perfusion on the liver and biliary tract.

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Effect of Fat on Plasma and Tissue Cholesterol Concentration of Hyper-Eu- and Hypothyroid Rats.* (28900)

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Numerous workers have observed that both metabolic activity and dietary fat have an effect on plasma cholesterol level. Hegsted *et al*(1) showed that in rats with normal metabolic activity, dietary unsaturated fat lowers plasma cholesterol when compared with animals fed saturated fat. Gerson *et al* (2) suggested that the action of unsaturated fats on the level of plasma cholesterol is due to the increased deposition of cholesterol in various organs and an increased fecal excretion. Hill *et al*(3) reported the effect of thyroid hormone and thyroid analogs on cholesterol metabolism in thyroidectomized rats and showed that the compound used lowers serum cholesterol levels when given in a non-toxic dosage. Ruegamer and Silverman(4), Duncan and Best(5) reported that administration of thyroid analogs to rats fed atherogenic diets prevented the elevation of cholesterol level in plasma and liver.

Although a great deal of work has been done with various fats and thyroid hormone and analogs on cholesterol metabolism, no report has shown the effect of the type of fat in the diet on cholesterol metabolism at the same time that the effect of metabolic activity is evaluated. It is obvious from the literature that both the type of fat and metabolic activity affect cholesterol metabolism by altering plasma cholesterol level. The present report shows the effect of different dietary fats at various metabolic activities.

Materials and methods. Rats were fed a purified diet which contained 25% (by weight) casein, 30% sucrose, and 20% fat either as cottonseed oil (Diet A) or as butter (Diet B). These diets also contained 4% cholesterol, 2% vitamin supplements (Fillios and Mann(6)), 3% salt mixture (Hegsted *et al*

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(7)), and 16% powdered cellulose.‡ All animals remained healthy and readily accepted the purified diet. No sign of dietary deficiency of vitamins, minerals, proteins or increased susceptibility to infection was noted throughout the experiment.

Metabolic hyperactivity (hyperthyroidism) in the rat was achieved by addition of 0.05% iodinated casein§ to the diet. Iodinated casein exerts the same characteristic effect on the metabolism as does administration of the thyroid hormone because it contains a mixture of precursors of thyroxine. Within one week this effect is seen by the greater physical activity of the animal, increased warmth of the tail and increased appetite. Additionally, some decrease of the plasma cholesterol level is observed. The production of metabolically hypoactive (hypothyroid) animals is carried out by mixing thiouracil (0.3%) in the diet. Under the influence of thiouracil the rats became hypothyroid within one week, characterized by sluggishness, scaliness of the tail and rapid development of hypercholesterolemia. These physiological effects of thiouracil can be completely prevented and reversed by administration of either a physiological dosage of thyroid hormone or iodinated casein.

Plasma total cholesterol was measured in duplicate by the method of Swartwout *et al* (8). Liver and adrenal tissues were saponified with 2% alcoholic potassium hydroxide and extracted with chloroform methanol 2:1. Duplicate determinations of tissue total cholesterol were done in the same manner as those of plasma cholesterol.

Prior to the experiment average dietary intake of energy was found to be 60-65 calories per day. A daily ration diet containing 50 calories was given. No significant weight changes (greater than 5% body weight) were observed during the experimental period. This maintenance of weight on a reduced caloric intake is probably due to a compensatory decreased physical activity of the animals. In the pre-experimental period (2 weeks) all groups received a mixed fat diet which derived 20% of the energy from cottonseed oil and 20% from butter. At day zero they were divided into 2 groups. One group was given Diet A while the other group received Diet B. This experimental period lasted 2 weeks. Blood samples were collected from the tail vein in capillary tubes and centrifuged immediately on day-zero and after one week on the experimental diet. At the end of the 2nd experimental week the rats were lightly anesthetized with surgical ether, blood was drawn by heart puncture, and liver and adrenals were immediately removed and quickly weighed and kept frozen until analyzed.

Statistical analyses were made on all results by calculating the mean (x) and the standard error of the mean (S.E.). The significance of difference was evaluated by the student "t" test.

Results. Experiment 1. Effect of type of dietary fat on plasma and tissue cholesterol concentration of hypothyroid rats. Diet A with thiouracil added was fed to 28 normal rats and diet B with thiouracil was fed to 29 normal rats for a period of 2 weeks. The changes in total plasma cholesterol can be seen in Fig. 1. A rapid increase in plasma cholesterol levels was observed in both groups, but the rats receiving butter had significantly higher levels of cholesterol at the end of the second week of experiment than the corresponding group receiving cottonseed oil.

