Cholesterol Content of Human Liver After Feeding of Corn Oil and Hydrogenated Coconut Oil.* (26479)

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The mechanism by which diets rich in unsaturated fats cause reduction of the serum cholesterol in human beings is not clear. It has been proposed that the reduction comes about through increased excretion of the total products of cholesterol catabolism(1), increased excretion of bile acids(2), or decreased synthesis of cholesterol(3). Another possibility, made plausible by observations in animals(4,5), is that cholesterol is shifted from the serum to the tissues. Avigan and Steinberg(5) found, for example, that rats fed corn oil showed a large rise in liver cholesterol, while rats fed coconut oil showed no significant change. Although the serum cholesterol in the animals fed corn oil was slightly lower than in those fed coconut oil, it was not lower than in the animals on a control diet, in contrast to the situation in man. Nevertheless, the hypothesis that unsaturated fats cause a shift of cholesterol from blood to liver seemed worth testing in human beings.

Methods. Twelve healthy male subjects with high normal levels of serum cholesterol were chosen from more than one hundred volunteers. The men were divided into 2 groups of 6 men each. Both groups were allowed to eat their institutional, American type diet *ad libitum*. Each man in Group I received, in addition, one ounce of hydrogenated coconut oil before each meal. Each man in Group II received a similar ration of corn oil. The experimental period was one month. Serum cholesterol was measured twice at the beginning and twice at the end of experiment, by the Abell method(6). A punch biopsy of the liver was obtained at beginning and end. In most instances 15-20 mg of wet liver were available for cholesterol analysis, which was done by a micro modification of the Abell method. The tissue specimens were first homogenized in 1 ml of water. Duplicate 0.05 ml aliquots were taken for protein nitrogen analysis by the method of Nayyar and Glick (7). Duplicate 0.2 ml aliquots were hydrolyzed and partitioned against 4 ml of petroleum ether, as in the Abell procedure for serum. Three ml portions of the petroleum ether extracts were evaporated to dryness in 12 ml conical centrifuge tubes. The residue was dissolved in 0.15 ml of Liebermann-Burchard color reagent, and 0.12 ml was removed for measurement of the optical density in microcuvettes in the Beckman DU spectrophotometer. The standard error of values obtained by this technic was 7%. Cholesterol concentrations were calculated in mg/mg of protein nitrogen, but are reported below in the more familiar units of mg/100 g of wet tissue, on the basis of a conversion factor obtained by analysis of macro specimens of normal human liver for protein nitrogen.

Results and discussion. Table I shows body weight, serum cholesterol concentration, and liver cholesterol concentration at beginning and end of experimental period for each subject. Body weights changed very little, in spite of the high caloric content of the dietary supplements. This failure of the subjects to gain weight is probably attributable to the fact that the oil was given just before meals, with an adverse effect on their appetites. Serum cholesterol concentrations after corn oil feeding dropped in all cases. Liver cholesterol concentrations also dropped, and the decrease was significant at the 0.05 level. In view of this finding, it appears unlikely that the fall in serum cholesterol produced by corn oil feeding in man is due to a shift of cholesterol from the blood to the liver.

Summary. Three ounces of corn oil daily were added to the diets of 6 men for a period of one month. Three ounces of hydrogenated coconut oil daily were added to the diets of a similar group of controls. Serum cholesterol

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TABLE I. Effect of Corn Oil and Hydrogenated Coconut Oil on Body Weight, Serum Cholesterol, and Liver Cholesterol.

Subject	Diet	Body wt Initial, lb	Body wt Final, lb	Body wt % change	Serum cholesterol Initial, mg %	Serum cholesterol Final, mg %	Serum cholesterol % change	Liver cholesterol Initial mg/100 g wet tissue	Liver cholesterol Final mg/100 g wet tissue	Liver cholesterol % change
1	Hydrog.	179½	180½	+ .6	261	301	+15	281	282	0
2	coconut	216¼	213½	−1.3	305	329	+ 8	343	315	− 8
3	oil	170½	167½	−1.8	316	316	0	1459	1218	−17
4		181½	180	− .8	267	278	+ 4	428	559	+31
5		143½	142½	− .7	280	264	− 6	353	534	+51
6		172	175	+1.7	265	273	+ 3	319	307	− 4
	Avg			− .4			+ 4*			+ 9*
7	Corn oil	219¾	215	−2.2	279	260	− 7	460	310	−33
8		172½	171	− .9	312	266	−18	910	545	−40
9		163¾	161	−1.7	294	272	− 7	331	349	+ 5
10		201¼	204	+1.4	272	245	−10	558	382	−32
11		204¼	204	− .1	325	298	− 8	531	410	−23
12		183¼	183½	+ .1	266	251	− 6	‡	763	‡
	Avg			− .6			− 9†			−25†

* Change from initial value not statistically significant.
† " " " " probably significant ($p < 0.05$).
‡ Although cholesterol was present, this specimen contained no detectable protein by the method used. It is assumed that the biopsy represented fat, rather than liver parenchyma.

concentration fell an average of 9% in the men fed corn oil, but did not change significantly in the controls. Liver cholesterol concentration, as measured by liver biopsy, fell an average of 25% in the men fed corn oil. No consistent effect was observed in the controls. It is concluded that the fall in serum cholesterol produced by corn oil feeding in man is probably not due to a shift of cholesterol from the blood to the liver.

1. Hellman, L., Rosenfeld, R. S., Insull, W., Jr., Ahrens, E. H., Jr., *J. Clin. Invest.*, 1957, v36, 898.

2. Gordon, H., Lewis, B., Eales, L., Brock, J. F., *Lancet*, 1957, v273, 1299.

3. Kinsell, L. W., Michaels, G. D., Walker, G., Conklin, J., *Circ.*, 1960, v22, 661.

4. Alfin-Slater, R. B., Schotz, M. C., Shimoda, F., Deuel, H. J., Jr., *J. Biol. Chem.*, 1952, v195, 311.

5. Avigan, J., Steinberg, D., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 814.

6. Abell, L. L., Levy, B. B., Brodie, B. B., Kendall, F. E., *J. Biol. Chem.*, 1952, v195, 357.

7. Nayyar, S. N., Glick, D., *J. Histochem.*, 1954, v2, 282.

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A Modified Plastic Petri Dish for Cell and Tissue Cultures.* (26480)

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For many years the 60 mm glass Petri dish has served as a standard culture vessel for maintenance of a variety of cell strains and tissue explants *in vitro*. Monolayer cultures have been maintained on the inside bottom of the Petri dish overlaid by a prescribed volume of liquid medium whose pH is maintained by the humidified 5% CO_2 environment of the incubator in which the culture dishes are placed. Observations of the growing cultures require an inverted microscope or replacement of the dish top with a device enabling one to bring the objective close enough so that the cells can be viewed with an ordinary microscope. Both sytems leave

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