intake of copper and that there is a close correlation between serum and liver copper concentrations.

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Bile Acid Metabolism, Dietary Fats, and Plasma Cholesterol Levels.* (24094)

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The discovery of plasma cholesterol lowering properties of ingested highly unsaturated fats(1,2) has stimulated extensive study concerning the possible mechanism by which this effect is mediated. Most research has been focused upon possible changes in plasma transport or tissue disposition of cholesterol, while little attention has been directed toward possible intra-intestinal effects of ingested unsaturated fats. However, recently Gordon *et al.*(3) have reported an increased excretion of bile salts in feces of individuals ingesting sunflower seed oil (iodine No. 135) as against those ingesting hydrogenated coconut oil (iodine No. 6). Recently, we observed(4) that when absorption of bile acids (but not of cholesterol) was diminished in rats by ileal reimplantation of the common bile duct, a marked reduction in plasma cholesterol concentration occurred. In the present study the effects of ingested highly unsaturated fats upon both the flow and constituents of bile and upon absorption of cholesterol itself are reported.

Methods. A. *Acute effect of ingested highly unsaturated fats upon intestinal absorption of cholesterol.* To determine the possible effect of a highly unsaturated fat upon intestinal absorption of cholesterol, a group of 9 healthy male adult rats (Long-Evans strain) were starved for 24 hours, then given 50 mg of cholesterol dissolved in 3 ml of soy bean oil (iodine No. 121; Liebermann-Burchard positive material = 4.6 mg/ml calculated as cholesterol). Another group of 5 rats was given the same amount of cholesterol in 3 ml of corn oil (iodine No. 120; L-B positive material = 11 mg/ml). For control purposes, one group of 5 rats was given 50 mg of cholesterol in 3 ml of warmed lard (iodine No. 59; L-B positive material = 1.2 mg/ml), and another group of 5 rats, the same amount of cholesterol in 3 ml of coconut oil (iodine No. 6; L-B positive material = 1.1 mg/ml). Immediately after feeding, all rats were subjected to cannulation of intestinal lymph duct(5) and lymph collected for 24 hours was analyzed for cholesterol(6). B.

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TABLE I. Effects of Ingestion of Unsaturated Fats on Cholesterol Absorption.

	No. of rats	Avg wt (g)	Intestinal lymph (24 hr) Avg vol (ml)	Total cholesterol mg/100 ml	Total cholesterol mg/24 hr
A. Rats given soybean oil (iodine No. 121; L-B + = 4.6 mg/ml)	9	276	46.9	51	22.8
			* 6.4	3.1	2.1
B. Rats given corn oil (iodine No. 120; L-B + = 11 mg/ml)	5	255	28.6	82	22.3
			* 8.4	5.1	2.6
C. Rats given lard (iodine No. 59; L-B + = 1.2 mg/ml)	5	302	24.9	58	14.0
			* 6.8	6.2	2.7
D. Rats given coconut oil (iodine No. 6; L-B + = 1.1 mg/ml)	5	265	25.4	38	8.9
			* 4.8	4.1	3.2

* S.E. of mean.

Chronic effect of ingested unsaturated fats upon (1) Plasma cholesterol and (2) Bile cholesterol and cholate. A group of 6 rats was fed 10% suspension of walnut oil† (iodine No.‡ 131.5; L-B positive material = 3 mg/ml) for 7 days. A second group of 6 rats was fed 10% suspension of coconut oil§ (iodine No. 6; L-B positive material = 1.1 mg/ml) for 7 days. A third group of 6 rats was fed a special fat and sterol free diet.‖ Rats on the oil diets each ingested approximately 65 ml of the suspension/day. Plasma samples were obtained at beginning and end of the 7 day period and analyzed for total cholesterol. The rats then were subjected to cannulation of their bile duct(7) and the 24 hour bile collection was analyzed for its cholesterol and cholate contents according to previously described methods(8).

