Failure of Corn Oil and Triparanol to Prevent Hypercholesterolemia and Atherosclerosis.* (28544)

MARK L. ARMSTRONG, WILLIAM E. CONNOR AND ROBERT S. MELVILLE
Veterans Administration Hospital and the Cardiovascular Research Laboratories, Department of Internal Medicine, College of Medicine, University of Iowa, Iowa City, Iowa

Diets high in polyunsaturated fat content have been recommended for treatment and prevention of human atherosclerosis(1,2). While such diets do indeed lower serum cholesterol concentration(3), an important question is whether this decrement of serum cholesterol actually leaves the body or moves into the tissues. The studies of Gerson *et al.*, in rats fed corn oil suggest that tissue cholesterol may increase as serum levels decrease (4). A preliminary report in man has indicated no loss of sterol from the body with corn oil feeding(5).

Another approach in the therapy of atherosclerosis has been to inhibit the biosynthesis of cholesterol in the liver(6). Reduction of serum cholesterol is achieved in this way by lessening the inflow of cholesterol to the blood from hepatic synthesis.

Hypercholesterolemia and atherosclerosis invariably occur in many animal species when cholesterol and fat are added to the diet(7). Current hypotheses about the pathogenesis of human atherosclerosis are likewise centered on the nutritional imbalance and hypercholesterolemia resulting from consumption of foods containing large amounts of cholesterol and fat. The purpose of the present study was to determine the extent to which experimental hypercholesterolemia and atherosclerosis can be inhibited by a combination of a high content of polyunsaturated fat in the diet and administration of a potent inhibitor of cholesterol biosynthesis.

Methods and materials. Male New Zealand white rabbits weighing between 2.5 and 3.2 kg were housed in individual cages and given a baseline diet of Ralston Purina® chow for one week. Five control animals were continued on the baseline diet without added cholesterol or fat for the duration of experiment. Other animals were assigned to one of the following regimens.

Exp. I.† Twenty rabbits were given a dietary supplement of 18% corn oil‡ by weight and 1% crystalline cholesterol.§ The corn oil was approximately 40% of the total calories. This amount of corn oil was selected to obtain a total fat intake comparable to the usual human fat consumption in the United States. Ten of these animals also received triparanol,‖ 0.05% by weight, dissolved in the cholesterol-oil supplement. The supplements were mixed thoroughly with the standard chow. Serum cholesterol concentration was measured by a modification(9) of the method of Zak(10). Determinations were made weekly during the first month, at the end of the second month, and at the end of the third month when the animals were autopsied.

Exp. II. In this study corn oil was fed in the same proportion as in Exp. I (18% by weight), dietary cholesterol was reduced and the dose of triparanol increased. Twenty rabbits were fed 0.25% cholesterol. Ten of these animals received 0.1% triparanol in the diet. Serum cholesterol level was measured twice monthly for the first 3 months and periodically thereafter until the animals were autopsied 7 months later.

At autopsy the liver and spleen of all animals in both experiments were examined grossly for lipid infiltration. Each aorta was graded 0 to 4 for visible atheromatous change (11). The grading was done without knowledge of the experimental regimen of the animals. In Exp. II the adrenal glands were weighed and paraffin sections were examined

* Supported by grants from Iowa Heart Assn., and Nat. Heart Inst., U.S.P.H.S.

† An abstract of results of Exp. I has been reported(8).

‡ Mazola Corn Oil, Division of Corn Products Company, New York.

§ Merck Sharp & Dohme, Philadelphia, Pa.

‖ Generously contributed by Dr. T. R. Blohm, W. S. Merrill Co., Cincinnati, Ohio.

