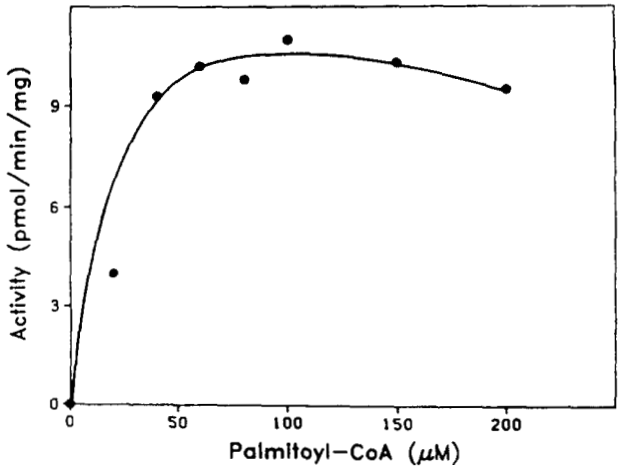

Fig. 4. Serine palmitoyltransferase activities with increasing concentrations of palmitoyl-CoA are presented. Assays were conducted using microsomes from a rabbit fed cholesterol for 9 weeks.

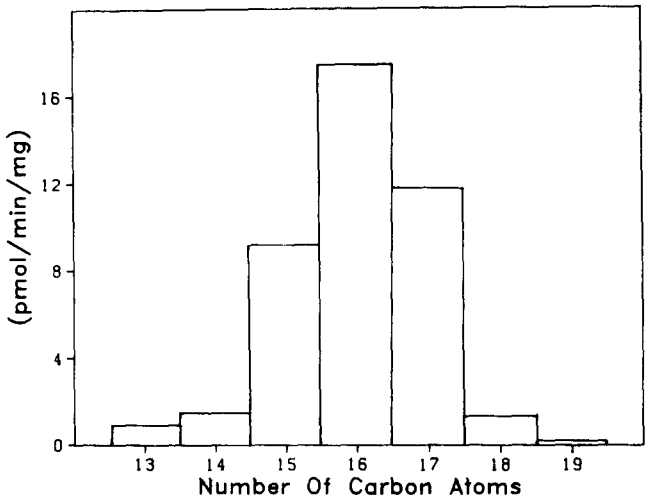

Fig. 5. Utilization of different saturated fatty acyl-CoA thioesters is presented. Assays were conducted using microsomes from a rabbit fed cholesterol for 9 weeks as described in the Materials and Methods section except for using different fatty acyl-CoAs.

expressed on a total cellular DNA basis. Activities, which were doubled by 1 week, were increased more than 4-fold by 12 weeks ($P < 0.01$) (**Table 4**).

Evaluations of arterial morphology

Several pieces of tissue were taken from different sites along the aorta in each of the rabbits and evaluated for gross and microscopic changes. Intimal proliferation, occurring initially in the arch and proximal thoracic areas, progressed over the duration of feeding to more distal regions of the aorta in the following order: distal thoracic, abdominal, then the bifurcation. Intercostal ostias where hemodynamic stress patterns occur were particularly susceptible to changes regardless of their location (33). Sections stained with hematoxylin and eosin (30), then viewed by light microscopy, revealed minor intimal changes by 3 weeks followed by a large increase in the number of cells in the arch and thoracic intima by 6 weeks, progressing further with the duration of feeding. Lipids, visualized by staining with Sudan IV, accumulated in the intima in parallel with cellular proliferation.

DISCUSSION

Serine palmitoyltransferase activity was measured in aorta from rabbits with different degrees of cholesterol-induced atherogenesis. A substantial increase in activity was found in the aorta of animals on the diet within 2 weeks of feeding. This change was prior to the detection by light microscopy of prominent cellular proliferation or sudanophilic lipid accumulation. Parker et al. (34) found by electron microscopy that cells do not begin to accumulate in the intima of cholesterol-fed rabbits until 2 to 4 weeks after feeding is initiated. The elevated serine palmitoyltransferase activity correlates with the early accumulation of arterial sphingomyelin (35). It also represents one of the initial changes associated with experimental atherogenesis. Its induction continued over the duration of feeding, with the increase in serum cholesterol and in proportion to the development of atheroma. A fourfold change was observed by 12 weeks. This is analogous to the fourfold increase in arterial sphingomyelin by 12 weeks in monkeys with experimental atherogenesis (4).

