Resolution of Aortic Atherosclerotic Infiltration in the Rabbit by Phosphatide Infusion.* (23300)

MEYER FRIEDMAN, SANFORD O. BYERS, AND RAY H. ROSENMAN
Harold Brunn Institute, Mt. Zion Hospital, San Francisco, Calif.

Earlier studies from this laboratory(1,2) have demonstrated that a *sustained* rise in the plasma phospholipid of either the rat or rabbit, induced by intravenous infusion of a suitable mixture of phosphatides, quickly leads to a hypercholesteremia in these species. A third study(3) also demonstrated that this phosphatide-induced hypercholesteremia could occur almost as well in the liverless as in the intact animal. This last finding of course indicated that the infused phosphatide was bringing into the blood, cholesterol from parts of the body other than the liver.

In view of these findings, it was thought advisable to determine whether a series of phosphatide infusions, administered to rabbits previously made hypercholesteremic and atherosclerotic by dietary ingestion of cholesterol, might alter the cholesterol content and extent of their aortic atherosclerotic process.

Methods. Two series of hypercholesteremic rabbits were studied. A very moderate hypercholesteremia was induced in the first series of 10 healthy male rabbits (avg age: 6 weeks; appx wt: 1500-1800 g) by placing them on Purina rabbit chow, containing 1% cholesterol and 2% cottonseed oil, for a period of 3 months. A very severe hypercholesteremia was induced in the second series of 14 rabbits by adding 3% cholesterol and 4% cottonseed oil to their Purina rabbit chow for a period of 3 months. At the end of this period, both series were returned to their regular rabbit chow diet. Plasma samples were taken before and then monthly after the high cholesterol feeding had been begun and analyzed for cholesterol(4). Monthly plasma samples also were obtained after the diet had been discontinued. Three months after cessation of excess cholesterol and oil feeding, the hypercholesteremia observed during the cholesterol feeding had disappeared. At this time, for control purposes, 4 rabbits of the second series were sacrificed and their aortas were examined for gross atherosclerosis and a segment of it was analyzed for cholesterol. This segment (1 cm in length) was taken at a site uniformly 3 cm distant from the semilunar valves. For additional controls, the aortas of 5 completely normal rabbits were analyzed for total cholesterol content. The average plasma cholesterol of the remaining rabbits of both series was calculated for only the period of high cholesterol feeding by averaging the 4 samples obtained just prior to, and for 3 successive months, respectively, while they were on the special diet. These latter values then were employed to divide the rabbits of each series into pairs, of which each member had approximately the same degree of previous hypercholesteremia. After this was done, a plastic cannula (external diameter: 1.5 mm) was inserted under ether anesthesia into the external jugular vein of each rabbit. The catheter then was filled with saline, temporarily sealed at its open end and, by tunnelling beneath the skin of the neck, was brought out into the right external auditory canal. Folding of the ear with adhesive then protected the catheter from accidental displacement. Approximately one week after insertion of the cannula, 5 of the 10 rabbits of the first series and 5 of the 10 rabbits of the second series were placed in restraining cages and given rapidly via their catheter, 15 ml of a 4% suspension of phosphatides† in 5% dextrose, normal saline solution, followed by continuous infusion of the same fluid at a rate of 5 ml/hour for 10 hours. The remaining paired rabbits of each series were similarly treated except they received only the dextrose, normal saline infusions. The animals were rested for 48 hours and then the

* Aided by grants from Life Insurance Medical Research Fund, Nat. Heart Inst., Alameda County Heart Ass'n. and Sterling-Winthrop Research Inst.

† "Lecithin (Animal) 90% Pure," purchased from Nutritional Biochemicals Corp., Cleveland, O.

TABLE I. Effect of Repeated Infusions of Phosphatide Suspension upon Atherosclerotic Infiltration and Cholesterol Content of Rabbit Aorta.

