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Received September 3, 1959. P.S.E.B.M., 1959, v102.

Absorption of Cholesteryl Methyl Ether and Its Effect on Cholesterol Absorption. (25359)

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Investigation of the role of esterification in absorption of cholesterol would indicate that during the absorptive process most of the cholesterol was esterified(1,2,3,4). However, Hernandez and associates(5) reported that epi-cholesterol, which is not esterified, was absorbed into the lymph of rats at about half the rate of cholesterol. This would suggest that esterification, although not obligatory, probably aids absorption. In addition, inhibition of cholesterol absorption by sitosterol is believed to be brought about by interference with its esterification(6). It was of interest to us therefore to study absorption of a sterol which could not be esterified. Accordingly, the methyl ether of cholesterol* was prepared and its absorption and effect on cholesterol absorption investigated in both rat and rabbit.

Methods. Cholesteryl-4-C^{14} methyl ether was prepared by the method of Stoll(7) scaled down to mg quantities. In the final reaction, approximately 100 mg of p-toluene sulfonic acid ester of cholesterol-4-C^{14} was refluxed in absolute methanol to yield 64 mg of the methyl ether of cholesterol-4-C^{14} M.P. 81-82° Fisher M.P. block (reported 84°), specific activity (SA) 8300 cpm/mg. A paper strip chromatogram by the method of Hansen and Dam(8) indicated presence of a single spot (phosphomolybdic acid indicator) Rf 0.95 far removed from the cholesterol location, Rf 0.58, which coincided with the single peak of radio activity when analyzed on an Atomic Accessories chromatogram scanner. It is of interest that this compound was quantitatively precipitated by digitonin and gave essentially the same color value as cholesterol with $FeCl_3$-conc. H_2SO_4 color reagent(9). Unlabeled cholesteryl methyl ether to be used for diluting the labeled compound was prepared by the same procedure. This was analyzed for O-CH_3 and contained 7.51% O-CH_3; calculated 7.73%. The thoracic lymph duct of adult male rats, weighing 165-200 g, of Wistar strain, and which had been maintained on Purina Lab. Chow was cannulated by the Bollman technic(10). The animals were allowed only 0.9% saline *ad lib.* The following morning, those animals which exhibited good lymph flow (greater than 40 ml in 16 hours) were given, by intubation, 1 ml of an emulsion containing the labeled sterol. The emulsion, essentially that described by Swell and associates(4) with omission of glucose, was prepared by homogenizing 50 mg of labeled sterol, 50 mg egg albumin,† 150 mg of sodium taurocholate,† 0.16 ml oleic acid USP and 1.8 ml 0.9% saline. The final volume contained approximately 25 mg sterol and 90,000 cpm/ml. Lymph was collected with addition of heparin and stored at 5°C until analyzed. To determine overall recovery of C^{14} activity, 0.1 ml samples of lymph were plated directly on aluminum planchets and counted in a Packard Flo-Window Counter. The remaining samples were lyophilized and pulverized for further work-up. A sample of lyophilized lymph was extracted with low boiling petroleum ether (30°-60°) by gentle refluxing for 1 hour. At least 80% of the radio activity was extractable by this method. An aliquot of the extract was applied to a silicic acid column for analysis of cholesterol esters, cholesteryl methyl ether and free cholesterol. This column was prepared as described(11), the extract ap-

* 3β methoxy, 5 cholestene as designated in *Chem. Abst.*

† Obtained from Fisher Scientific Co.

plied and sterols eluted with chloroform-petroleum ether, 1:1. One ml fractions were collected, and analyzed for radio activity by direct plating and for sterol by the $FeCl_3$ colorimetric method(9). Male, Dutch Belted rabbits approximately 1.5 kg weight, were divided into groups of 6 and fed sterol supplemented diets. These diets were prepared by spraying Wayne rabbit pellets with an ether or chloroform solution of sterol and then air drying. Composition of diets is indicated in Table II. At weekly intervals blood samples from 3 rabbits/group were obtained for cholesterol analysis using the Trinder(12) saponification and extraction procedure, followed by $FeCl_3$ colorimetric method(9). At termination of experiment, the aorta including the arch and heart were examined grossly for evidence of plaque formation. These were scored from 0 to 3 depending upon involvement of the arch and thoracic aorta. The average of these scores gave the composite atheroma value.

Results. These studies may be divided into 2 aspects: absorption in the rat *via* lymph and absorption in the rabbit as a dietary supplement.

Absorption of methyl ether of cholesterol-4-C^{14} in the rat. Table I shows recoveries obtained on oral administration of labeled sterol. The data indicate that the methyl ether of cholesterol was absorbed, although to a lesser extent than cholesterol.

