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Effect of infusions of phosphatides upon the atherosclerotic aorta *in situ* and as an ocular aortic implant*

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SUMMARY

Rabbits fed cholesterol- and oil-supplemented diet were carefully paired for equality of plasma cholesterol concentration during the feeding period and a subsequent 30 days of normal diet. The normal diet was continued and one of each pair of rabbits was then subjected to intravenous infusion of mixed phosphatides of animal or soybean origin twice weekly for an average of 11 infusions. At the end of this time the aortas of the infused rabbits showed markedly less atherosclerosis than their paired controls, both grossly and when assessed on the basis of cholesterol content. When implants of atherosclerotic aorta were made into the anterior chamber of the eyes of normal host rabbits, infusions of phosphatide did not accelerate the decline in cholesterol content of the implants or affect the normal cholesterol content of the host rabbit's own aorta. When normal rabbit aorta was implanted into normal host rabbits thereafter fed a diet enriched in cholesterol and oil, infusion of phosphatide had no effect on atherosclerosis of the host's own aorta. However, a marked increase was observed in sudanophilia and cholesterol content of the aortic implants in those rabbits receiving infusions. Apparently the lipid dynamics of ocular aortic implants differs from that of the aorta *in situ*. Infusions of phosphatide can alleviate atherosclerosis when a cholesterol-free dietary is followed but may worsen atherosclerosis when a cholesterol-free diet is not followed.

The sustained infusion of various phosphatides has been observed (1, 2) to elevate promptly the level of plasma cholesterol. One source of this cholesterol appears to be the cholesterol already present in various extravascular tissues (3). This suggests that under proper circumstances, experimental elevation of plasma phospholipid content leads to a loss of cholesterol from various tissues to the blood.

These findings raised the possibility that chronic infusion of the atherosclerotic rabbit with a suitable mixture of phosphatides might lead to a removal of some of the cholesterol present in the atherosclerotic plaques of the animal. Accordingly, a series of atherosclerotic rabbits was infused with a relatively crude mixture of phosphatides derived from animal brain. The results (4) suggested that such infusions indeed led to a diminution in both the size and the cholesterol content of previously formed atherosclerotic infiltrations. In view of these results, this phenomenon was investigated more extensively, employing relatively pure suspensions of "lecithin" derived from vegetable sources.

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METHODS

The Infusion of Various Phosphatide Suspensions into Atherosclerotic Rabbits. Three separate groups of young male rabbits were fed a Wayne rabbit chow diet enriched with 2 per cent cholesterol and 2 per cent cottonseed oil for 3 months. They were then returned to a diet of only Wayne rabbit chow for a period of not less than 30 days. Heparinized plasma for analysis of cholesterol content was obtained monthly from these rabbits while on the enriched diet, and again 30 days after their return to the Wayne chow diet. Rabbits were paired as experimental and control when found to have similar plasma cholesterol levels during the cholesterol feeding period and also the same plasma

cholesterol value 30 days after cessation of cholesterol feeding. It should be stressed that in our experience, if a pair of rabbits is desired that exhibits the same amount of aortic atherosclerosis, not only should their average plasma cholesterol content during the cholesterol feeding be essentially similar, but also the "fall-off" level 30 days after cessation of feeding should be the same. Thus if one of a pair of rabbits exhibits the same average plasma cholesterol as its mate during the cholesterol feeding period, but remains

TABLE 1. EFFECT OF INFUSION OF VARIOUS PHOSPHATIDES UPON THE ATHEROSCLEROTIC AORTA OF THE RABBIT

No. of Rabbits	Average Weight	Average Plasma Cholesterol (mg./100 ml.)*		No. of Infusions	Aorta		
		During Cholesterol Feeding	One Mo. After Cholesterol Feeding		Degree of Atherosclerosis	Cholesterol	Total Lipid
	g.				*0–5*	*g./100 g.*	*g./100 g.*
A. Rabbits Infused with Crude Phosphatide (Animal Brain)							
Infused–6	2867	995 *802–1270*	167 *71–404*	8 *5–16*	3.3 *2.0–5.0*	6.69 *1.1–14.2*	34 *19.5–42.3*
Control–6	3087	958 *738–1174*	213 *91–485*	8† *5–16*	4.8 *4.5–5.0*	12.8 *9.5–17.2*	38.0 *27.2–49.2*
B. Rabbits Infused with Soybean Phosphatide (Sterling-Winthrop)							
Infused–7	3373	585 *241–777*	203 *41–420*	13 *12–13*	2.7 *1.5–4.0*	4.18 *1.3–6.9*	—
Control–7	3164	568 *489–1277*	187 *26–104*	0	4.5 *3.5–5.0*	5.31 *1.97–7.11*	—
C. Rabbits Infused with Soybean Phosphatide (Cutter)							
Infused–6	3176	909 *440–1333*	68 *23–104*	12 *11–12*	2.3 *1.0–3.0*	5.04 *3.45–7.1*	—
Control–6	2076	908 *489–1277*	72 *26–104*	0	4.0 *2.0–5.0*	6.49 *2.74–9.9*	—
Totals							
Infused–19	3151	822	149	11	2.7	5.34‡	—
Control–19	2796	799	159	—	4.6	8.05‡	—

