deposition of cholesterol in the liver. Also, in all experiments, rats fed a saturated fat supplemented diet always had higher cholesterol levels in plasma and lower cholesterol levels in the liver than did animals fed the unsaturated fat diet.

It seems reasonable to suggest some yet unclarified effect of dietary fat upon the distribution of cholesterol in the animals. It is highly suggestive that in rats the various fats effect the partition of cholesterol between the plasma and the liver, which may explain in part the mechanism of the lowering effect of plasma cholesterol by unsaturated fat.

Summary. A series of experiments was undertaken in an attempt to demonstrate the effects of dietary fats on plasma and liver cholesterol concentration in hyper-, eu- and hypothyroid rats. The lowering effect of unsaturated fat on plasma cholesterol seems to be independent of the thyroid state of the animal; that is, regardless of whether the animal is hyper- eu-, or hypothyroid, cholesterol levels of plasma are always lower when animals are fed cottonseed oil than when they are fed the same amounts of butter. In contrast to these effects on plasma, concentration of cholesterol in liver was always higher in animals which were fed unsaturated fat at any of the thyroid states.

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Effect of Monoglycerides on Absorption of Cholesterol from the Intestine and Turnover Rate of Cholesterol Esters in Plasma and Liver of the Rat.* (28901)

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The mechanism by which cholesterol is absorbed from the intestine has been intensively investigated in the past decade. Swell *et al* (1,2) and Treadwell *et al*(3) suggested that absorption of cholesterol proceeds mainly in conjunction with its esterification in the intestinal mucosa. They reported good evidence to support the theory that cholesterol (exogenous and endogenous) passes into the intestinal mucosa as free cholesterol and there it becomes esterified with certain long chain fatty acids. Their *in vivo* studies have been substantiated indirectly by the *in vitro* studies of Murthy *et al*(4,5) who showed that the intestinal mucosa preferentially esterified cholesterol with unsaturated fatty acids.

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TABLE I. Composition of Emulsified Diet.

Ingredient	Quantity
Fat	20 g
Dried skim milk solids	10 "
Cornstarch	5 "
Fresh rat-bile	25 ml
5% Dextrose in normal saline solution	55 "

These cholesterol esters are then incorporated into the intestinal lymph, which enters the blood circulation *via* the thoracic duct. The fate of the absorbed cholesterol from here on is not well understood but it is believed that cholesterol (as esters) then enters the exchangeable cholesterol pool of the liver(6). Klein(7) suggested that one possible effect of the dietary polyunsaturated fat is to esterify a large portion of cholesterol in the metabolic pool with polyunsaturated fatty acids and these cholesterol esters may have a faster renewal rate when compared with cholesterol esterified with saturated fatty acids.

This report shows the influence of various monoglycerides on rate of absorption of cholesterol from the small intestine and the partition of cholesterol between plasma and liver of the rat.

Methods. A surgical procedure was developed by which blood samples from the rat could be obtained at any desired time or time interval. A mid-abdominal incision was made on male albino rats and a polyethylene tube (No. 20) was introduced into the stomach through a small hole. After closing the abdominal wall, another incision was made in the anterior region of the neck and the descending part of the aorta was cannulated (8). This tube was filled with physiological saline and the free end was flame-sealed. After these surgical procedures the rats were placed in restraining cages and allowed to recover for a period of 3 days.

An emulsified diet (Table I) was injected into the stomach through the gastric cannula. Glyceryl-monostearate, monooleate or monolinoleate was the source of fat in each diet used.

All rats received 10 ml of 5% glucose in saline through the gastric cannula on the first postoperative day. On the second day, a 5 g portion of the formula diet was given. On the 3rd postoperative day 3 μc, (60 mc/mM or about 28 μg) of cholesterol-4-C^{14} were included in the 5 g of formula diet. Blood samples were collected from the aortic cannula in capillary tubes at time intervals over a period of 18 hours. The plasma was analyzed for cholesterol esters and free cholesterol by quantitative glass paper chromatography(9). Radioactivity was measured by liquid scintillation counting of samples isolated by glass paper chromatography(10). Animals were sacrificed at 9, 12 and 18 hours after administration of the labeled cholesterol. Their livers were removed and analyzed for cholesterol and its ester in a similar manner as for plasma. All determinations were done in duplicate and the average of the two results is reported. Mean value ($\bar{x}$) and standard error (SE) were calculated for all groups.

