Phosphatidyl Cholines of Aorta from Rabbits Fed Cholesterol[1] (36095)

DAVID N. SKURDAL AND W. E. CORNATZER

Guy and Bertha Ireland Research Laboratory, Department of Biochemistry, University of North Dakota School of Medicine, Grand Forks, North Dakota 58201

Buck and Rossiter (1) have reported an increased concentration of lecithin and sphingomyelin in human atheromas. In rabbits fed 1 g of cholesterol/100 g of Purina rabbit chow for 5 months, an increased concentration and rate of synthesis from $^{32}PO_4$ of lecithins and sphingomyelins occurred in the aorta (2).

Roscoe and Riccardi (3) have reported similar increased concentrations of lecithin and sphingomyelin in aorta of male Dutch belted rabbits fed 1% cholesterol in rabbit pellets. Lecithins can be fractionated by thin-layer chromatography into different fractions with silica gel impregnated with $AgNO_3$ (4). Fraction 1 contains higher polyunsaturated (decosahexaenoic, 22:6) fatty acids and represents the lecithins synthesized by the ethanolamine pathway (5). Fractions 3 and 4 contain oleic, linoleic, and arachidonic and are synthesized by the choline pathway (5). The concentration of these various lecithins in aorta or in atheroma from animals fed cholesterol has not been determined.

Methods. Male and female New Zealand white rabbits were fed ground Purina rabbit pellets containing 1% cholesterol for 14 weeks. All animals were allowed food and water *ad libitum*.

Rabbits fed a similar diet for 12 weeks produce atheroma lesions in the aorta (3). At the end of the dietary regime, the control and the cholesterol-fed rabbits were sacrificed; and the aorta was removed, blotted dry, and weighed. Serum levels of cholesterol were determined (6). The aortas were homogenized with ice-cold methanol in a Potter-Elvehjem homogenizer with a Teflon pestle. The lipids were extracted with chloroform–methanol as described by Folch *et al.* (7). The total phospholipid phosphorus was determined (8, 9) on an aliquot of the chloroform solution. Phosphatidyl cholines and sphingomyelin were isolated from the lipid extract by thin-layer chromatography by the method of Parker and Peterson (10) using solvent chloroform:methanol:acetic acid:water, 65:25:4:1.4 (v/v/v/v). Phospholipids were identified by comparisons with purified standards. Plates were sprayed with 0.008% rhodamine 6G solution and viewed under ultraviolet light to identify and outline the band of gel containing the phospholipids. The silica gel containing the phosphatidyl cholines and sphingomyelins were scraped into a flask containing 20 ml of chloroform–methanol (2:1; v/v). The phosphatidyl cholines and sphingomyelins were eluted from the gel by filtration of the chloroform–methanol solution with the aid of a sintered-glass funnel (medium porosity). The gel was washed twice with chloroform–methanol–water (200:97:3) and once with methanol. Quantitative recovery of the phospholipids were possible with this elution procedure. Recovery values represented 94% of the total phosphorus. The filtrate was washed with 0.2 vol of 0.04% calcium chloride solution. Dilute solutions of the phospholipids extracts in chloroform were stored under dry nitrogen at −18°. On an aliquot of the chloroform solution, the total phosphatidyl choline phosphorus and total sphingomyelin phosphorus were determined (8, 9). Fractionation of the phosphatidyl choline fractions were carried out by thin-layer chromatography on silica gel H impregnated with silver nitrate (4). The phosphatidyl choline fractions were identified by spraying with 0.01% methanolic solution of 2, 7-dichlorofluorescein and viewing under ultraviolet light (4). The phosphatidyl choline fractions were scraped into tubes containing

[1] Supported in part by a grant from U.S. Atomic Energy Commission AT(11-1)-1513.

TABLE I. Rabbit Aorta Phospholipids.