Figures in italics indicate range.
* Cholesterol concentrations prior to infusion are included to show equality of exposure to atherosclerogenic infiltration.
† Infusions of dextrose solution (5%).
‡ t value equals 3.4.

significantly higher 30 days after its removal from the cholesterol diet, almost invariably such a rabbit exhibits far more aortic atherosclerosis than its paired mate. In other words, the rate of decline of a previously high plasma cholesterol is inversely proportional to the amount of excess cholesterol stored in the animal's tissues, including its aorta, during the prior cholesterol feeding (5).

After the rabbits of each group were paired, one of each pair was subjected to a series of 5 to 16 intravenous infusions of various phosphatide suspensions maintained for 6 hours and given twice a week. Plasma samples were obtained usually before and at the end of each infusion period and analyzed for cholesterol and phospholipid (4). At the end of the series of infusions, the infused and its paired control rabbit were sacrificed. The aorta was exposed, inspected grossly, and graded on a scale from 0 to 5 (4). Then a section of the aorta extending 10 cm. from the insertion of the semilunar valves was obtained routinely from both the infused and control rabbits and analyzed for its cholesterol content (4).

Group A consisted of six pairs of rabbits. One of each pair received 5 to 16 infusions of a crude mixture of phosphatides derived from animal brain.[1] Approximately 60 to 80 ml. of a 5 per cent suspension in 5 per cent dextrose solution was infused over the 6-hour period. The paired control rabbits received similar infusions of only 5 per cent dextrose. Little or no adverse reaction was observed during or after the infusions. The phosphatide mixture was analyzed for both its sterol and phospholipid content (2).

Group B consisted of seven pairs of rabbits. One of each pair received 12 to 13 6-hour infusions of a mixture of phosphatides derived from soybean (Sterling-Winthrop) similarly made up to 5 per cent and suspended in 5 per cent dextrose solution; the control rabbits were not infused.

Group C consisted of six pairs of rabbits treated exactly like those of Group B except that one of each pair received a mixture of phosphatides derived from soybean obtained from a different manufacturing source (Cutter Laboratories, Inc.).

The Infusion of Various Phosphatide Suspensions into Normal Rabbits Bearing Corneal Atherosclerotic Aortic Implants. Three different groups of normal rabbits were employed in this experiment. Three closely similar segments of the atherosclerotic aorta of a rabbit previously fed cholesterol were implanted into the anterior eye chamber of pairs of rabbits of each group, according to the Higginbotham technique (6). Then one of each pair of rabbits was subjected to a series of 4 to 13 infusions of the same phosphatide mixtures employed above. One of each pair of Group A rabbits (five pairs) received infusions of the animal brain phosphatide.[2] One of each pair of Group B rabbits (21 pairs) was infused with the purified "lecithin" prepared by Sterling-Winthrop. One of each pair of Group C rabbits (six pairs) was infused with the Cutter-prepared "lecithin" suspension. At the end of the series of infusions, the rabbits were sacrificed, the implants removed, a small section removed for Sudan IV staining, and the remainder analyzed for cholesterol content according to previously described methods (7). In five pairs of rabbits of Group B a 5-cm. section of each rabbit's own aorta was removed and analyzed for cholesterol.

The Infusion of a "Lecithin" Suspension into Rabbits Bearing Corneal Aortic Implants During the Ingestion of a Cholesterol and Oil-Enriched Diet. Three segments of a normal rabbit's aorta were implanted into the anterior eye chamber of each of 10 normal rabbits. One week later the 10 rabbits were allowed to ingest a diet enriched with 2 per cent cholesterol and 2 per cent cottonseed oil for 30 days. In addition, 5 of the 10 rabbits were infused for 8 hours every 4 days, for a total of 8 infusions, with the purified soybean "lecithin" suspension prepared by Sterling-Winthrop. Blood samples obtained at the beginning and at the end of the second, third, and fourth weeks were analyzed for plasma total cholesterol and phospholipid. At the end of the 30-day feeding period all rabbits were sacrificed, and the aortic implants were removed and studied as described above. In addition, each rabbit's own aorta was inspected and graded, and a 10-cm. segment taken for cholesterol analysis.

