

The Antihypercholesteremic Effect of Essential Fatty Acids in Hypercholesteremic Dogs

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HANSEN and Wiese¹ demonstrated the dog's need for dietary unsaturated fats. These same investigators² showed serum cholesterol values were lower in dogs fed unsaturated fatty acids than in dogs fed butter. Tsai et al.³ reported later serum cholesterol values were lower in dogs fed vegetable oils.

The availability of naturally hypercholesteremic dogs afforded us an opportunity to study, under natural dietary conditions, the antihypercholesteremic effects of unsaturated fatty acids and of vegetable oils.

MATERIALS AND METHODS

Naturally hypercholesteremic dogs were obtained by screening our stock colony for dogs which had abnormal eye grounds and hypercholesteremia. The dogs were housed individually in metabolic cages, with water constantly available. They were fed twice a day, once about 11:00 A.M. and again about 4:30 P.M. In addition to one can of prepared dog food, they were given mineral-enriched whole milk supplements as indicated in the figures. Food and milk supplements were supplied on a per kilo basis and were adequate to maintain constant weights of the dogs. Plasma cholesterol levels (method of Anderson and Keys⁴) were determined weekly on each dog throughout each test period. Test materials were administered to dogs in capsule form. The control dogs received an equiv-

alent number of empty capsules. Three experiments were performed in which soybean oil, linoleic acid or ethyl linoleate were tested for their antihypercholesteremic effects. The experimental details of each test are presented with the results.

Linoleic acid was prepared in our laboratory from safflower seed oil by the method of Swern and Parker.⁵ Ethyl linoleate was prepared from linoleic acid using acid catalyzed esterification. The soybean oil emulsion was prepared to contain 60 per cent fat in an aqueous medium. The analytical data obtained on the fatty acids and fat used in these experiments are presented in Table I.

RESULTS

Experiment 1

The antihypercholesteremic effect of orally administered linoleic acid was compared with an equivalent amount of unsaturated fatty acids in soybean oil in naturally hypercholesteremic dogs (Fig. 1). Neither substance, administered orally once daily with the morning meal at a dosage of 200 mg./kg. body weight/day, had antihypercholesteremic effects in these dogs during a one-week period. When the levels of linoleic acid or equivalent unsaturated fatty acids administered as soybean oil were doubled to 400 mg./kg./day once daily, a significant decrease in plasma cholesterol was observed in the dogs receiving linoleic acid (group 2) but not in those fed soybean oil (group 3). When the unsaturated fatty acid dosage was reduced to 200 mg./kg./day during the last two weeks of the experiment, the plasma cholesterol values tended to remain

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Presented at the Seventh Annual Deuel Conference on Lipids, February 20-22, 1959, Death Valley, California.

FIG. 1. Antihypercholesteremic effect of orally administered linoleic acid or its equivalent as soybean oil emulsion in dogs. Starting average plasma total cholesterol value 162 mg. per 100 ml.

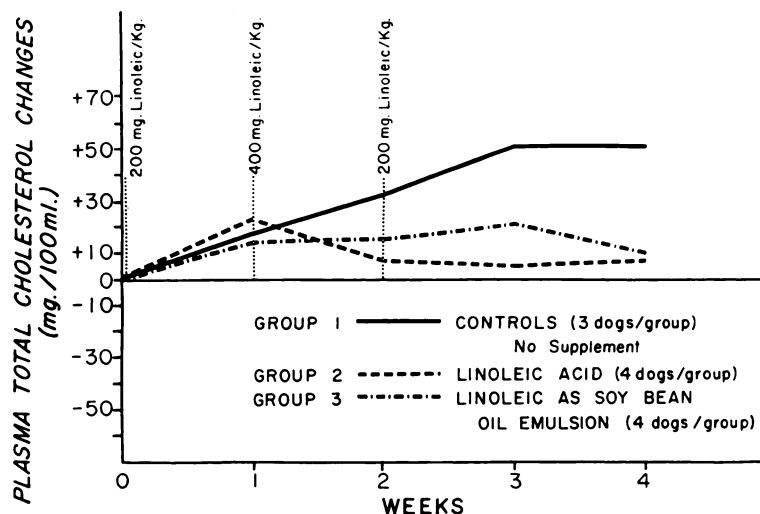


TABLE I

Composition of Unsaturated Fatty Acids, Fatty Acid Esters and Soybean Oil Fed to Dogs

Material	Purity* (%)	Iodine Value		Peroxide Value‡
		Theory	Found†	
Linoleic acid§, ¶ PJC-3-253-A	95.5	181	178	0.0
Ethyl linoleate** PJC-3-289-B	94.6	164.5	163.0	0.0
PJC-4-20-A	96.9	163.5	163.5	
Soybean oil (Lot No. 587)				
Oleic acid	24.8		127	0.95
Linoleic acid	47.2			
Linolenic acid	7.12			
Saturated fatty acids	16.6			
Free fatty acids††	<0.1			

* Method Cd-7-48, Official and Tentative Methods of American Oil Chemists Society.

† Method Cd-1-25, Official and Tentative Methods of American Oil Chemists Society.

‡ Method Cd-8-53, Official and Tentative Methods of American Oil Chemists Society.

§ Linoleic acid was isolated from safflower oil according to the method of Swern and Parker.⁵ It was purified finally by molecular distillation through a 2-inch, Rota-film molecular still. Digitalin precipitable material <0.005 per cent.