Results. Table I indicates almost twice as much sterol found in lymph after administration of cholesterol with either of the highly unsaturated fats as compared with that found when cholesterol is administered in either lard or coconut oil. That this sterol is cholesterol is indicated by the finding that lymph contained the same quantity of Liebermann-Burchard positive material regardless of whether cholesterol was given in soybean oil, containing 4.6 mg/ml plant sterol, or in corn oil with over twice this amount of sitosterols.

Table II indicates that as early as 7 days, continued ingestion of a highly unsaturated fat resulted in a plasma cholesterol significantly lower than in rats given either a relatively saturated fat or a diet containing neither fat nor sterol. Excretion of bile cholesterol also was significantly higher in these rats fed walnut oil. Total amount of cholate excreted by rats given walnut oil was twice as great as that observed in rats ingesting a sterol free diet and 26% greater than that excreted by rats given coconut oil.

Discussion. The present results indicate that, whatever mechanism effects a reduction in plasma cholesterol level of the animal fed highly unsaturated fats, does not involve a hindrance to intestinal absorption of sterol, because the latter is not hindered and may well be enhanced by administration of such fats.

If bile cholesterol excretion can be employed, as we think it can be, as index of hepatic rate of turnover of cholesterol(9), then it is clear that following ingestion of a highly unsaturated fat, a marked increase in cholesterol turnover occurs. This present finding confirms the similar conclusion reached by Avigan and Steinberg(10) who measured the hepatic turnover of cholesterol with radioactive carbon.

The increased rate of cholesterol turnover after administration of unsaturated fat is not followed by any increase in cholesterol content

† Walnut oil, 100 g; casein, 120 g; emulsifying agent, 3 g; NaCl, (20%), 35 ml; KCl (20%), 47 ml; distilled H_2O, 960 ml.

‡ Determined by Dr. George D. Michaels.

§ Exactly similar to † except 100 g of coconut oil substituted for walnut oil.

‖ Cane sugar, 7100 g; Na Caseinate, 2,500 g; NaCl, 150 g; Salts W., 200 g (Nutritional Biochemicals Corp.) Vit. mixture "Litrison" (R) (Hoffmann La-Roche).

TABLE II. Effect of Ingestion of Unsaturated and Saturated Fats upon Plasma Cholesterol and Bile Cholesterol and Cholate.

No. of rats	Avg wt			Plasma cholesterol (mg/100 ml)			Bile†				
								Total cholesterol		Total cholate	
	Beg.	7 days	21 days	Beg.	7 days	21 days	Vol	mg/100 ml	mg/24 hr	mg/100 ml	mg/24 hr
Walnut oil (iodine No. 131.5; L–B + = 3 mg/ml)											
6	276	237	—	56	43	—	11.8	17.7	2.1	459	58
				* 3.1	2.0				.16		3.7
Coconut oil (iodine No. 6; L–B + = 1.1 mg/ml)											
6	260	224	—	60	62	—	10.1	16.3	1.6	436	43
				* 2.4	4.2				.05		2.5
Sterol-free diet											
6	272	259	—	58	52		12.2	9.6	1.2	331	25
				* 3.2	1.7				.17		2.1

* S.E. of mean. † Bile collected for 24 hr, after 7 days on respective diets.

of plasma(11) and indeed, not always followed even by increase in content of cholesterol in the liver(12). This of course suggests that the newly formed cholesterol is being converted further. In view of the fact that the principal end product of cholesterol metabolism in the body is bile acid(13), the implication is strong that the liver of the animal fed highly unsaturated fats is producing an increased amount of bile acids. This possibility is greatly strengthened by the present findings of an increased biliary excretion of bile acids after feeding of walnut oil and by the recent observation of Gordon *et al.*(3) that there is an increased fecal excretion of bile acids in persons fed sunflower seed oil. This last observation suggests either that an excess of bile acid is being excreted or that less is being reabsorbed. It therefore seems likely that enhanced removal of bile acid from the body provokes increased cholesterol turnover. This suggestion is reinforced by the finding of Beher and Baker(14) that *feeding* of cholic acid greatly *reduces* rates of synthesis and mobilization of cholesterol in the rat.