TABLE I. Effect of Corn Oil and Triparanol on Serum Cholesterol and Aortic Atherosclerosis.*

Experiment	Triparanol (dose)	Serum cholesterol (mg/100 ml) Basal diet	Serum cholesterol (mg/100 ml) After treatment	Atherosclerosis (grade)	Aortic sterol content (mg/g dry wt)	Hepatic sterol content (mg/g dry wt)
I (1% cholesterol 18% corn oil)	0	45 ± 6	2885 ± 339	1.51 ± .31		
	.05%	39 ± 6	2782 ± 190	1.73 ± .37		
Difference			−103‡	+.22‡		
II (.25% cholesterol 18% corn oil)	0	63 ± 7	596 ± 97	1.89 ± .38	21.3 ± 2.0	86.9 ± 37.9
	.1 %	53 ± 6	462 ± 94	.78 ± .15	13.0 ± 3.5	49.5 ± 19.6
Difference			−134‡	−1.11†	−8.3‡	−37.4†
Control (no added cholesterol or corn oil)	0	68 ± 21	43 ± 14	0	4.5 ± 0.3	9.0 ± 1.5

* Mean values ± S.E. † P <.02 by the t-test. ‡ P >.1 by the t-test.

microscopically after fat staining.

The sterol content of the aorta was estimated in Exp. II in terms of Liebermann-Burchard positive material. A uniform length of aorta from aortic valve to diaphragm was excised and extraneous tissue removed. The specimen was then dried at 105°C until it reached a constant weight. It was then ground to a fine powder and extracted in 20 parts 2:1 chloroform-methanol overnight. Final extraction was accomplished by bringing the mixture to a boil. The residual material was extracted 2 more times to make certain that all of the sterol was obtained by the third extraction. The chloroform-methanol extracts of the aortas were evaporated to dryness and sterol content measured by a modification of the method of Abell *et al.* (12). Hepatic sterol was determined similarly.

Results. Exp. I. Rabbits fed the diet containing the 1% cholesterol and 18% corn oil for 3 months developed pronounced hypercholesterolemia and extensive atherosclerosis. Addition of 0.05% triparanol to this regimen had no effect on either the serum cholesterol level or gross aortic atheromatosis (Table I, Fig. 1). Moderate to severe lipid infiltration of liver and spleen occurred in both groups, generally in parallel with the aortic involvement. Minor lipid deposits in the iris were found in both groups. The mean weight gain was somewhat less in the triparanol-fed rabbits (9.6% *vs* 19.9% for the rabbits given only cholesterol and corn oil).

Exp. II. Serum cholesterol levels of rabbits fed 0.25% cholesterol and 18% corn oil increased from 63 mg% to 596 mg% over the 7-month period of study. Atheromatous lesions were also formed (Table I, Fig. 1). Addition of 0.1% triparanol to this regimen may have decreased the resulting hypercholesterolemia slightly, but the difference was not significant. The triparanol-treated animals had somewhat less aortic atherosclerosis as graded visually. These animals also had a smaller content of aortic sterol, statistically not significant. Hepatic sterol in the corn oil-cholesterol-fed group increased to 86.9 mg/g from a control value of 9.0 mg/g. The animals given triparanol had 49.5 mg/g of hepatic sterol.

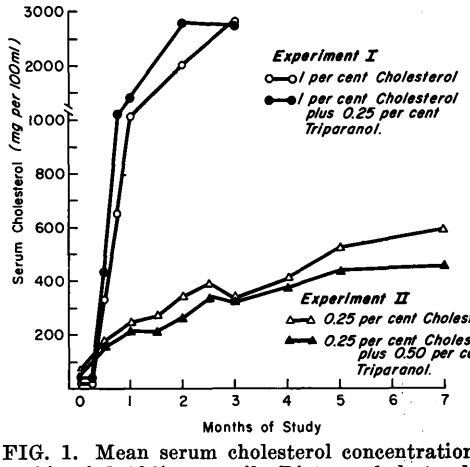

FIG. 1. Mean serum cholesterol concentrations of rabbits fed 18% corn oil. Dietary cholesterol was given at 2 levels with and without triparanol.

The mean difference in adrenal weights between the 2 groups of Exp. II was less than 2%. On microscopic examination, the cortices of the adrenal glands of both groups had similar zonal widths. The triparanol-treated animals had conspicuously less fat accumulation in the zona fasciculata, an indication of drug effect(13). No iris deposits were found in either group. The lipid infiltration of liver and spleen varied from minimal to moderate and was generally less than in Exp. I.