¶ Infrared study identified this material as natural *cis,cis*-octadecadien-9,12-oic acid.

** Ethyl linoleate was prepared by *p*-toluenesulfonic acid catalyzed esterification of linoleic acid and was purified finally by one molecular distillation.

†† Method Ca-5-40, Official and Tentative Methods American Oil Chemists Society.

at a lower level than was observed in the control dogs.

Experiment 2

When linoleic acid was administered orally to dogs at a level of 400 mg./kg./day twice a day at feeding time in the morning and again in the afternoon, a steady drop of plasma total cholesterol occurred during the four-week test period. This downward trend continued throughout the eleven-week observation period (Fig. 2). The total drop in plasma cholesterol values for the dogs treated with linoleic acid (group 5) averaged -69 mg. per 100 ml. (-81 and -57 mg. per 100 ml.), while the control dogs (group 4) averaged +7 mg. per 100 ml. (+56, -5 and -29 mg. per 100 ml.).

Experiment 3

This experiment tested the ability of the ethyl ester of linoleic acid to reduce plasma cholesterol. Ethyl linoleate, administered orally at a level of 200 mg./kg./day twice daily to experimental dogs, caused a consistent reduction of plasma total cholesterol (Fig. 3). The plasma cholesterol-lowering effect of ethyl linoleate was evident even when whole milk was added to the diet. The downward trend in plasma cholesterol levels continued in the dogs treated with ethyl linoleate for at least four weeks after the supplementation of

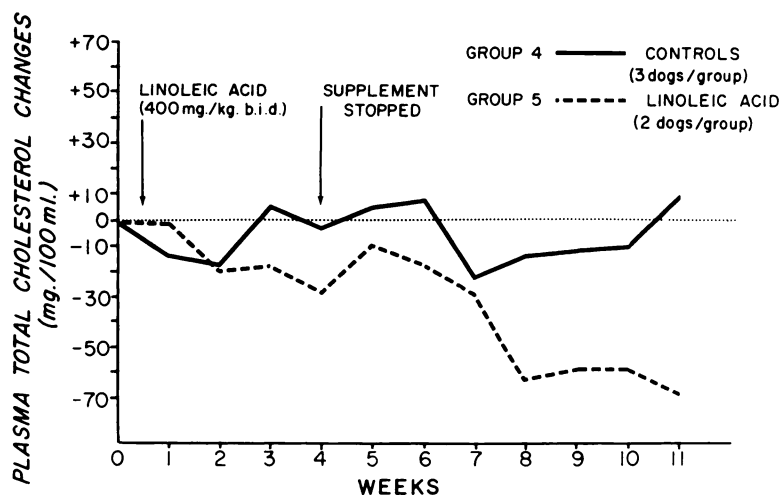


FIG. 2. Antihypercholesteremic effect of linoleic acid when administered orally at a level of 400 mg./kg. twice a day to dogs. Starting average plasma total cholesterol value 190 mg./100 ml.

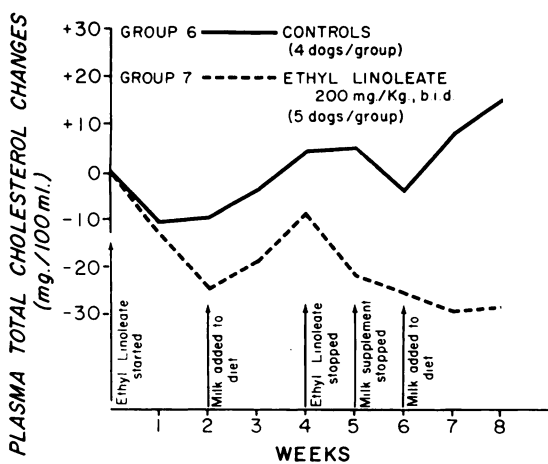


FIG. 3. Effect of ethyl linoleate on plasma total cholesterol in dogs when administered orally at a level of 200 mg./kg. twice a day. Starting average plasma total cholesterol value 186 mg./100 ml.

the ester had been stopped. These persistent postadministration antihypercholesteremic effects of ethyl linoleate were similar to those seen in experiment 2 when linoleic acid was tested.

COMMENTS

The cholesterol values of the naturally hypercholesteremic dogs used in this study may be considered to be moderately elevated or high, if one considers cholesterol levels in dogs on controlled diets (80 to 120 mg. per

100 ml.)⁶ as representing normal plasma cholesterol values for dogs. Deuel⁷ points out that essential fatty acids are required for the normal distribution and metabolism of cholesterol. Bloor⁸ noted earlier that the unsaturated fatty acids were important for the transportation and metabolism of cholesterol. In the present study both linoleic acid and ethyl linoleate, essentially free of sterols, were effective agents for reducing plasma total cholesterol; thus the identity of essential fatty acids as an active antihypercholesteremic component of vegetable oil seems established. Our observations are in accord with the findings of others who have shown that unsaturated fatty acids have beneficial effects on cholesterol metabolism.⁹⁻¹⁵

SUMMARY

Naturally hypercholesteremic dogs serve as useful animals in determining the value of unsaturated fatty acids for reducing plasma total cholesterol. Unsaturated fatty acids (linoleic or ethyl linoleate) bring about a significant and consistent reduction in plasma cholesterol.

ACKNOWLEDGMENT

We wish to thank Dr. Blaine Sutton, Organic Chemistry, for the preparation of linoleic acid and ethyl linoleate.

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