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Time of occurrence of changes in the liver's capacity to utilize acetate for fatty acid and cholesterol synthesis after fat feeding*

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SUMMARY

The time relationships in the changes in hepatic lipogenesis and cholesterogenesis induced by the feeding of corn oil and lard have been studied. A pronounced decrease in the capacity of the liver to convert acetate carbon to fatty acids was observed as early as 1 hour after fat administration. The increase in the liver's capacity to incorporate acetate carbon into cholesterol developed slowly after fat feeding; the earliest change was observed in 12 hours. The restoration of lipogenesis to normal, following the withdrawal of fat, was more rapid than that of cholesterogenesis. The time changes in hepatic lipogenesis and cholesterogenesis induced by fat feeding were correlated with the time of appearance of exogenous fat in the liver.

Recent investigations have shown that the capacity of rat liver to utilize acetate for lipid synthesis is altered by inclusion of fat in the animal's diet. The liver's capacity to convert acetate carbon to fatty acids was measurably decreased when as little as 2.5 per cent fat was added to the diet. When 15 per cent was added, the liver retained only 10 per cent of its original capacity to convert acetate to fatty acids. Fat feeding had an opposite effect on hepatic cholesterogenesis; the presence of 15 per cent fat in the diet resulted in a three- to fourfold increase in the liver's capacity to incorporate acetate carbon into cholesterol. The fats tested were lard, corn oil, a vegetable oil, and a hydrogenated vegetable oil. They were of equal value in augmenting cholesterogenesis and in reducing lipogenesis. In the experiments under consideration, the fat content of the diet was not excessive—in no case did it exceed 15 per cent—and the period of feeding was kept short, namely, 3 days. Addition of the fats to the diets did not alter the levels of glucose, fatty acids, and cholesterol of plasma nor the glycogen, fatty acids, and cholesterol contents of the liver (1, 2).

In the present study we have examined the time relationships of the changes in hepatic lipogenesis and cholesterogenesis of rats fed fat by stomach tube. It is shown that the change in lipogenesis develops rapidly, in a matter of a few hours, but that about a day is required, after the start of fat feeding, before a measurable change in cholesterogenesis is observed.

I. Experiments with Liver Slices

EXPERIMENTAL

For 3 days before the start of the experiment, and continuing for its duration, male rats of the Long-Evans strain (200 to 250 g.) were fed, *ad libitum*, a synthetic diet containing 50 per cent glucose, 37 per cent casein (vitamin-free, Nutritional Biochemical Corp.), 6 per cent salts (3), 2 per cent defatted liver (VioBin), 5 per cent cellulose, and an adequate mixture of the B vitamins (4). Water was available to the animals at all times. At the intervals indicated by arrows in Figures 1 to 3, 2.0 ml. of corn oil or melted lard was fed by stomach tube, after which the rats were returned to their cages immediately and allowed free access to the high glucose, fat-free diet. The in-