Identity of sterol-C^{14} in lymph. Petroleum ether extracts of lyophilized lymph were analyzed on silicic acid columns as described above. Preliminary studies with authentic samples of cholesterol esters, cholesteryl methyl ether and free cholesterol located these compounds in the folowing 1 ml fractions: 2-5, 8-13, and 22-28 respectively. Typical chromatograms of lymph extracts from cholesterol-4-C^{14} or cholesteryl-4-C^{14} methyl ether fed animals are shown in Figs. 1 and 2 respectively. Cholesterol-4-C^{14} appeared in lymph primarily in the esterified form. Appreciable dilution with endogenous cholesterol esters was observed since cholesterol administered possessed a SA of 3900 cpm/mg, while that recovered from lymph had a SA of 1300 cpm/mg. However, in the case of the methyl ether of cholesterol-4-C^{14} there was no significant change in specific activity between administered and recovered compound.

TABLE I. Absorption of Sterols in the Rat as Measured by Recovery of C^{14} Activity in Lymph.

Sterol administered*	Lymph vol, ml†	% C^{14} recovered
Methyl ether of cholesterol-4-C^{14}	106 (56-152)	19.4 (16.9-24.6)
Cholesterol-4-C^{14}	107 (51-158)	35.5 (31.6-37.9)

* Administered orally, as an emulsion containing approximately 25 mg sterol and having 90,000 cpm. 4 rats/group.

† Twenty-four hr lymph collection. Values in parentheses are ranges.

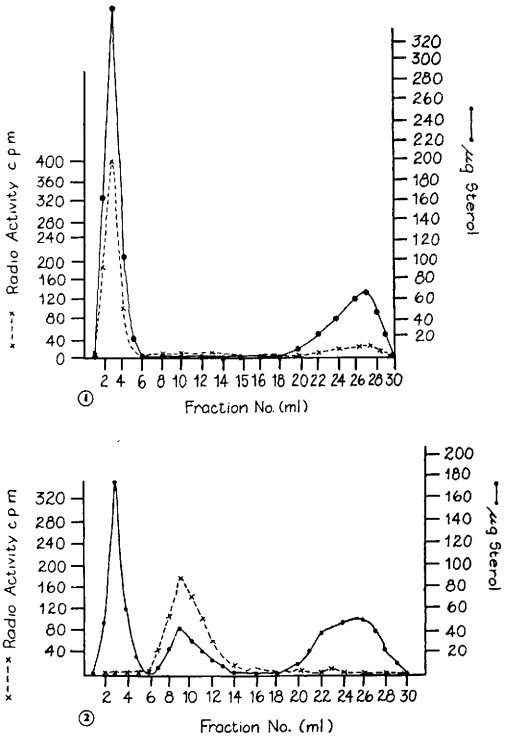

FIG. 1. Silicic acid column analysis of petroleum ether extract of lyophilized lymph from rat fed cholesterol-4-C^{14} (SA 3900 cpm/mg). Fractions 2-5, cholesterol ester (SA 1300 cpm/mg). Fractions 20-30, free cholesterol (SA estimated at less than 300 cpm/mg).

FIG. 2. Silicic acid column analysis of petroleum ether extract of lyophilized lymph from rat fed the methyl ether of cholesterol-4-C^{14} (SA 3700 cpm/mg). Fractions 7-12, cholesteryl methyl ether (SA 4000 cpm/mg).

Effect of cholesteryl methyl ether on cholesterol-4-C^{14} absorption in the rat. Lymph duct cannulated rats were prepared as described and fed 1 ml of test emulsion by intubation. The emulsion was prepared as de-

TABLE II. Effect of Cholesteryl Methyl Ether on Cholesterol Absorption in the Rabbit.

Group*	Diet	Avg serum cholesterol, mg % 2nd wk	4th wk	6th wk	8th wk	Atheroma‡
A	1% cholesteryl methyl ether	46.0 ± 71†	67.7 ± 15	62.3 ± 39	80 ± 64	.1
B	3% cholesteryl methyl ether + 1% cholesterol	542 ± 335	727 ± 329	685 ± 214	890 ± 245	1.75
C	1% cholesterol	916 ± 763	1192 ± 940	1188 ± 885	1070 ± 406	2.4
D	Pellets without supplement	35.0 ± 10.3	29.6 ± 9.5	25.3 ± 15	30 ± 15.8	.2

* 6 rabbits/group. † Stand. dev. ‡ Avg of both scores for the arch and thoracic aorta.

scribed above but modified so that each ml contained 10 mg cholesterol-4-C^{14} (100,000 cpm) and 40 mg of cholesteryl methyl ether. Lymph was collected for 24 hours and recovery of radioactivity determined as described. In a group of 4 rats the average recovery of administered C^{14} activity was 33.7% with a range of 27.5 to 38.8%. This data would indicate that as much as 4-fold quantities of cholesteryl methyl ether did not appreciably inhibit absorption of cholesterol.