The gross nutritional status of the animals in the 2 groups was similar. The mean gain in body weight was 6.9% for the animals fed cholesterol and corn oil, and 1.9% for those given triparanol in addition. Control animals fed only Purina® chow had no hypercholesterolemia or atherosclerosis.

Discussion. In the presence of cholesterol in the diet, corn oil did not block the development of hypercholesterolemia and atherosclerosis. This was true at 2 levels of cholesterol intake, 1% and 0.25%. Gross atheroma formation was similar in the 2 experiments despite the difference in amounts of cholesterol fed. In Exp. II the animals were fed a lower cholesterol intake over a longer period. Since Anitschkow's report in 1912, experimental atherosclerosis has invariably been observed in the rabbit fed cholesterol dissolved in a liquid vegetable oil high in polyunsaturated fat content(14). The amount of vegetable oil fed has usually been at the 2.5 or 5% level, although in one study it was fed at the 12% level(15).

The amount of corn oil given in our study was large, 18% by weight or about 40% of the total calories. Because of its rich content of polyunsaturated fat, this oil is commonly recommended in diets for treatment of human atherosclerosis. In the rabbit even the large supplement of corn oil used in the present study did not prevent either hypercholesterolemia or atherosclerosis. Merrill showed that 10% linoleic acid in the diet exaggerated the atherosclerosis produced in the rabbit fed 2% cholesterol, probably because of enhanced cholesterol absorption(16). Atherosclerosis has also occurred in rabbits fed a diet containing shrimp(17). The polyunsaturated fatty acid content of this shellfish did not retard the formation of arterial lesions since this food also has a high cholesterol content.

Triparanol, a compound recently withdrawn from clinical use because of side effects(18), was used in this study as a prototype of agents that inhibit cholesterol biosynthesis in the liver. It had the capacity to reduce tissue and plasma sterol levels in rats(19). Furthermore, when 0.25% cholesterol was added to the chow, hepatic cholesterol was lower in those rats also given triparanol. Although depression of cholesterol biosynthesis by dietary cholesterol(20) would presumably diminish the inhibiting action of triparanol, Herndon and Siperstein found that a triparanol effect (as indicated by demosterol levels in serum and aorta) occurred in rabbits fed 2% cholesterol with 10% lard(21). Using another inhibitor, phenylethylacetate, Milhaud and Aubert reported that cholesterol biosynthesis was decreased to a greater extent when rats were also fed polyunsaturated fat(22). Nevertheless, in our study the combination of a potent inhibitor of cholesterol biosynthesis and a very large amount of corn oil did not prevent atherosclerosis.

With a different experimental design, Curran and Costello found less sterol in the rabbit aorta when endogenous cholesterol synthesis was inhibited by vanadium(23). Their animals were given a cholesterol-free diet along with vanadium after a period of cholesterol feeding. Perhaps inhibitors of cholesterol biosynthesis might block the accumulation of sterols in the arteries at lower levels of cholesterol feeding. Our studies suggest that the level of dietary cholesterol would have to be much lower than those ordinarily used in experimental atherosclerosis.

Summary and conclusions. 1. A diet high in polyunsaturated fat derived from corn oil did not prevent the development of hypercholesterolemia and atherosclerosis in rabbits fed cholesterol at the 1% and 0.25% levels. Aortic and hepatic sterol content increased greatly. 2. When triparanol, a potent inhibitor of cholesterol biosynthesis, was added to the corn oil-cholesterol feeding, it had no effect on the resulting hypercholesterolemia and atherosclerosis at the 0.05% level. Triparanol

did appear to reduce aortic atherosclerosis slightly at a dose of 0.10% and under conditions of a lessened intake of dietary cholesterol. 3. The evidence to date is poor that atherosclerosis can be controlled by the use of either polyunsaturated oils in the diet or pharmacologic agents to inhibit cholesterol synthesis as long as significant amounts of cholesterol are absorbed in the body.