RESULTS

The Effect of Phosphatide Infusions on Aortic Infiltration of Atherosclerotic Rabbits. Following each infusion of "lecithin" derived from either animal or vegetable sources, the plasma phospholipid and cholesterol promptly rose, as described previously (1, 2). Thus in a typical infusion of the six rabbits of Group A, the average serum cholesterol and phospholipid rose in 6 hours from 42 and 92 mg. per 100 ml., respectively, to 110 and 857 mg. per 100 ml. These lipid elevations usually disappeared 24 to 48 hours after each infusion. There was no discernible tendency for the plasma cholesterol response to increase or decrease

[1] "Ninety per cent pure" animal "lecithin," Nutritional Biochemicals Corp., Cleveland, Ohio.

[2] See footnote 1.

with repeated phosphatide injections, nor did the initial injection provoke a markedly different response than did subsequent injections.

The aortas of the atherosclerotic rabbits given repeated infusions of "lecithin" suspensions, whether derived from animal or vegetable sources, exhibited markedly less gross atherosclerosis than their paired controls (Table 1). Thus the average gross degree of aortic atherosclerosis in the 19 treated rabbits was adjudged as 2.7, and that of the 19 controls, 4.6. Upon analysis, the average cholesterol content of the aortas of the treated animals also was less, being 5.34 g. per

TABLE 2. EFFECT OF INFUSIONS OF VARIOUS PHOSPHATIDES UPON THE OCULAR-IMPLANTED ATHEROSCLEROTIC AORTA OF THE RABBIT

No. of Rabbits	Average Weight	No. of Infusions	Implantation	Aorta Implant			Aorta *in Situ*
				Initial Cholesterol Content	Terminal Cholesterol Content	Presence of Terminal Sudanophilia	Cholesterol
	g.		*days*	*g./100 g.*	*g./100 g.*	*per cent*	*g./100 g.*
A. Rabbits Infused with Crude Phosphatide (Animal Brain)							
Infused–5	3124	9 *8–10*	49 *42–57*	6.97*	3.59 *2.35–4.78*	80	—
Control–5	3062	0	49 *42–57*	6.97*	3.12 *1.31–5.56*	80	—
B. Rabbits Infused with Soybean Phosphatide (Sterling-Winthrop)							
Infused–21	3061	9 *3–13*	43 *22–67*	7.95 *5.13–12.9*	4.06 *0.99–6.7*	76	0.419 *0.388–0.496*
Control–21	2954	0	43 *22–67*	7.95 *5.13–12.9*	3.92 *0.6–9.34*	71	0.396 *0.37–0.427*
C. Rabbits Infused with Soybean Phosphatide (Cutter)							
Infused–6	2784	6 *3–7*	41 *32–44*	5.37 *4.59–6.17*	1.85 *1.34–3.03*	0	—
Control–6	2890	0	41 *32–44*	5.37 *4.59–6.17*	2.17 *1.11–4.50*	0	—
Average of All							
Infused–32	3019	8	44	7.31	3.57	62	
Control–32	2959	0	44	7.34	3.47	59	

Figures in italics indicate range.
* All implants taken from single area of aorta; therefore no range of cholesterol content.

100 g. of dry weight as compared to 8.05 g. per 100 g. of dry weight of the control aortas—a significant difference ($t = 3.4$). The aortic total lipid content, however, of the infused rabbits of Group A failed to exhibit a significant decline.

The Effect of Phosphatide Infusions upon Corneal Atherosclerotic Aortic Implants. The expected decline (7) in the cholesterol content and sudanophilia of atherosclerotic aortic implants many weeks after their deposition was observed once more in both the infused and the control rabbits. However, this disappearance, as Table 2 clearly depicts, was not accelerated by the infusion of any of the three types of phosphatide suspensions. It is of interest also that the cholesterol content of the normal aorta of the host animal (Table 2, B) was not significantly altered by as many as 10 infusions of phosphatide.

The Effect of Phosphatide Infusions upon the Cholesterol Infiltration of the Aorta and Corneal Implant of Cholesterol-Fed Rabbits. The five rabbits ingesting excess cholesterol for 4 weeks, during which time they also received 7 infusions of phosphatide, failed to exhibit any significant change in either the gross degree of aortic atherosclerosis or in the aortic cholesterol content as compared to the controls (Table 3). However, a marked change was observed in the corneal implants. Of the implants of the infused rabbits, 40 per cent exhibited some sudanophilia as compared to 7 per cent in the controls. Similarly, the average cholesterol content of the implants in the infused animals was over twice as much as was found in the controls.