Expt. condition	Sex	No. of animals	Body wt (kg)	Aorta wt (mg of wet wt)	Serum cholesterol (mg)	Aorta: Total phospholipid-P (μg)	Aorta: Total phospholipid-P (μg/g of wet wt)	Aorta: Total phosphatidyl choline-P (μg)	Aorta: Total phosphatidyl choline-P (μg/g of wet wt)	Aorta: Phosphatidyl choline (%) total lipid-P (%)	Aorta: Total phosphatidyl choline-P fractions (μg) 1 and 2	3	4	Aorta: Total sphingomyelin-P (μg)	Aorta: Sphingomyelin (%) total lipid-P (%)
Control	M	5	3.65 ±0.5	366.3 ±274.	49.8	189.6 ±42.8	548.9 ±161.1	38.4 ±4.6	111.8 ±25.4	21.9 ±7.9	21.4 ±2.6	11.4 ±1.6	8.9 ±1.6	25.4 ±3.0	14.4 ±4.5
1% cholesterol	M	4	2.78 ±0.1	389.8 ±127.	438.2 ±212.8	251.2 ±114.7	634.2 ±119.4	140.3[b] ±54.8	355.6[b] ±65.8	57.8[b] ±14.7	68.3[d] ±32.4	34.3[d] ±18.1	21.4[c] ±7.6	108.6[b] ±34.5	46.9[b] ±13.8
Control	F	7	3.30 ±0.3	300.9 ±42.1	66.6 ±26.6	161.4 ±26.7	541.2 ±84.7	35.0 ±4.5	117.0 ±10.5	22.0 ±3.3	15.5 ±6.0	10.4 ±4.2	9.7 ±3.4	25.1 ±2.9	15.8 ±3.0
1% cholesterol	F	6	2.90 ±0.1	333.6 ±37.4	499.8[b] ±105.6	227.1[c] ±46.8	678.8[d] ±104.5	100.1[b] ±40.6	296.6[b] ±107.3	42.4[b] ±10.1	51.3[b] ±4.8	31.1[b] ±8.6	19.5[c] ±7.8	74.0[c] ±40.8	31.0[b] ±11.3

[a] Numbers preceded by ± are standard deviations.

[b] The test of significance was applied to the difference between mean value for control and the cholesterol fed animals. Probability for occurrence of this difference was: $p < .01$; [c] $p < .02$; [d] $p < .05$.

15 ml of chloroform:methanol, 2:1 (v/v), and filtered with sintered-glass funnel (medium porosity) to collect the lipid silica gel. The lipids were extracted from the gel with 15 ml of chloroform:methanol:water, 200:97:3 (v/v/v); methanol–water, 97:3 (v/v); and methanol. Recovery values represented 98% of the phoshorus applied to silica gel impregnated with silver nitrate. Samples were taken for phosphorus analyses for the phosphatidyl choline fractions (8, 9).

Results and Discussion. 541 μg of total phosphatidyl-P/g of aorta (wet wt) was observed in normal female New Zealand strain of rabbits 3 months of age. This concentration is three times that reported for 3 month old female rabbits by Eisenberg *et al.* (11). However, the authors did not give the strain of rabbits. Zilversmit and McCandless (12) have reported a concentration of 172 μg of total phospholipid-P/g of aorta (wet wt). However, they did not give the sex of the New Zealand rabbits. A concentration of 678 μg of total phosphatidyl-P/g of aorta (wet wt) was observed (Table I) in females and 634 μg of total phosphatidyl-P/g of aorta (wet wt) for males fed 1% cholesterol in the diet for 3 months. This value is similar to 663 μg of total phospholipid-P/g of aorta (wet wt) for New Zealand rabbits fed 1% cholesterol for 3 months reported by Zilversmit and McCandless (12). However, they did not give the sex of the animals.

Eisenberg *et al.* (11) reports that in female rabbits (the strain of animal not given), 3 months of age, the lecithin-P represents 37.1% of the total phospholipid-P and the total aortic phospholipid-P was 153.5 μg of P/g of wet weight. According to these values the total phosphatidyl choline-P/g of wet weight would be 56.94 μg. The data in Table I for a similar age and sex gives 117 μg of total phosphatidyl choline-P/g of wet weight and the phosphatidyl choline-P represents 22% of total phospholipid-P.