Procedure	No. of rabbits	Avg plasma cholesterol (mg/100 ml) During cholesterol feeding	Avg plasma cholesterol (mg/100 ml) Beginning of exp.	Aorta Atherosclerosis	Aorta Cholesterol (g/100 g)
Normal rabbits	5	—	64 ± 6 (52–84)	0	.4 ± .03 (.35–.44)
Hypercholesteremic					
Series I					
(a) Dextrose infused (control)	5	175 ± 14.2 (151–205)	72 ± 7.5 (65–92)	.8 (.5–1.5)	.60 ± .02 (.49–.72)
(b) Phosphatide infused	5	187 ± 15.6 (147–280)	76 ± 6.4 (66–88)	0	.40 ± .02 (.37–.55)
Series II					
(a) Not infused (control)	4	1119 ± 42 (821–1329)	72 ± 6.8 (58–82)	5	16.1 ±1.2 (12.9–18.4)
(b) Dextrose infused (control)	5	942 ± 52 (759–1081)	68 ± 7.2 (62–78)	4.4 (4–5)	12.8 ±2.9 (4.7–22.7)
(c) Phosphatide infused	5	1006 ± 61 (802–1270)	71 ± 6.2 (58–76)	1.4 (.5–3)	3.1 ± .91 (.8–6.2)

infusion was again repeated. All rabbits of the first series received 4, and those of the second series, 5 infusions. Plasma samples usually obtained before and at the end of each infusion were analyzed for total cholesterol and phospholipid(5). At the end of the series of infusions, each of the treated rabbits and its paired, untreated control were sacrificed. The entire aorta of each was assessed visually for degree of aortic atherosclerosis according to an arbitrary scale of 0-5. A grade of 1 was employed to describe an aorta having 20% of its total internal surface involved in the atherosclerotic process; a grade of 2, to describe an involvement of 40%, etc. Thus a grade of 5 was given to an aorta exhibiting almost total involvement. In addition, a segment of aorta was obtained, air dried, and analyzed for its total cholesterol content(6).

Results. The infusion of phosphatide was found, as previously reported(1,2), to elevate both the average plasma phospholipid and cholesterol. Thus in a typical infusion, the average plasma phospholipid of the rabbits of both series was raised from 110 to 750 mg/100 ml and the cholesterol from 58 to 120 mg/100 ml. This hypercholesteremia, however, was a temporary phenomenon and usually disappeared within the succeeding 48-hour period. The animals appeared to exhibit no ill effects from the repeated phosphatide infusions.

When the 5 treated rabbits of the first series and their paired controls were autopsied and their aortas examined, it was immediately apparent that in each pair, the treated rabbit exhibted no detectable atherosclerotic infiltration (Table I). However, 4 of the 5 paired rabbits receiving only dextrose-saline solution exhibited a slight but significant degree of infiltration in the arch of the aorta and about the orifices of the intercostal arteries. The cholesterol analyses of these aortas confirmed the gross findings (Table I) in that the aortas of the treated rabbits contained considerably less cholesterol than those of their paired controls. Indeed, in this first series no significant difference, either in gross appearance or in cholesterol content, was observed between the aortas of the treated animals and those of normal rabbits.

Similar changes were observed in the aortas of the treated rabbits of the second series. Whereas the group of 4 control rabbits sacrificed before the infusion and the group of 5 rabbits given only dextrose infusion both exhibited intense and widespread confluent atherosclerotic infiltration of the aorta, extending in most instances from the semilunar valves down to the bifurcation, the aortas of the 5 treated rabbits (infused with phosphatide suspension) exhibited only scattered and