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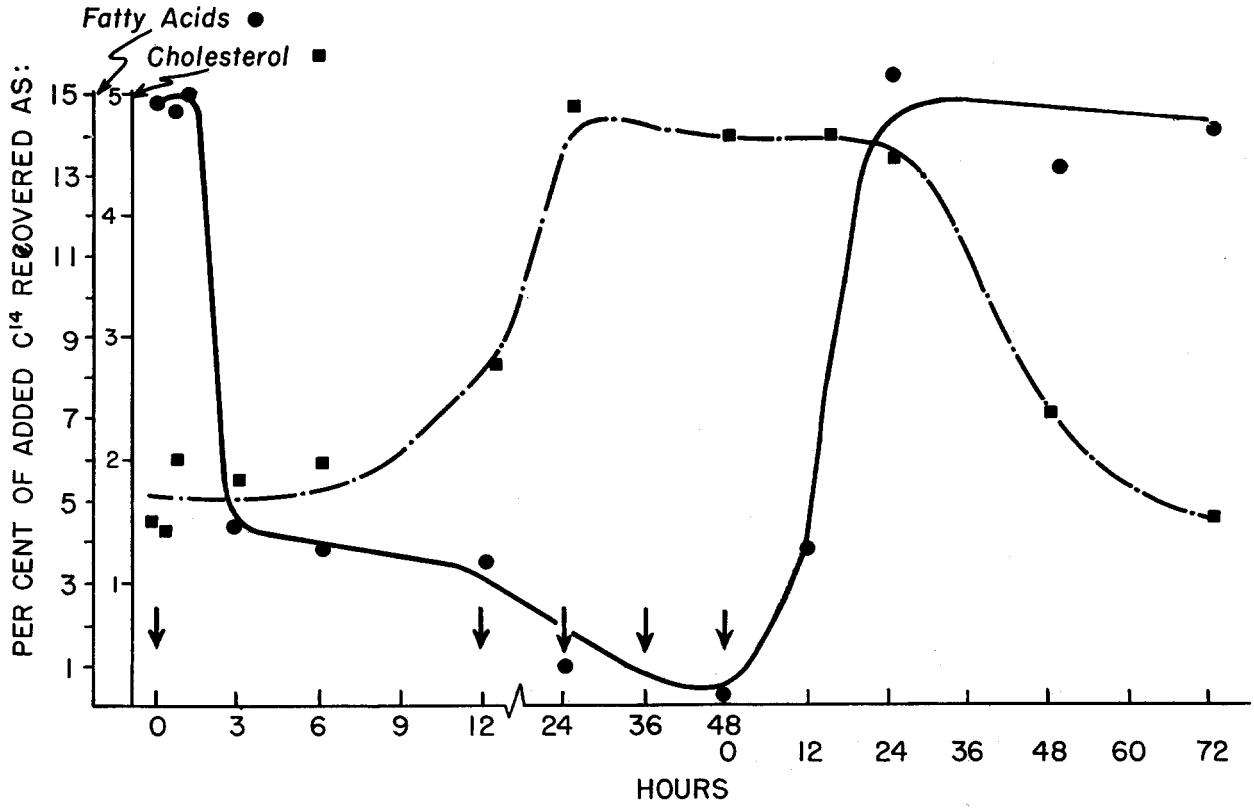

FIG. 1. Effect of ingested corn oil on hepatic lipogenesis and cholesterogenesis (experiment 1) of rats that had been fed a fat-free diet. At the times indicated by the arrows, each rat received by stomach tube 2 ml. of corn oil. Each point is the average of closely agreeing results obtained with 4 rats.

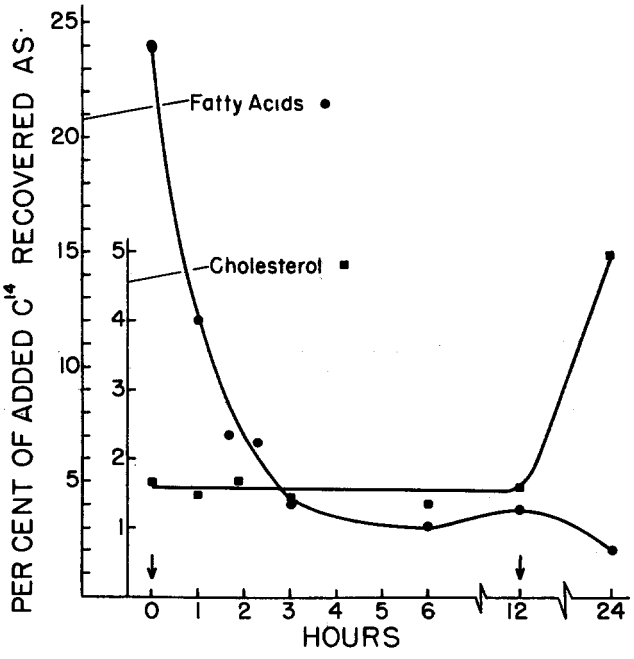

FIG. 2. Effect of ingested corn oil on hepatic lipogenesis and cholesterogenesis (experiment 2) of rats that had been fed a fat-free diet. At the times indicated by the arrows, each rat received by stomach tube 2 ml. of corn oil. Each point is the average of closely agreeing results obtained with 3 rats.