In this connection it is of interest that when an animal is induced to excrete more bile acid as occurs in the hyperthyroid state(15) or when bile acid reabsorption is interfered with (4), plasma cholesterol promptly falls. It is our suggestion that a similar mechanism is in play in the observed reduction in plasma cholesterol of the subject ingesting highly unsaturated fats.

Summary. 1. Administration of highly unsaturated fats to the rat effected a greater intestinal absorption of cholesterol than similar administration of saturated fats. 2. Following continued ingestion of highly unsaturated fat, there was evidence that the fall in plasma cholesterol observed was accompanied by a marked increase in excretion of both cholesterol and bile acid in the bile. 3. The possible relationship of reduction in plasma cholesterol to the observed change in bile cholesterol and bile acid excretion is discussed.

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Incorporation and Conversion of Lysine-2-C^{14} in Rat Biopsy-Connective Tissue. (24095)

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The amino acids, hydroxyproline and hydroxylysine, are found chiefly in collagen and are responsible in part for stability of the fiber(1,2). Hydroxyproline cannot be incorporated directly into the peptide chain of collagen, but a portion of the proline becomes hydroxylated following its incorporation(3). Presumably lysine and hydroxylysine are involved in collagen formation in a similar manner because only labeled lysine fed to an animal is incorporated into collagen and a portion appears as hydroxylysine(4).

The present report deals with incorporation, distribution and rate of turnover of C^{14}-labeled lysine in rat connective tissue obtained by sponge implantation technic(5). Conversion of labeled lysine to hydroxylysine in connective tissue of different ages and from both sexes is also presented.

Methods. Sprague-Dawley rats of approximately 6 months of age and weighing 200 to 250 g were used. All animals included were given a single intraperitoneal injection of 1 ml of neutralized dl-lysine-2-C^{14} monohydrochloride (9.45 mg or 0.024 mc) in physiological saline. Three polyvinyl sponges (Ivalon†) were implanted subcutaneously along dorsum of rat. Previous histological work indicates growth of normal appearing connective tissue into the interstices of the sponge(5). The fact that rate of collagen formation in sponge biopsy(6) was similar to that found by Abercrombie *et al.* in wound healing in the rat(7) suggests comparable metabolic rates for the 2 connective tissues. Incorporation of labeled lysine and formation of tagged hydroxylysine were studied in connective tissue of 2 male and 2 female rats, 21 days after sponge implantation and in one male and one female rat 300 days after implantation. The tissues were removed 16 hours following intraperitoneal injection. Sponge-tissues were weighed, homogenized with 10 ml of physiological saline in VirTis "45" Macro Homogenizer‡ for 5 minutes with rheostat reading at 50, and centrifuged at approximately 15,000 x g at 5°C for 1 hour to obtain the saline-soluble protein. The residue was washed with distilled water and centrifuged at approximately 15,000 x g at 5°C for 30 minutes, and the wash discarded. The resultant residue was shaken with 5 ml of 0.5 M acetic acid at 5°C for three 24-hour periods in 12 ml plastic tube with rubber stopper. After each 24-hour period, the plastic tubes were centrifuged at 90,000 x g in a Spinco Model E ultracentrifuge for 1 hour and the acetic acid-soluble protein decanted. The 3 acetic acid extracts were combined. Protein of the saline-soluble fraction was precipitated with 4 volumes of 10% trichloroacetic acid. The trichloroacetic acid precipitate of the saline, the acetic acid-soluble protein and the residue were hydrolyzed with 6 N HCl in sealed pyrex tubes at 110°C for 16 hours. Nitrogen was determined by Conway microdiffusion technic(8) and converted to a protein value by factor 6.25. An aliquot of the hydrolysate was evaporated to dryness on steam bath to remove excess HCl, neutralized and again evaporated to dryness. The neutralized samples were dissolved in citrate buffer, pH 5.0, to concentration of 15-20 mg

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