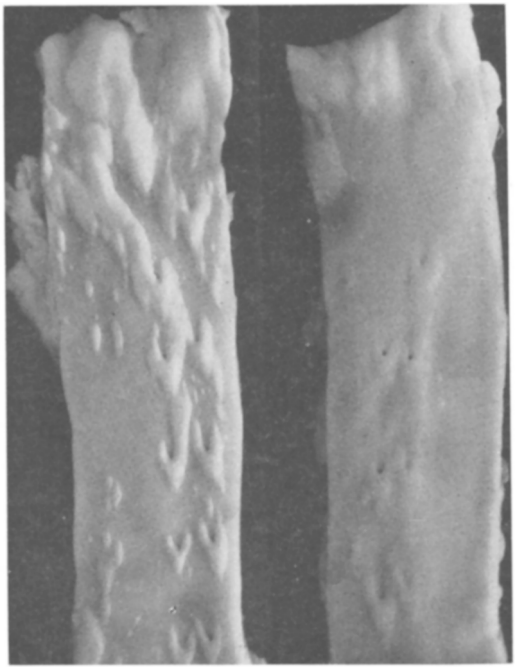

FIG. 1. Aortic segments of rabbit No. 912 (left) and rabbit No. 920 (right). Avg plasma cholesterol content of rabbit No. 912 during period of cholesterol feeding was 1174 mg/100 cc and that of rabbit No. 920 was 1159 mg/100 cc. Rabbit No. 920 received 5 infusions of phosphatide suspension. Rabbit No. 912 received only dextrose-saline infusion.

far less exuberant plaques (Fig. 1). As was expected from the gross findings, the average cholesterol content of the aortic segments of the control rabbits infused with dextrose-saline solution was 4 times as great as that found in comparable aortic segments of the rabbits treated with phosphatide.

Discussion. The difference observed in the present studies, between the aortas of previously hypercholesteremic rabbits given infusions of phosphatide suspension and those of the controls, was marked and striking. In the first series of rabbits made moderately hypercholesteremic, neither atherosclerotic infiltration nor excess cholesterol in the aorta could be detected after four infusions of phosphatide. However, 4 of the 5 paired controls exhibited some atherosclerosis and excess cholesterol in their aortas.

Perhaps even more dramatic changes were observed in the second series of rabbits. These rabbits, previously made intensely hypercholesteremic and then infused with phosphatide, exhibited far less atherosclerotic infiltration and far less excess cholesterol in their aortas than was observed in the aortas of control rabbits of a similar degree of hypercholesteremia. This was true regardless of whether these latter rabbits were examined before the period of phosphatide infusions or after a comparable period of infusions with dextrose-normal saline solution. In view of these findings, infusions of suitable phosphatide suspensions appear to us to have possible therapeutic value.

Apparently, as we suggested in a preceding study(3), the *temporary* hypercholesteremia induced in our rabbits during the infusion of phosphatide represents a mobilization of cholesterol in plasma, derived in part at least from their aortic deposits of cholesterol. This excess cholesterol in turn probably was taken up by their livers(6), converted to bile acids (7) and hence excreted.

Summary. Intermittent intravenous infusion of a phosphatide emulsion into 2 series of previously hypercholesteremic rabbits appeared to effect a marked resolution of their atherosclerotic infiltration and cholesterol deposit as judged by the findings in paired, untreated animals.

We wish to express our thanks for the technical assistance of Betty Tsai, Warren Hayashi and Clarence Omoto.

1. Friedman, M., and Byers, S. O., *Am. J. Physiol.*, 1956, v186, 13.

2. ———, PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 452.

3. Byers, S. O., and Friedman, M., *ibid.*, 1956, v92, 459.

4. Saifer, A., and Kammerer, O. F., *J. Biol. Chem.*, 1946, v164, 657.

5. Zilversmit, D. B., and Davis, A. K., *J. Lab. and Clin. Med.*, 1950, v35, 155.

6. Friedman, M., Byers, S. O., and Gunning, B., *Am. J. Physiol.*, 1953, v172, 309.

7. Byers, S. O., and Biggs, M. W., *Arch. Biochem. and Biophys.*, 1952, v39, 301.

Received April 26, 1957. P.S.E.B.M., 1957, v95.