Experiment 2. Effect of type of dietary fat on plasma cholesterol of hyperthyroid rats. Table I indicates the effect on the plasma cholesterol of rats whose diets differed only in the type of fat in the diet. These rats had iodinated casein added to their daily rations. By the end of the second week on these diets, the group that received butter had significantly higher plasma cholesterol levels than the group receiving cottonseed oil.

Experiment 3. Effect of type of dietary fat on plasma cholesterol level of rats when their thyroid activity is changed from hypoactive to hyperactive state. For a period of

‡Celluflour was obtained from Chicago Dietetic Supply House in Chicago.

§ Obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

TABLE I. Effect of Type of Fat on Plasma Cholesterol Levels of Rats with Different Thyroid Activities.

Exp No.*	Type of fat in diet	Drug supplement	No. of rats	Plasma cholesterol (mg/100 ml) Day-zero	1 wk	2 wk	"P" value†
2	Cottonseed oil (A)	Iodinated casein	17	76 ± 7	89 ±11	75 ± 4	P: <.05
	Butter (B)	" "	18	71 ± 7	82 ± 4	95 ± 9	
3	Cottonseed oil (A)	Iodinated casein	29	180 ±11	147 ± 7	109 ± 5	P: >.05
	Butter (B)	" "	30	185 ±17	152 ± 9	124 ± 7	
4	Cottonseed oil (A)	Thiouracil	9	104 ±14	101 ± 9	79 ± 6	P: <.01
	Butter (B)	"	10	104 ± 9	121 ±12	129 ±11	

* See text for other details of experiments.
† Probability of difference between effect of cottonseed oil and butter on plasma cholesterol concentration of the end of 2nd week of the experiment.

TABLE II. Effect of Type of Fat on Concentration of Cholesterol in Liver and in Adrenal of Rat.*

Exp No.	Type of fat in diet	Drug supplement	No. of rats	Liver Wt (g)	Liver Cholesterol (mg/g)	"P" value†	Adrenal Wt (mg)	Adrenal Cholesterol (mg/g)
6	Cottonseed oil (A)	Iodinated casein	10	12.2 ± 1.1	13.9 ± .9	P: <.01	24 ± 3	97.8 ± 7.5
	Butter (B)	" "	10	12.7 ± 1.9	5.1 ± .6		23 ± 5	109.0 ± 7.8
7	Cottonseed oil (A)	Thiouracil	10	10.9 ± 1.8	10.2 ±1.2	P: <.02	22 ± 3	67.1 ± 8.1
	Butter (B)	"	9	11.3 ± 2.1	6.2 ± .8		20 ± 3	84.5 ± 9.4

* See text for other details of experiments.
† Probability of difference between effect of cottonseed oil and butter on liver cholesterol concentration at the end of 2nd week of experiment.

2 weeks all of these animals received a mixed fat diet containing thiouracil. At day-zero half of these rats received diet A and iodinated casein; the other half of these rats received diet B and iodinated casein. At the end of the second experimental week plasma cholesterol levels of all animals decreased (Table 1). Although there is some difference between the two groups, this difference was not statistically significant.

Experiment 4. Effect of type of dietary fat on plasma cholesterol level of rats when their thyroid activity is changed from hyperactive to hypoactive state. Nineteen rats received the diet containing iodinated casein and mixed-fat for 2 weeks prior to beginning of the experiment. At this time 9 rats were changed to a diet containing thiouracil and diet A and 10 rats were changed to a diet B containing thiouracil. At the end of the second week the rats receiving the diet containing butter had significantly higher plasma cholesterol levels than the group consuming diets containing cottonseed oil (Table I).