1. Am. Heart Assn. Central Comm., *Circulation,* 1961, v23, 133.

2. A.M.A. Council on Foods and Nutrition, *J.A.M.A.,* 1962, v181, 139.

3. Ahrens, E. H., Jr., Hirsch, J., Insull, W., Peterson, M. L., in *Chemistry of Lipides as Related to Atherosclerosis,* ed. Page, I. H., Charles E. Thomas, 1958, 222.

4. Gerson, T., Shorland, F. B., Adams, Y., *Biochem. J.,* 1961, v81, 584.

5. Spritz, N., Grundy, S., Ahrens, E. H., Jr., 55th *Ann. Meet. Am. Soc. Clin. Invest.,* April, 1963.

6. Steinberg, D., Avigan, J., Feigelson, E. B., *J. Clin. Invest.,* 1961, v40, 884.

7. Katz, L. N., Stamler, J., *Nutrition and Atherosclerosis,* Lea and Febiger, 1958, 61.

8. Armstrong, M. L., Connor, W. E., Melville, R. S., *Circulation,* 1961, v24, 1062.

9. Connor, W. E., Hodges, R. E., Bleiler, R. E., *J. Clin. Invest.,* 1961, v40, 894.

10. Zak, B., *Am. J. Clin. Path.,* 1957, v27, 583.

11. Katz, L. N., Stamler, J., *Experimental Atherosclerosis,* Charles C Thomas, 1953, 135.

12. Anderson, J. T., Keys, A., *Clin. Chem.,* 1956, v2, 145.

13. King, W. M., *Prog. in Cardiovasc. Dis.,* 1960, v2, 504.

14. Anitschkow, N., *Arteriosclerosis, A Survey of the Problem,* ed., Cowdry, E. V., Macmillan, 1933, 271.

15. Steiner, A., Varsos, A., Samuel, P., *Circ. Res.,* 1959, v7, 448.

16. Merrill, J. M., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 268.

17. Connor, W. E., Rohwedder, J. J., Hoak, J. C., *J. Nutrition,* 1963, v79, 443.

18. A.M.A. Council on Drugs, *J.A.M.A.,* 1962, v181, 145.

19. Gould, R. G., *Prog. in Cardiovasc. Dis.,* 1960, v2, 492.

20. Morris, M. D., Chaikoff, J. M., Fells, S., Abraham, S., Fansah, N. O., *J. Biol. Chem.,* 1957, v224, 1039.

21. Herndon, J. H., Jr., Siperstein, M. D., *Circ. Res.,* 1963, v12, 228.

22. Milhaud, G., Aubert, J. P., *J. Atheroscler. Res.,* 1961, v1, 162.

23. Curran, G. L., Costello, R. L., *J. Exp. Med.,* 1956, v103, 49.

Received June 4, 1963. P.S.E.B.M., 1963, v113.

Production of Mercaptoethanol Sensitive, Slowly Sedimenting Antibody in the Duck.*† (28545)

HOWARD M. GREY‡ (Introduced by F. J. Dixon)

Division of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, Calif.

Recent studies have indicated the presence of slowly sedimenting, mercaptoethanol sensitive antibody in the antisera of certain non-mammalian vertebrates(1-3). The present study was performed in order directly to compare the mercaptoethanol sensitivity of antibody produced in the duck and rabbit exposed to identical immunization schedules and to ascertain the relation between this antibody and the mercaptoethanol resistant 7S gamma globulin antibody of the mammal.

Materials and methods. Domesticated mallard ducks, both male and female, weighing 1.5 to 2 kg and albino male rabbits weighing 2.5 to 3 kg were used. Groups of 3 ducks and 3 rabbits were immunized with Armour & Co. bovine serum albumin (BSA) by: a) Intramuscular injection of 40 mg BSA in saline, and b) intramuscular injection of 8 mg alum precipitated BSA. All groups were reinjected on day 21 with the same dose and preparation of BSA as was given initially. Serum samples

* Publication No. 49 from Division of Experimental Pathology, Scripps Clinic and Research Foundation.

† This work was supported in part by an Atomic Energy Commission Contract and U.S.P.H.S. grants.

‡ Present address: Rockefeller Institute, New York.