Results. The rate of appearance of radioactivity both in plasma (Fig. 1) and in liver (Fig. 2) was much more rapid in the rats which received either glyceryl-monooleate or glyceryl-monolinoleate than in those which received glyceryl-monostearate. Samples of plasma and liver were analyzed for concentration of individual cholesterol esters and free cholesterol and the average values are shown in Table II.

Rats which received glyceryl-monolinoleate as the sole dietary fat, and radioactive cholesterol, showed an interesting distribution pattern of radioactivity in the cholesterol es-

TABLE II. Average Concentration of Individual Cholesterol Esters and Free Cholesterol in Plasma and Liver of 20 Randomly Selected Male Rats Weighing Between 200 and 250 g.

	Plasma, mg/100 ml	Liver, mg/100 g
Cholesteryl palmitate*	8.4 ± .13	14.8 ± 1.6
" oleate	12.8 ± .21	22.0 ± 1.9
" linoleate	18.5 ± .33	19.5 ± 2.7
" arachidonate	27.2 ± .42	20.3 ± 2.2
Free cholesterol	39.4 ±2.70	248.2 ±65.0

* The glass fiber paper chromatographic method does not separate cholesteryl palmitate from cholesteryl stearate. Gas chromatographic analysis was done which indicated the cholesteryl palmitate fraction contains 5-6% cholesteryl stearate. With this information in mind the saturated cholesterol esters will be discussed in the text as cholesteryl palmitate.