Since the specific activity of serine palmitoyltransferase is lower than those for subsequent enzymes of ceramide synthesis (15), its increase should provide a larger pool of precursors available for the formation of sphingomyelin. In support of this view, aortic concentrations of ceramide are low in normal aorta (0.020 mg per g of tissue), but are doubled in atherosclerotic aortas (0.042 mg/g) (14). Furthermore, Portman and Alexander (14) found that, in the absence of an acceptor molecule such as sphingosylphosphorylcholine, ceramide was the major alkali-stable product from [^{14}C]palmitoyl-CoA using homogenates of squirrel monkey aorta (14). Concurrent induction in the other enzymes of long-chain base synthesis is not precluded by this hypothesis, e.g., when elevation in serine palmitoyltransferase is sufficient to render their activities comparatively slower and additional synthesis of sphingomyelin is required. In either case, several processes may contribute to an increased demand for long-chain bases. Some of these are described in relation to the two major types of cells (smooth muscle and macrophage) participating in the development of atheromas, infra vide.

At initial periods of atherogenesis in experimental models, macrophages are present predominately at superficial layers of the lesions, later migrating towards the subendothelial space (36). These cells apparently employ the surface-active properties of phospholipids in dispersing and phagocytizing cholesterol to reduce its sclerosing effect (37-40). Sphingomyelin, which displays a preferential affinity for cholesterol relative to other phospholipids

TABLE 2. The relative activities of serine palmitoyltransferase with different fatty acyl-CoA thioesters

Fatty Acyl-CoA	Chain-Length and Unsaturation	Relative Activity
		%
Palmitoyl-CoA	16:0	100
Palmitelaidoyl-CoA	*trans*, 16:1	65
Palmitoleoyl-CoA	*cis*, 16:1	13
Stearoyl-CoA	18:0	1
Elaidyl-CoA	*trans*, 18:1	< 1
Oleoyl-CoA	*cis*, 18:1	< 1
Linoleoyl-CoA	*cis*, 18:2	3

All of the assays were conducted using 200 μM fatty acyl-CoA.

TABLE 3. The concentration of cholesterol in serum from control and experimental rabbits

Time on the Diet	Serum Cholesterol in Rabbits Pair-Fed Purina Chow only	Serum Cholesterol in Rabbits Fed Purina Chow Supplemented with 2% Cholesterol
weeks	*mg/dl*	*mg/dl*
0	80 ± 12.0	ND
3	73 ± 8.3	211 ± 22.9
6	80 ± 11.8	777 ± 82.1
9	73 ± 1.4	826 ± 56.7
12	74 ± 6.5	2454 ± 475.4

Fresh blood was obtained by heart puncture immediately after killing. The data represent the mean ± SE for two to six animals.

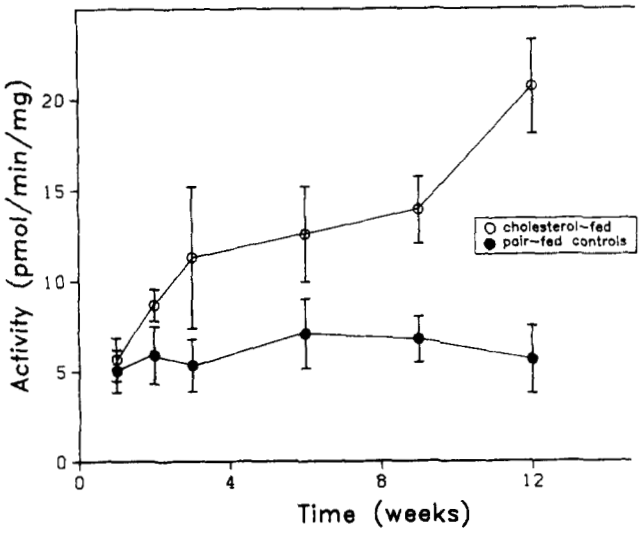

Fig. 6. Serine palmitoyltransferase activities in rabbits pair-fed a standard Purina chow (lower curve) and the same formula supplemented with 2% cholesterol (ascending curve) are presented. Each point represents the mean activity ± SE from three or four rabbits.

based on differential scanning calorimetry and its removal of cholesterol from L5178Y cells in tissue culture, mitigates the fibrogenic response in aorta to cholesterol (38, 41, 42). Thus, sphingomyelin may be synthesized by activated macrophages to aid in solubilizing, packaging, or subsequently exocytosing the large quantities of phagocytosed cholesterol, e.g., as a part of the apoE-phospholipid particle through formation of the HDL_c complex (43, 44). By enhancing the partitioning of cholesterol from the aorta to circulation, followed by removal out of circulation via liver filtration, cholesterol's effectiveness as an inflammatory stimulus would be reduced, thereby circumventing ensuing events such as fibrosis (37-40, 43, 44).