tubation of the corn oil and lard did not reduce measurably the rate of ingestion of the high glucose diet. Groups of fat-fed rats and their corresponding controls (these received 2.0 ml. of saline, instead of the fat, by intubation) were killed at various intervals thereafter. Their stomachs were examined and found to be distended with food, most of which was identified as the glucose diet. Their livers were rapidly removed, weighed, and sliced. Duplicate 500 ± 5 mg. portions were incubated, with shaking, for 3 hours at 37°C in 5.0 ml. of Krebs-Henseleit bicarbonate buffer (pH 7.3 to 7.4) (5), to which had been added 2 μmoles of acetate-1-C^{14} as the sodium salt. The methods for assaying the C^{14} content of the expired CO_2, as well as the incorporation of C^{14} into cholesterol and fatty acids, have been reported previously (1, 2).

RESULTS

The Experiments with Corn Oil. The results of two experiments with corn oil feedings are shown in Figures 1 and 2. The earliest interval after corn oil feeding at which a measurable decline was noted in the liver's capacity for incorporating acetate carbon into fatty acids was 3 hours in experiment 1 (Fig. 1) and about 1 hour in experiment 2 (Fig. 2). In experiment

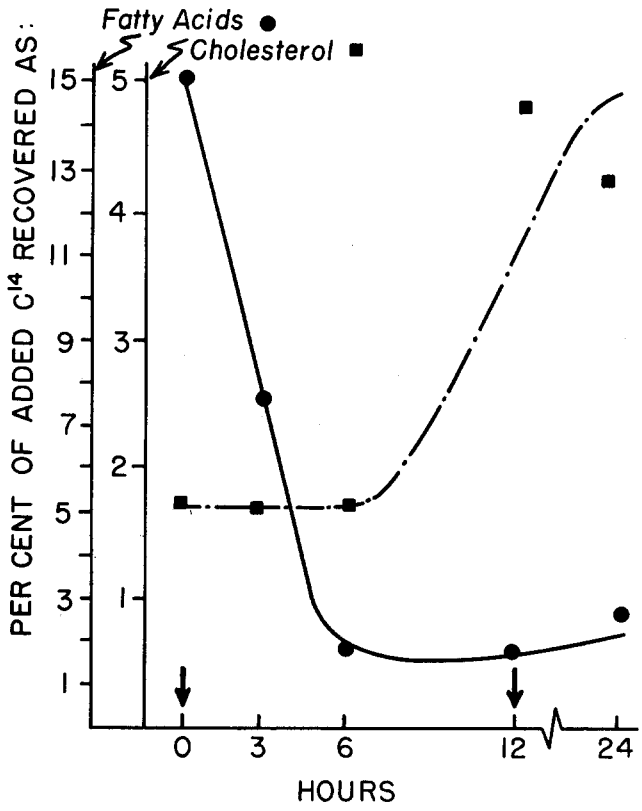

FIG. 3. Effect of ingested lard on hepatic lipogenesis and cholesterogenesis of rats that had been fed a fat-free diet. At the times indicated by the arrows, each rat received by stomach tube 2 ml. of melted lard. Each point is the average of closely agreeing results obtained with 3 rats.

1 the control rats were killed at intervals of 0.5 hour to 5 days after the first intubation of saline; in these rats the C^{14}-fatty acid recoveries (not recorded in Fig. 1) ranged from 12 to 16 per cent. At the end of 48 hours, by which time the fat-fed rats had received five administrations of corn oil, the livers had almost completely lost the capacity to form fatty acids from added acetate. The decline in C^{14}-fatty acid recoveries was also pronounced in experiment 2, which lasted 24 hours, with each rat receiving one or two feedings (2.0 ml. each) of corn oil. The C^{14}-fatty acid values found at 0 and 12 hours were 24 and 4 per cent, respectively.