Effect of dietary cholesteryl methyl ether on cholesterol absorption in the rabbit. This was studied by feeding rabbits a diet in which cholesterol and/or cholesteryl methyl ether was incorporated in pellets as described. Table II shows serum cholesterol values obtained during experiment and the final group atheroma index on termination. There is no significant difference between groups B and C in both serum cholesterol level and atheroma when evaluated by the Rank method of Wilcoxon(13). It is of interest to note that cholesteryl methyl ether alone had relatively slight effect on serum cholesterol.

Discussion. Absorption of cholesteryl methyl ether is an example of sterol absorption without esterification. Cholestenone(14) and epicholesterol(5) cited above, are other instances of this phenomenon which would indicate that esterification is not indispensable although it probably speeds absorption.

Studies in lymph duct cannulated rats indicated appreciable absorption of cholesteryl methyl ether and one would therefore expect significant elevation of serum sterol levels in the rabbit fed 1% cholesteryl methyl ether. That this did not occur may be indicative of a species difference between rabbit and rat with respect to absorption of cholesteryl methyl ether or that it may be rapidly metabolized or removed from the circulation soon after absorption. The answer to this question awaits further investigation.

Recently Blomstrand and Ahrens(15) reported cleavage of ether linkage of chimyl alcohol during absorption in man which confirmed earlier report in rats(16). This is of interest since no evidence of ether cleavage was noted in absorption of cholesteryl methyl ether reported here.

Summary. Oral administration of the methyl ether of cholesterol-4-C^{14} to lymph duct cannulated rats resulted in approximately 20% recovery of C^{14} activity in a 24 hour lymph collection as compared to 35% recovery in the case of cholesterol-4-C^{14}. Simultaneous administration of cholesteryl methyl ether with cholesterol-4-C^{14} had no effect on cholesterol-4-C^{14} absorption in the cannulated rat. In the rabbit, dietary cholesteryl methyl ether had negligible effect on sterol levels when given alone or in conjunction with cholesterol.

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Received September 4, 1959. P.S.E.B.M., 1959, v102.

Pressures in the Common Hepatic Duct of the Rat. (25360)

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The first measurements of pressure in the common hepatic duct in the rat were reported by Mann and Foster(1), and the sphincteric action of the intraduodenal portion of the duct in animals including the rat was later investigated by Mann(2). Reports on pressure in the extrahepatic bile ducts of other animals followed(3-6), but measurements of the secretory pressure of bile in albino rats were not reported until Friedman, Byers and Michaelis (7) included observations on a small series as part of a study on production and secretion of cholesterol in mammals. In these studies pressures were measured with the water manometer. Kelsey and Beard(8) measured pressures in the common bile duct of human beings with a strain-gauge manometer. Since this manometer is more convenient for continuous recording than the water manometer, we repeated some of previous studies of hepatic duct pressures in the normal rat and studied also the pressure after certain induced hepatic injuries.

Methods. Adult albino rats of Sprague-Dawley strain were used. Rats weighed between 200 and 400 g. The rats selected for study were of 3 types: (a) rats with normal livers, (b) rats with hepatic cirrhosis induced by inhalation, in a closed chamber, of 25 mg of carbon tetrachloride/minute in 50 liters of air, for 6 hours, 3 times a week for 6 weeks and once a week for additional 4 weeks and (c) rats with passive venous congestion of the liver induced by application of a cellophane band around the inferior vena cava above the liver 4 weeks previously. The rats were fasted routinely for 16 hours before any observation of pressure was made. Under ether anesthesia the common hepatic duct of each rat in the study was isolated and ligated at its midpoint. Two lengths of polyethylene tubing (P.E. 50) with internal diameter of 0.58 mm were introduced into the duct; the first was directed toward the liver and the second toward, but not entering, the opening of duct into the duodenum. The 2 tubes were brought out through the skin. Free flow of bile into the duodenum could be restored by joining the ends of the tubes with a cuff of polyethylene tubing. After operation the rats were immobilized in a Bollman cage(9) and initial recordings of pressure were made at time intervals varying from a half hour to 16 hours after operation. During the intervening period the animals were allowed free access to drinking water. The pressure in the common hepatic duct was recorded by means of a strain-gauge manometer (Statham P6-4G-250, 0-4 P.S.I.) equipped with a 3-way stopcock. This system was filled with water, and all air excluded from the system before the test. The ends of the polyethylene tubes leading from hepatic duct, were connected to the stopcock, and the strain-gauge output was recorded on a photokymograph. The pressure transducer was referred to the level of the exit of the tubes through the skin of the animal, which closely approximated the openings