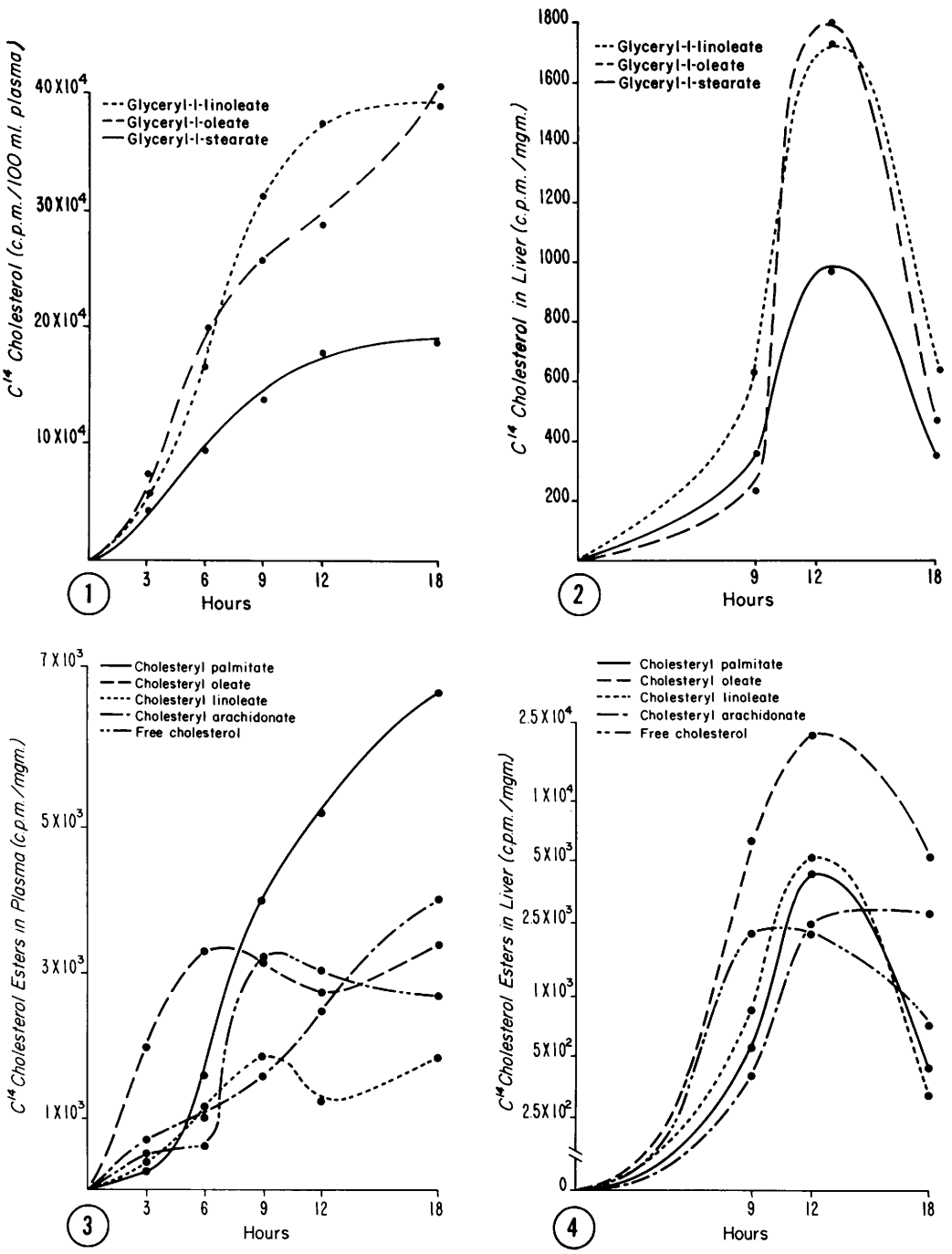

FIG. 1. Effect of giving rats an emulsified diet containing one of various monoglycerides as the sole source of fat upon absorption of cholesterol-4-C^{14} from the intestine as indicated by appearance of radioactivity in plasma.

FIG. 2. Effect of giving rats an emulsified diet containing one of various monoglycerides as the sole source of fat upon absorption of cholesterol-4-C^{14} from the intestine as indicated by appearance of radioactivity in the liver.

FIG. 3. Distribution of cholesterol-4-C^{14} among the cholesterol esters and free cholesterol

TABLE III. Summary of Specific Activity Data for Individual Cholesterol Esters and Free Cholesterol in Plasma and Liver of Rats Following Administration of an Emulsified Diet Containing Cholesterol-4-C^{14} and Glyceryl Monolinoleate as Sole Source of Fat.

Time, hr	No. of rats	Cholesteryl palmitate*	Cholesteryl oleate*	Cholesteryl linoleate*	Cholesteryl arachidonate*	Free cholesterol*
			Plasma			
3	3	0	203 ± 203	1,797 ± 652	168 ± 168	380 ± 380
6	8	2,634 ± 966	1,056 ± 492	2,385 ± 879	870 ± 323	1,852 ± 568
9	8	4,391 ± 2,040	1,838 ± 524	3,464 ± 971	2,497 ± 594	3,210 ± 337
12	7	4,868 ± 1,430	4,084 ± 1,275	3,005 ± 1,690	2,796 ± 815	3,694 ± 1,522
18	3	6,121 ± 2,267	3,599 ± 1,212	4,425 ± 687	2,005 ± 1,240	3,147 ± 583
			Liver			
9	4	8,108 ± 1,774	9,318 ± 1,277	8,564 ± 4,512	8,532 ± 5,610	533 ± 231
12	2	26,221 ± 7,702	38,600 ± 14,450	56,410 ± 12,500	6,995 ± 591	1,254 ± 890
18	3	2,432 ± 1,427	7,272 ± 2,090	2,800 ± 482	2,098 ± 1,070	790 ± 55

* Counts per min per mg.

ters of their plasma. Up to about 9 hours after administration of radioactive cholesterol the specific activity (counts per minute per milligram of cholesterol) of the various cholesterol esters showed a somewhat similar increase but beyond 9 hours less and less of the absorbed cholesterol remained as cholesteryl linoleate. Meanwhile the specific activity of cholesteryl palmitate steadily increased. Specific activities of cholesteryl oleate and cholesteryl arachidonate are also lower than that of cholesteryl palmitate. These interrelations between cholesterol esters and free cholesterol are shown in Table III.