DISCUSSION

In earlier studies (2, 3) the hypercholesterolemic effects of various phosphatide infusions were seemingly due to the mobilization and entrance into the blood of already formed cholesterol present in various tissues. There was little or no evidence that such infusions altered the endogenous rate of cholesterol synthesis. For example, prior deprivation of the animal from dietary cholesterol (a procedure supposedly capable of increasing the rate of endogenous cholesterol synthesis) tended to reduce the hypercholesterolemic effect usually attained during phosphatide infusion. Conversely, prefeeding of cholesterol heightened the hypercholesterolemic effect of such infusion. The results obtained in the present study are in general agreement with these earlier findings. However, work now in progress in this laboratory indicates that the hyper-

TABLE 3. EFFECT OF INFUSION OF SOYBEAN PHOSPHATIDES UPON THE OCULAR-IMPLANTED AORTA AND AORTA *in Situ* OF THE CHOLESTEROL-FED RABBIT

No. of Rabbits	Average Weight	Average Plasma Cholesterol*	Average Plasma Phospholipid*	Aorta Implant†		Aorta *in Situ*	
				Sudanophilia	Cholesterol	Aver. Degree Atherosclerosis	Cholesterol
	g.	*mg./100 ml.*	*mg./100 ml.*	*per cent*	*g./100 g.*	*0–5*	*g./100 g.*
A. Cholesterol-Fed Rabbits Infused with Soybean Phosphatides							
5	3053	842	385	40	5.05	0	0.89
Standard error		±26.8	±20.6	—	±1.3		±0.14
B. Control Cholesterol-Fed Rabbits							
5	2705	447	264	7	2.12	0	0.81
Standard error		±101	±39	—	±0.35		±0.1

* Represents average of plasma analyses at beginning, second, third, and final week of feeding period.
† Original cholesterol content of aorta implant was 0.91 g./100 g. of dry weight.

cholesterolemic effect of phosphatide infusion into normally fed animals may be diminished, though not abolished, by administration of a drug that strongly inhibits cholesterol synthesis.

The infusion of phosphatides of either animal or vegetable source into the normocholesterolemic but atherosclerotic rabbit ingesting a cholesterol-free diet invariably induced a temporary hypercholesterolemia during such infusions. Following a series of such infusions, the aortas of such animals exhibited far less atherosclerosis and significantly less cholesterol than the untreated controls. It is our belief that during each infusion, some of the cholesterol making up the hypercholesterolemia observed was extracted from aortic atherosclerotic depots.

In earlier studies (8) we have called attention to the quantitatively different sequence of lipid dynamics that takes place in both the normal and atherosclerotic ocular-implanted aorta, as compared to the aorta *in situ*. First, a far greater degree of lipid infiltration takes place in the ocular-implanted aorta than in the animal's own aorta when this animal is fed excess cholesterol. Second, when the animal is removed from a high cholesterol diet, the lipid infiltration rapidly diminishes in the ocular implant but remains unchanged in the aorta *in situ*. This rapid disappearance of lipid from the ocular-implanted atherosclerotic aorta could not be hastened by the present series of phosphatide infusions.

When animals implanted with ocular fragments of normal aorta were placed upon a high cholesterol diet and also given frequent infusions of phosphatide suspensions, such animals, as was expected (1, 2), exhibited a greater degree of hypercholesterolemia than their uninfused controls. The aortas of both the infused and control animals, when examined 30 days later, failed to show either gross atherosclerosis or any excess cholesterol in the aorta *in situ*. However, again as expected (8), the ocular implants already were heavily impregnated with cholesterol and were sudanophilic. It was of great interest that the cholesterol content and sudanophilia of the implants of the infused animals were far greater than those of the controls. Apparently the infusion of phosphatide simultaneously with the ingestion of excess cholesterol, by inducing a greater degree of hypercholesterolemia, led to a greater cholesterol deposit in the ocular-implanted aorta.

These latter findings appear to us to suggest two things: First, that the lipid dynamics of the implanted aorta and that of the aorta *in situ* are different, and one is not necessarily an index of the other's processes; second, the possibility that phosphatide infusions carried on at the same time that excess cholesterol is taken in the diet may, by the ability to maintain or prolong hypercholesterolemia derived from this dietary cholesterol, actually worsen, rather than alleviate, cholesterol deposition in various tissues. In this sense, then, phosphatide, as we suggested earlier (3), may act as a double-edged sword. Certainly the infusion of phosphatide into human subjects ingesting or absorbing large amounts of cholesterol might be expected to induce a hypercholesterolemia derived in part not from tissue depots of cholesterol but from dietary sources. If this is the case, its infusion might have deleterious rather than therapeutic effects.

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