In addition to the presence of macrophages, smooth muscle cells are found in early and advanced lesions during atherogenesis (45). Denudation of the endothelium lining the lumen of the aorta, contributing to a loss of down-regulation of medial smooth muscle growth, coupled with induced proliferation by a variety of mitogenic substances, stimulates the migration of these cells to the intima concurrent with their division (46). Their lipid metabolism is focused on the requirements of membranogenesis during cellular proliferation. Parker and Odland (47) reported a substantial increase in plasma membrane, its vesicles, and intracellular organelles of myo-intimal cells from atherosclerotic rabbit. These cells displayed increased phospholipid synthesis (34). Consequently, membranogenesis necessary for intracellular organelle construction or mitosis would increase the demand for sphingomyelin long-chain bases over normal maintenance levels.

We propose that the constituent cells (smooth muscle and endothelial) of the aortic intima may develop a reduced level of regulation over long-chain base synthesis due to the irregular processing of low density lipoproteins (LDL) under atherogenic conditions. Cholesterol accumulates in parallel with sphingomyelin within the arterial intima during experimental atherogenesis. The metabolism of these lipids appears to be coordinated through cooperative mechanisms since under several different conditions, each resulting in increased sphingomyelin concentrations, the amounts of cholesterol also increase (42). Gatt and Bierman (48) and Kudchodkar, Albers, and Bierman (49) have demonstrated that internalized sphingomyelin alters the ability of human skin fibroblasts to bind and utilize LDL. They suggested that reduced LDL receptor expression and increased incorporation of acetate into sterols such as cholesterol is influenced by intracellular sphin-

TABLE 4. The activities of microsomal serine palmitoyltransferase in control and experimental rabbits as expressed on a DNA basis

Group	Number of Rabbits	Activity	Significance
		pmol/min per mg DNA	
Control rabbits fed the Purina chow only	2	8.8 ± 1.4	
Rabbits fed the cholesterol-supplemented diet for 1 week	3	20.0 ± 11.7	$P < 0.30$
Rabbits fed the cholesterol-supplemented diet for 12 weeks	3	36.8 ± 4.6	$P < 0.01$

DNA was measured in the initial homogenates as described in reference 27. The values represent the mean ± SE.

gomyelin concentrations. Furthermore, a level of regulation over sphingomyelin long-chain base synthesis was reported to occur through the receptor-dependent LDL pathway, and may be at the level of serine palmitoyltransferase analogous to the regulation of cholesterol synthesis by the 3-hydroxy-3-methylglutaryl-CoA reductase (50).

Thus, if LDL receptor expression is reduced or absent, as in aorta from Watanabe heritable hyperlipidemic rabbits or familial hypercholesterolemic individuals, or in response to elevated LDL-cholesterol concentrations through dietary manipulation (51), serine palmitoyltransferase activity would increase in situ as its down-regulation is removed, resulting in increased synthesis of long-chain bases leading to the accumulation of intracellular sphingomyelin. This sequence of events would reinforce the cell's inability to maintain proper regulation over its cholesterol and sphingomyelin concentrations, thus contributing to the foam-cell morphology characteristic of atherogenic aorta.

The authors thank Michael Hehmann, David Stemerman, and Doug Rodriguez for their skilled technical assistance, and Dr. Alfred H. Merrill, Jr. for his valuable suggestions.

Manuscript received 17 December 1985.