When one determines the specific activities of the individual cholesterol esters and free cholesterol of the liver, one finds a completely different picture. In Table III one observes a sharp rise in specific activity of cholesteryl linoleate by the end of 12 hours while the activity of cholesteryl palmitate falls below it. These observations suggest that some relationship exists between the dietary fat and the cholesterol ester fatty acids appearing in the liver. In this case glyceryl-monolinoleate was fed and the specific activity of cholesteryl linoleate in the liver appeared to be the highest at a time when the specific activity of cholesteryl linoleate was lower than that of cholesteryl palmitate in the plasma.

In a similar experiment in which glyceryl-monooleate was used as the dietary fat, examination of the specific activity data of cholesterol esters in the plasma revealed that there was a steady rise in specific activity of cholesteryl palmitate while the specific activities of the unsaturated cholesterol esters were consistently lower after 9 hours (Fig. 3). The cholesterol esters in the livers of rats fed glyceryl-monooleate showed a very high peak of specific activity of cholesteryl oleate while nearly all of the other cholesterol esters have much lower specific activity peaks (Fig. 4). These data suggest, as was seen in the previous experiment, that there is a close relationship between the unsaturation of the dietary fatty acids and the unsaturation of cholesterol esters appearing in the liver.

The feeding of glyceryl-monostearate as the sole source of dietary fat resulted in data similar to the previously described 2 experiments. The specific activity of cholesteryl palmitate in the plasma was approximately twice that of the cholesteryl oleate or linoleate and slightly higher than cholesterol arachidonate after 18 hours and this difference was demonstrated as early as the third hour after administration of the formula diet containing glyceryl-monostearate and the labeled cholesterol. These observations are summarized in Table IV. Analyses of cholesterol

in plasma of rats as a function of time following administration of an emulsified diet containing 3 μc of cholesterol-4-C^{14} and glyceryl-monooleate as the sole source of fat.

FIG. 4. Distribution of cholesterol-4-C^{14} among the cholesterol esters and free cholesterol in the liver of rats as a function of time following administration of an emulsified diet containing 3 μc of cholesterol-4-C^{14} and glyceryl-monooleate as the sole source of fat.

TABLE IV. Summary of Specific Activity Data for Individual Cholesterol Esters and Free Cholesterol in Plasma and Liver of Rats Following Administration of an Emulsified Diet Containing Cholesterol-4-C^{14} and Glyceryl Monostearate as Sole Source of Fat.

Time, hr	No. of rats	Cholesteryl palmitate*	Cholesteryl oleate*	Cholesteryl linoleate*	Cholesteryl arachidonate*	Free cholesterol*
			Plasma			
3	3	1,828 ±1,470	1,721 ± 860	755 ± 640		473 ± 270
6	8	715 ± 390	1,020 ± 370	982 ± 240	358 ± 135	672 ± 250
9	8	2,564 ± 880	762 ± 470	1,268 ± 480	1,028 ± 390	1,413 ± 78
12	7	2,350 ±1,000	1,674 ± 620	447 ± 250	2,045 ± 840	1,862 ± 620
18	3	2,303 ± 900	1,408 ± 950	1,133 ± 540	2,150 ± 490	1,940 ± 580
			Liver			
9	4	1,141 ± 182	963 ± 96	333 ± 158	3,418 ± 901	91 ± 51
12	2	8,510 ± 560	6,270 ± 446	3,170 ± 276	4,160 ± 112	163 ± 23
18	3	0	545 ± 460	0	38 ± 16	437 ± 70

* Counts per min per mg.

esters in the liver found the highest specific activity of cholesterol in cholesteryl palmitate, which again suggests that there is a close relationship between saturation of the dietary fat and turnover rate of the cholesterol esters of the liver. The specific activity of all other cholesterol esters in the liver were below that of cholesteryl palmitate.

Discussion. These results have shown that the degree of unsaturation of dietary fat in the intestinal lumen affects the absorption of cholesterol from the intestine. Unsaturated fat (glyceryl-monooleate or glyceryl-monolinoleate) enhanced the absorption of cholesterol as compared with saturated fat (glyceryl-monostearate).

