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Effects of Saturated and Unsaturated Fat on Cholesterol Metabolism in the Rat. (23887)

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Studies in several laboratories have established the remarkable effects of unsaturated fats in lowering serum cholesterol levels in man, *e.g.*(1,2). The mechanism by which these fats act is not yet known. While effects in animals may be different in some respects from those in humans, the extra parameters that can be evaluated make animal studies worthy of exploration. Previous animal studies on effects of dietary fat have frequently been complicated by simultaneous feeding of cholesterol, of cholesterol-containing animal fat, or other atherogenic factors. In our studies a comparison is made of effects of 2 vegetable oils, corn oil and coconut oil, on rate of cholesterol biosynthesis and on distribution of cholesterol in body pools in rats on a cholesterol-free diet.

Methods. Adult male rats of Osborne-Mendel strain were fed *ad libitum* with ground Purina chow containing 20% by weight of corn oil or coconut oil. Both oil-containing diets were consumed at about same rate, supplying animals with same amount of calories. The rats given these diets had a caloric intake higher by about 35% than control rats on Purina chow alone. At an exactly defined time before sacrifice, animals were injected intraperitoneally with a tracer amount of labeled compound serving as cholesterol precursor. The rats were anesthetized with ether, killed by bleeding and livers were removed and chilled. Aliquots of liver, serum or whole adrenal glands were extracted with alcohol-acetone 1:1, saponified and the non-saponifiable fraction counted in the Packard scintillation counter. Free and esterified cholesterol in tissues were determined according to Sperry and Webb(3).

Results. Table I shows effect of diet on cholesterol levels in serum. Except in Exp. I, the shortest one, both coconut oil and corn oil fed rats had higher serum cholesterol levels than controls. Comparison of corn oil fed animals with coconut oil fed animals shows that in all experiments, irrespective of feeding period, total serum cholesterol of corn oil fed rats was lower. In 2 experiments the P value of this difference was between 0.05 and 0.1. However, the difference was never very large compared with that observed by others in man. Livers from these animals were analyzed for cholesterol content and here the effects of diet were marked (Table II). Cholesterol concentration in liver of corn oil fed animals was almost twice that in controls. The effect of feeding coconut oil was much smaller and not always significant. Of particular interest was the fact that almost all the increase was in the normally small cholesterol ester fraction. There were only small differences between groups in concentration of free cholesterol.

TABLE I. Effect of Diet on Cholesterol Concentration of Rat Serum.*

Exp.	Duration of feeding (days)	Serum cholesterol (mg %) Control diet	Coconut oil diet	Corn oil diet
I	17	68.5 ± 6.9†	77.6 ± 13.6	63.4 ± 3.2
II	20	75.1 ± 7.9	87.0 ± 10.0	82.4 ± 10.7
III	32	68.8	79.9	76.6
IV	80	60.1 ± 6.3	81.1 ± 10.9	67.7 ± 8.9

* Each figure represents mean value for 6 rats.
† ± stand. dev.

TABLE II. Effect of Diet on Cholesterol Content of Rat Liver.

Exp.		Cholesterol concentration (mg/g wet tissue) Control diet	Coconut oil diet	Corn oil diet
I	Free cholesterol	2.29	2.04	2.53
	Esterified "	.07	.57	1.93
II	Free "	2.03	1.90	2.28
	Esterified "	.28	.46	1.45
IV	Free "	1.94	1.89	2.24
	Esterified "	.11	.16	1.59

TABLE III. Effect of Sterol-Free Linoleic Acid on Liver Cholesterol Levels.*

	Linoleic acid diet	Linoleic acid diet + sterols
Liver free cholesterol (mg/g)	2.11	2.05
" esterified " (")	1.37	1.01
Serum total " (mg/%)	72.7	73.1

* Animals were on respective diets 20 days. Each figure represents mean value for 6 rats.

We next investigated the role of vegetable sterols in changes brought about by corn oil feeding. One group of rats received a diet containing sterol-free linoleic acid preparation. A second group received linoleic acid in same amount together with mixed sterols prepared from commercial corn oil. The results shown in Table III clearly indicate that, like crude corn oil, purified linoleic acid itself leads to accumulation of esterified cholesterol. This finding rules out the possibility that the sterol appearing in liver is to any significant extent contributed by vegetable sterols in oil. Addition of sterols did not significantly increase the amount of cholesterol accumulating in liver nor did it affect total serum cholesterol. This strongly suggests that unsaturated fatty acids rather than sterols in corn oil are responsible for changes in lipid composition of liver.