REFERENCES

1. Buck, R. C, and R. J. Rossiter. 1951. Lipids of normal and atherosclerotic aorta. *Arch. Pathol.* **48:** 224–237.
2. Steele, J. M., and H. J. Kayden. 1955. The nature of phospholipids in human serum and atheromatous vessels. *Trans. Assoc. Am. Physicians.* **68:** 249–254.
3. Bottcher, C. J. F. 1963. Phospholipids of atherosclerotic lesions in the human aorta. *In* Evolution of the Atherosclerotic Plaque. R. J. Jones, editor. Chicago University Press, Chicago. 109–116.
4. Portman, O. W., and R. D. Illingworth. 1976. Arterial metabolism in primates. *Primates Med.* **9:** 145–223.
5. Hakomori, S. I. 1981. Glycosphingolipids in cellular interaction, differentiation, and oncogenesis. *Annu. Rev. Biochem.* **50:** 733–764.
6. Smith, E. B. 1960. Intimal and medial lipids in human aorta. *Lancet.* **1:** 799–803.
7. Newman, H. A. I., E. L. McCandless, and D. B. Zilversmit. 1961. The synthesis of ^{14}C-lipids in rabbit atheromatous lesions. *J. Biol. Chem.* **236:** 1264–1268.
8. McCandless, E. L., and D. B. Zilversmit. 1956. The effect of cholesterol on the turnover of lecithin, cephalin, and sphingomyelin in the rabbit. *Arch. Biochem. Biophys.* **62:** 402–410.
9. Zilversmit, D. B., M. L. Shore, and R. F. Ackerman. 1954. The origin of aortic phospholipid in rabbit athermatosis. *Circulation.* **9:** 581–585.
10. Chobanian, A. V., and W. Hollander. 1966. Phospholipid synthesis in the human arterial intima. *J. Clin. Invest.* **45:** 932–938.
11. Karlsson, K-A. 1970. On the chemistry and occurrence of sphingolipid bases. *Chem. Phys. Lipids.* **5:** 6–43.
12. Chernick, S., P. A. Srere, and I. L. Chaikoff. 1949. The metabolism of arterial tissue. II. Lipide syntheses: the formation in vitro of fatty acids and phospholipides by rat artery with ^{14}C and ^{32}P as indicators. *J. Biol. Chem.* **179:** 113–118.
13. Morin, R. J. 1968. Phospholipid composition and synthesis in male rabbit aortas. *Metabolism.* **17:** 1051–1058.
14. Portman, O. W., and M. Alexander. 1970. Metabolism of sphingolipids by normal and atherosclerotic aorta of squirrel monkeys. *J. Lipid Res.* **11:** 23–30.
15. 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.
16. 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.
17. Merrill, A. H., Jr., D. W. Nixon, and R. D. Williams. 1985. Activities of serine palmitoyltransferase (3-ketosphinganine synthase) in microsomes from different rat tissues. *J. Lipid Res.* **26:** 617–622.
18. Merrill, A. H., Jr., and Williams, R. D. 1984. Utilization of different fatty acyl-CoA thioesters by serine palmitoyltransferase from rat brain. *J. Lipid Res.* **25:** 185–188.
19. Stoffel, W. D., 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.
20. Ullman, M. D., and N. S. Radin. 1972. Enzymatic formation of hydroxy ceramides and comparison with enzymes forming non-hydroxy ceramides. *Arch. Biochem. Biophys.* **152:** 767–777.
21. Singh, I. 1983. Ceramide synthesis from free fatty acids in rat brain: function of NADPH and substrate specificity. *J. Neurochem.* **40:** 1565–1570.
22. 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.
23. Morrison, W. R., and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride–methanol. *J. Lipid Res.* **5:** 600–608.
24. Mendershausen, P. B., and C. C. Sweeley. 1969. Chemistry and metabolism of sphingolipids. Chemical synthesis of 2-amino-1-hydroxyoctadecan-3-one (3-ketosphinganine). *Biochemistry.* **8:** 2633–2635.
25. Bligh, E. A., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem.* **37:** 911–917.
26. Bensadoun, A., and D. Weinstein. 1976. Assay of proteins in the presence of interfering materials. *Anal. Biochem.* **70:** 241–250.
27. Brunk, C. F., K. C. Jones, and T. W. James. 1979. Assay for nanogram quantities of DNA in cellular homogenates. *Anal. Biochem.* **92:** 497–500.
28. Sottocasa, G. L., B. Kuylenstierna, L. Ernster, and A. Bergstrand. 1967. An electron-transport system associated with the outer membrane of liver mitochondria. *J. Cell Biol.* **32:** 415–438.
29. Moorehead, W. R., and H. G. Biggs. 1974. 2-Amino-2-methyl-1-propanol as the alkylating agent in an improved continuous-flow cresolphthalein complexone procedure for calcium in serum. *Clin. Chem.* **20:** 1458–1460.
30. Lillie, R. D., and H. M. Fuller. 1976. Histopathologic Technique and Practical Histochemistry. 4th ed. McGraw-Hill, New York. 69, 208–210, and 566–567.