The feeding of cholesterol in the diet for 14 weeks raised the serum cholesterol over sixfold. The data of Table I show that the feeding of cholesterol in the diet in both male and female rabbits increased the concentration of total lecithin-P and sphingomyelin-P in the aorta. Roscoe and Riccardi (3) have observed a similar increase in the concentration of phosphatidyl choline and sphingomyelin in the aorta of male Dutch rabbits fed 1% cholesterol in the diet.

The use of $^{32}PO_4$ and acetate-1-^{14}C incorporation into aorta lecithin suggest that these phospholipids of atheromatous lesions are synthesized *in situ* (2, 13).

The total phosphatidyl cholines of the aorta were fractionated into three fractions. The concentration of the phosphatidyl cholines of normal aorta are different from those found in liver microsomes of control rabbits. Ninety-five percent of the total lecithin-P is found in fractions 3 and 4 of rabbit liver microsomes (14). In the aorta only 52% of the total lecithin-P is found in these two fractions. The data in Table I show that a significant increase occurs in all lecithin fractions following the administration of 1% cholesterol in the diet for 14 weeks. A greater increase occurred in fractions 1–2, and 3. This would suggest that cholesterol may be affecting their biosynthetic pathway. The change in concentration of these phosphatidyl cholines and/or their biosynthetic pathway in the membranes of the aorta during the development of the atheroma lesions could represent part of the pathogenesis of this process.

Summary. Male and female New Zealand white rabbits were fed ground Purina rabbit pellets containing 1% cholesterol for 14 weeks. A significant increase in the total phosphatidyl choline-P, percentage of total lipid-P, total sphingomyelin-P, and percentage of lipid-P occurred in the aorta of the animals fed cholesterol. The total phosphatidyl cholines of the aorta were fractionated into four fractions by thin-layer chromatography on silica gel H impregnated with silver nitrate. A significant increase occurred in all the phosphatidyl choline fractions of the aorta from the animals fed cholesterol. A greater increase occurred in fractions 1–2 and 3.

1. Buck, R. C., and Rossiter, R. J., Arch. Pathol. 51, 224 (1951).

2. McCandless, E. L., and Zilversmit, D. B., Arch. Biochem. Biophys. 62, 402 (1956).

3. Roscoe, H. G., and Riccardi, B. A., J.

Atheroscler. Res. **10**, 123 (1969).

4. Arvidson, G. A. E., J. Lipid Res. **6**, 574 (1965).

5. Rytter, D., Miller, J. E., and Cornatzer, W. E., Biochim. Biophys. Acta **152**, 418 (1968).

6. Kingsley, G. R., and Schaffert, R. R., J. Biol. Chem. **180**, 315 (1949).

7. Folch, J., Lees, M., and Sloan-Stanley, G. H., J. Biol. Chem. **226**, 297 (1957).

8. Miller, J. E., and Cornatzer, W. E., Proc. Soc. Exp. Biol. Med. **118**, 948 (1965).

9. Fiske, C. H., and Subbarow, Y., J. Biol. Chem. **66**, 375 (1925).

10. Parker, F., and Peterson, N. F., J. Lipid Res. **6**, 455 (1965).

11. Eisenberg, S., Stein, Y., and Stein, O., Biochim. Biophys. Acta **176**, 557 (1969).

12. Zilversmit, D. B., and McCandless, E. L., J. Lipid Res. **1**, 118 (1959).

13. Newman, H. A. I., McCandless, E. L., and Zilversmit, D. B., J. Biol. Chem. **236**, 1264 (1961).

14. Miller, J. E., and Cornatzer, W. E., Lipids **4**, 102 (1969).

Received Sept. 1, 1971. P.S.E.B.M., 1972, Vol. 139.