Rate of *in vivo* hepatic synthesis of cholesterol from 1-C^{14}-acetate was compared in rats under various dietary conditions. The results are given in the first 2 lines of Table IV, both as total counts/g of liver and as specific radioactivity. Coconut oil feeding did not influence total counts incorporated but corn oil increased it significantly. Since there was a higher concentration of cholesterol in livers of corn oil fed animals the specific radioactivity of cholesterol remained the same as in the 2 other groups. The possibility had to be considered that different dietary fats might be metabolized at different rates, affecting to a different degree the size of pools of 2 carbon fragments, which serve as precursors of the cholesterol molecule. To eliminate this possible effect of diet on rate of incorporation of labeled acetate, the study was repeated using tritiated water as the cholesterol precursor. As seen in Table IV, there was again an increase in total count incorporation in corn oil-fed animals.

Rate of isotope incorporation into cholesterol of serum and of adrenals is shown in Table V. In serum both total non-saponifiable lipid counts and specific radioactivity of cholesterol were markedly higher in corn oil-fed rats than in the other 2 groups. Total count incorporation from T_2O into adrenal non-saponifiable lipid was also markedly increased in corn oil-fed rats.

Distribution of cholesterol in cell fractions was also investigated. In cell-free liver homogenates of oil-fed rats there was a marked increase in a fat-rich fraction floating at density 1.006 on ultracentrifugation. Most of the excess cholesterol over amount found in control-fed animals was present in this layer. This "free fat" fraction increased also after cholesterol feeding as reported by other authors(4).

TABLE IV. Incorporation of 1-C^{14}-acetate and T_2O into Nonsaponifiable Lipids of Rat Liver.

Exp.	Precursor	Non-saponifiable lipid radioactivity (cpm/g liver) Control	Coconut	Corn	Cholesterol spec. radioactivity (cpm/mg) Control diet	Coconut oil diet	Corn oil diet
I	1-C^{14}-acetate	1524 ± 480*	1478 ± 848	3528 ± 1378	643	584	685
IV	"	438 ± 204	389 ± 86	693 ± 346	218	192	183
II	T_2O	161 ± 38	181 ± 97	225 ± 98	88	92	78

* ± stand. dev.

TABLE V. Incorporation of 1-C^{14}-acetate and T_2O into Nonsaponifiable Lipids of Rat Serum and Adrenals.

Tissue	Exp.	Precursor	Non-saponifiable lipid radioactivity			Cholesterol spec. radioactivity (cpm/mg)		
			Control	Coconut	Corn	Control diet	Coconut oil diet	Corn oil diet
			(cpm/ml)					
Serum	I	1-C^{14}-acetate	152 ± 33*	179 ± 66	266 ± 72	220	250	416
			(cpm/mg tissue)					
Adrenals	II	T_2O	1.33 ± .45	1.02 ± .41	2.72 ± 1.44			

* ± stand. dev.

Discussion. Our results clearly indicate that feeding rats corn oil, or linoleic acid, the major component of corn oil, increases markedly the *liver* cholesterol pool. At the same time the serum cholesterol is to a small, but significant extent lower than in rats receiving coconut oil. Both oil-fed groups have higher serum cholesterol levels than controls. The increased accumulation of cholesterol in livers of rats fed with unsaturated oils was also observed by Grunbaum *et al.*(5), who used sunflower oil, and Brown and Lewis(6), for soybean oil. The latter authors also report some tendency for lower serum cholesterol where the hepatic cholesterol was highest.

The present data also show that on feeding corn oil, the overall rate of C^{14}-labeled acetate or T_2O incorporation increases, which probably represents an elevated net synthesis/unit time. If a steady state is prevailing, and this is probably a fair assumption in long term experiments, the rate of excretion or breakdown of cholesterol must also be increased in corn-oil fed animals. Recent studies by Hellman and coworkers suggest that this is the case in man(7). The finding of an increased rate of incorporation of isotopic precursors into non-saponifiable lipids in livers rich in cholesterol seems to contradict the prevailing theory of homeostatic control of cholesterol synthesis in this organ. One should note, however, that in livers of cholesterol-fed rats the concentration of free cholesterol is markedly increased over the controls(4), whereas in corn oil-fed animals the amount of this fraction is only very slightly elevated (Table II). On feeding corn oil the size of cholesterol pool in rats increases but at the same time the partition within this pool between plasma and liver is affected by some unknown mechanism in such a way that there is less cholesterol in blood. If qualitatively a similar situation exists in man one should, perhaps, look at the change in partition of cholesterol between the 2 compartments as a key to understanding of the effect of unsaturated fats on serum cholesterol. Information on this point would certainly be of importance in any final evaluation of dietary therapy of hypercholesterolemia.

Summary. 1. Rats fed a diet containing 20% corn oil have significantly lower concentrations of serum cholesterol than those fed equal amounts of coconut oil but both high-fat diets lead to elevation of serum cholesterol above that seen on control diets. 2. There is a very marked increase in esterified cholesterol of livers of rats fed corn oil whereas coconut oil-fed rats show no significant changes in liver cholesterol compared to controls. 3. Rate of incorporation of 1-C^{14}-acetate or of T_2O is higher in corn oil-fed rats than in others. 4. Some implications with respect to the mechanism by which unsaturated fats alter cholesterol metabolism are discussed.

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