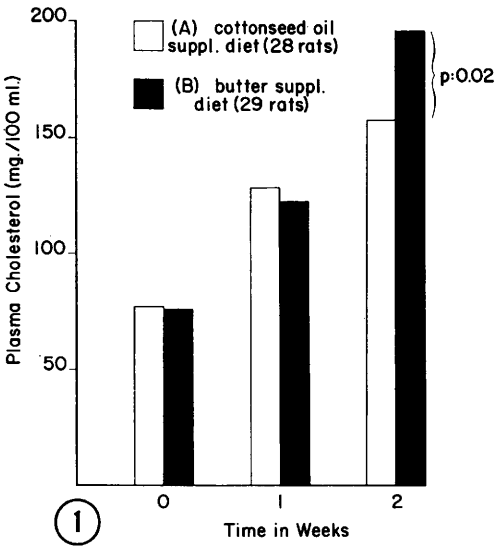
Experiments 5-6-7. Effect of dietary fat and thyroid activity of the animal on cholesterol concentration of liver and adrenals. The tissue total cholesterol concentration showed a surprisingly different type of response to the fat in the diet. A higher cholesterol concentration was observed in the livers of rats fed the polyunsaturated fat as compared with the rats maintained on a saturated fat diet (Exp. 5). However, the adrenal total cholesterol concentration was the same in both groups. Concentration of cholesterol in the livers of animals which received diet A was about twice that in the livers of the group of animals which consumed diet B (Fig. 2). The adrenal cholesterol concentration of these same groups showed no significant difference.

The data shown in Table II demonstrate that the average cholesterol concentration in the livers of 10 rats which received diet A was more than twice that of the group of 9 rats that had received diet B. In this experiment (Exp. 6) both groups had received iodinated casein in the diet to induce a hypermetabolic state.

Even the rats that had been given a diet containing thiouracil showed an increased

cholesterol concentration in the liver as an effect of feeding unsaturated fat (Exp. 7). In this case again a difference was noted. These data are also represented in Table II.

There was no difference in weight of the livers or adrenals in any of these metabolically different groups or any difference due to diet A or B. This is possibly explained by the brevity of the experiment inasmuch as 2 weeks may have been too short a time to develop significant change in the weight of these organs.



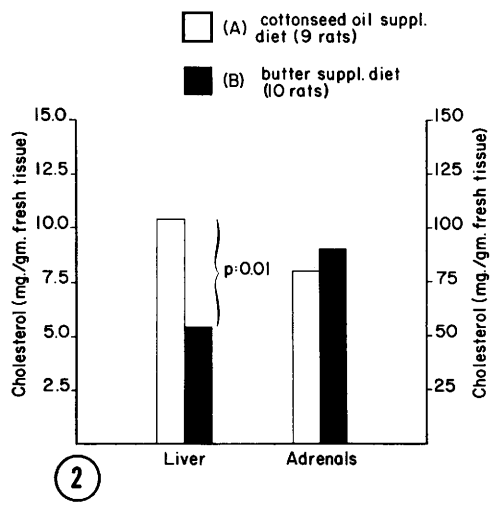


FIG. 1. Effect of type of dietary fat on plasma cholesterol concentration of hypothyroid rats, see text for other details.

FIG. 2. Effect of type of dietary fat on cholesterol concentration of liver and adrenals of rats with normal thyroid activity at the end of 2nd week of the experiment.

Discussion. Some significant relationships have been found between dietary fat and cholesterol level in plasma and in the liver. The cholesterol level of plasma was lower as a result of feeding rats unsaturated fat in the form of cottonseed oil regardless of the different levels of thyroid activity induced by iodinated casein or thiouracil. This is in agreement with the reports of numerous investigators on euthyroid animals (normometabolic)(1,9) and euthyroid men(10,11,12,13). Our data suggest that unsaturated fat in the diet lowers plasma cholesterol at any thyroid level. These observations raise the question of the effectiveness of the regulation of metabolic activity in controlling cholesterol metabolism. A hypothyroid animal fed a diet containing unsaturated fat may have a plasma cholesterol level similar to that of another animal which was euthyroid but received saturated fat diet. One may also visualize that an euthyroid rat may have as low a level of cholesterol in plasma while consuming unsaturated fat as a hyperthyroid animal which is consuming a diet rich in saturated fat. This suggests the possibility that the dietary fat has a predominant influence in regulating the plasma cholesterol level as compared with the possible control of cholesterol metabolism by thyroid hormone alone. These observations further suggest a different action of dietary fat and activity of the thyroid in regulating plasma cholesterol.

This possibility is further supported by data obtained from analysis of the cholesterol concentration of the livers of the 3 experimental groups at various thyroid levels (hyper-eu, and hypothyroid) which received the same type of dietary fat. There is little or no difference in cholesterol content of the liver regardless of the thyroid state of the animal, but there is a great difference in cholesterol concentration in the liver when groups of rats are fed different kinds of dietary fats. There is twice as high a cholesterol concentration in the livers of rats receiving an unsaturated fat diet as compared with a diet high in saturated fat. Regardless of thyroid activity, dietary fat can influence the

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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