A close relationship exists between the saturation (or unsaturation) of dietary fat in the intestine and the type of cholesterol esters appearing in the liver. In the experiments in which only one kind of fatty acid, such as linoleic acid, was available in the intestine the highest specific activity appeared in the cholesteryl linoleate fraction of the cholesterol esters in the liver. In another case when only stearic acid was available in the intestine, the saturated cholesterol ester fraction of the cholesterol esters showed the highest specific activity in the liver.

All of these data are consistent with the suggestion that cholesterol esters are transported in chylomicrons of larger size and are immediately filtered out of the plasma by the liver. The esters are then either released into the blood stream or processed for further metabolic use in the liver. In the specific activity data of the cholesterol esters in plasma at 12 or 18 hours after administration of the radioactive cholesterol one finds an unexpected interrelationship among the individual cholesterol esters. Generally the specific activity of cholesteryl palmitate and cholesteryl oleate tend to remain higher as a function of time as compared with the polyunsaturated cholesterol esters, such as cholesteryl linoleate and cholesteryl arachidonate. This feature remained constant in spite of different amounts of radioactive cholesterol absorbed from intestine, due to the effect of feeding monoglycerides with varying degrees of saturation. The higher specific activity of the saturated cholesterol esters as compared to the more unsaturated esters suggests the preferential specificity of the cholesterol ester hydrolytic enzyme for unsaturated cholesterol esters.

Recently, Deykin and Goodman(11) reported the isolation of specific cholesterol esterase enzyme from the liver which readily hydrolyzed cholesteryl linoleate and cholesteryl oleate and very slowly hydrolyzed cholesterol palmitate or stearate. Their *in vitro* observations are in agreement with those reported above, which suggests that the polyunsaturated esters of cholesterol in the liver and the plasma are more rapidly hydrolyzed than others. If this hypothesis is correct, one would expect that as time goes on rats fed polyunsaturated fat will accumulate more and more free cholesterol in the liver or excrete greater amounts into the bile duct. The rapid disappearance of the polyunsaturated

cholesterol esters from the blood is possibly related to an increased turnover rate of cholesterol in the liver. This possibility is supported by the fact that the group fed polyunsaturated fat showed a higher rise of specific activity of free cholesterol in the serum than did the group of rats fed saturated fats.

Finally the data herein reported are not fully explicable on the basis of a 2-step enzymatic reaction in the liver, in which the hydrolysis of cholesterol esters of intestinal origin is followed by the reesterification of free cholesterol by a fatty acid other than the dietary source. For example when monolinoleate was the dietary source of fatty acid, the specific activity of the cholesteryl linoleate after 12 hours was about 56,000 CPM/mg and about 38,000 CPM/mg was observed for cholesterol oleate. In contrast the specific activity of free cholesterol was only about 1200 CPM/mg after 12 hours. Thus, it appears that free cholesterol could not have been an obligatory intermediate. These data are consistent with a hypothesis that the interconversion of the cholesterol esters is mediated by a transesterase. Recently Lopez *et al* have obtained experimental support of this hypothesis(12).

Summary. Absorption and transport of cholesterol were studied in rats with gastric and venous cannula. These studies were made on individual experimental animals by periodically sampling the blood of the same animal. Absorption of cholesterol from the intestine depends upon the degree of saturation of the dietary fat available in the intestine. More cholesterol is absorbed when polyunsaturated fat is present than when saturated fat is present in the intestinal lumen. Analyses of individual cholesterol esters in the plasma of rats show a gradual increase in the specific activity (counts per minute per milligram) of the cholesteryl palmitate fraction and to some degree the cholesteryl oleate as a function of time, while, on the other hand, the cholesteryl linoleate and cholesteryl arachidonate showed a gradual decrease in specific activity within a few hours after administration of the radioactive cholesterol. This pattern was observed regardless of whether glyceryl-monostearate, glyceryl-monooleate or glyceryl monolinoleate was fed and this suggests that the polyunsaturated cholesterol esters such as cholesteryl linoleate or cholesteryl arachidonate have a faster turnover rate than cholesteryl oleate or cholesteryl palmitate which have a relatively slow turnover rate.

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