31. Snell, E. E., S. J. Di Mari, and R. N. Brady. 1970. Biosynthesis of sphingosine and dihydrosphingosine by cell-free systems from *Hansenula cifferi*. *Chem. Phys. Lipids.* **5:** 116–138.
32. Hurst, R. D., K. Lund, and R. W. Guynn. 1981. Enzymatic assays of L-serine in biological material. *Anal. Biochem.* **117:** 339–345.
33. Fry, D. L. 1973. Response of the arterial wall to certain physical factors. *In* Atherogenesis: Initiating Factors. R. Porter and J. Knight, editors. Associated Scientific Publishers, New York. 121–125.
34. Parker, F., J. W. Ormsby, N. F. Peterson, G. F. Odland, and R. H. Williams. 1966. *In vitro* studies of phospholipid synthesis in experimental atherosclerosis. *Circ. Res.* **19:** 700–710.
35. Portman, O. W., M. Alexander, and C. A. Maruffo. 1967. Nutritional control of arterial lipid composition in squirrel monkeys. Major ester classes and types of phospholipids. *J. Nutr.* **91:** 35–46.
36. Watanabe, T., M. Hirata, Y. Yoshikawa, Y. Nagafuchi, H. Toyoshima, and T. Watanabe. 1985. Role of macrophages in atherosclerosis: sequential observations of cholesterol-induced rabbit aortic lesion by the immunoperoxidase technique using monoclonal antimacrophage antibody. *Lab. Invest.* **53:** 80–90.
37. Ahrens, E. H., and H. G. Kunkle. 1949. The stabilization of serum lipid emulsions by serum phospholipids. *J. Exp. Med.* **90:** 409–424.
38. Adams, C. W. M., O. B. Bayless, M. Z. M. Ibrahim, and M. W. Webster, Jr. 1963. Phospholipids in atherosclerosis: the modification of the cholesterol granuloma by phospholipid. *J. Pathol. Bacteriol.* **86:** 431–436.
39. Spain, D. M., and N. Aristizabal. 1962. Rabbit local tissue response to triglycerides, cholesterol, and its esters. *Arch. Pathol.* **73:** 82–85.
40. Hirsch, E. F., and S. Weinhouse. 1940. Role of fixed tissue phagocytes in lipid metabolism. *Arch. Pathol.* **30:** 1079–1084.
41. Friedman, M., S. O. Byers, and R. H. Rosenman. 1957. Resolution of aortic atherosclerotic infiltration in the rabbit by phosphatide infusion. *Proc. Soc. Exp. Biol. Med.* **95:** 586–588.
42. Demel, R. A., J. W. C. M. Jansen, P. W. M. Van Dijck, and L. L. M. Van Deenan. 1977. The interaction of cholesterol with different classes of phospholipids. *Biochim. Biophys. Acta.* **465:** 1–10.
43. Haberland, M. E., and J. A. Reynolds. 1973. Self-association of cholesterol in aqueous solution. *Proc. Natl. Acad. Sci. USA.* **70:** 2313–2316.
44. Goldstein, J. L., and M. S. Brown. 1977. The low-density lipoprotein pathway and its relation to atherosclerosis. *Annu. Rev. Biochem.* **46:** 897–930.
45. Aqel, N. M., R. Y. Ball, H. Waldmann, and M. J. Mitchinson. 1985. Identification of macrophages and smooth muscle cells in human atherosclerosis using monoclonal antibodies. *J. Pathol.* **146:** 197–204.
46. Ross, R., and J. A. Glomset. 1976. The pathogenesis of atherosclerosis. *N. Engl. J. Med.* **295:** 369–377.
47. Parker, F., and G. F. Odland. 1966. A correlative histochemical, biochemical and electron microscopic study of experimental atherosclerosis in the rabbit aorta with special reference to the myo-intimal cell. *Am. J. Pathol.* **48:** 197–239.
48. Gatt, S., and E. L. Bierman. 1980. Sphingomyelin suppresses the binding and utilization of low-density lipoproteins by skin fibroblasts. *J. Biol. Chem.* **255:** 3371–3376.
49. Kudchodkar, B. J., J. J. Albers, and E. L. Bierman. 1983. Effect of positively charged sphingomyelin liposomes on cholesterol metabolism of cells in culture. *Atherosclerosis.* **46:** 353–367.
50. Verdery, R. B., and R. Theolis, Jr. 1981. Regulation of sphingomyelin long-chain base synthesis in human fibroblasts in culture. *J. Biol. Chem.* **257:** 1412–1417.
51. Goldstein, J. L., T. Kita, and M. S. Brown. 1983. Defective lipoproteins receptors and atherosclerosis. *N. Engl. J. Med.* **309:** 288–296.