No significant change in the liver's capacity to synthesize cholesterol occurred during the first 6 hours after the administration of 2.0 ml. of corn oil. A slight rise in C^{14}-cholesterol recoveries was noted at 12 hours (Fig. 1), and at 24 hours the values for C^{14}-cholesterol had more than doubled (Figs. 1 and 2).

Corn oil was last fed at the 48-hour interval (Fig. 1). Some restoration of the liver's capacity to synthesize fatty acids was observed 12 hours later. By the time 24 hours had elapsed after the last feeding, the liver had acquired its initial capacity to form fatty acids.

A delayed response in modification of the liver's capacity to synthesize cholesterol was also observed after the corn oil feedings stopped. The heightened capacity for cholesterol synthesis induced by fat feeding was still evident 24 hours after the last administration of corn oil. By 48 hours, however, the values for C^{14}-cholesterol recoveries were about the same as those observed before the corn oil feedings.

The Experiment with Lard. The results of this experiment are shown in Figure 3. The rapid loss in the liver's capacity to synthesize fatty acids and the delayed increase in its capacity for cholesterogenesis are again evident.

II. Experiments with Intact Animals

EXPERIMENTAL

Male Long-Evans rats weighing 200 to 250 g. were used. In the first experiment the feeding of the fat-free diet containing 50 per cent glucose was begun 3 days before the first administration of fat, and was continued until the rats were killed 3 and 12 hours after a single intubation of 2.0 ml. of corn oil. In the second experiment the rats were killed 72 hours after they were transferred to a diet containing 50 per cent glucose and 15 per cent corn oil. All control rats were fed the fat-free, high glucose diet throughout. Exactly 20 minutes before the rats and their respective controls were killed, they were injected intraperitoneally with 0.5 ml. of 0.9 per cent saline containing 2 μmoles of 1-C^{14}-labeled sodium acetate. The livers were rapidly excised and weighed, and aliquots were analyzed for C^{14}-fatty acids and C^{14}-cholesterol.

RESULTS

The results are recorded in Table 1. A reduction in the C^{14} incorporated into fatty acids by the livers of the intact rats was observed as early as 3 hours after the start of fat feeding, but not until more than 12 hours had elapsed were the C^{14}-cholesterol recoveries augmented. The C^{14}-cholesterol recoveries found for the livers of rats that had ingested the fat-containing diet for 72 hours were about four times those observed with the controls fed the fat-free diet.

III. Time of Appearance of Fed Fat in the Liver

The rapidity of the changes induced in hepatic lipogenesis by fat feeding raised the question of how soon

the administered fat reaches the liver. Tripalmitin-1-C^{14} was dissolved in 2.0 ml. of corn oil, and the mixture was introduced by intubation into the stomachs of rats that had been fed the fat-free, high glucose diet for 3 days. They were killed at different intervals, and their livers were excised and assayed for C^{14}-lipids. Lipids were extracted with a 3 : 1 ethanol-ether mixture, and aliquots of the extract were mounted directly on aluminum planchets. The results are shown in Figure 4. C^{14}-activity was found in the lipid fraction of the liver of rats killed as early as 1 hour after intubation. If it is assumed that the C^{14} is a measure of the transport of corn oil, it can be calculated that in 1 hour about 5 mg. of the administered oil had reached the liver, which contained about 300 mg. of fatty acids. It is of interest that the most pronounced fall in lipogenic capacity of the liver occurred at a time when so little of the administered fat had found its way into the liver. The mere presence of exogenous fat, rather than the accumulation of large amounts of it, seems to be associated with the change in hepatic lipogenesis.

DISCUSSION

Our earlier observation that fat feeding induced a change in the liver's capacity to incorporate acetate carbon into fatty acids and cholesterol is fully confirmed by the experimental findings presented here. The rapidity of the change in the lipogenic capacity of the liver, as well as the very small amounts of administered fat that reach the liver by the time the changes in lipogenic capacity are first detected, favors the idea that fatty acid synthesis in the liver is under homeostatic regulation.

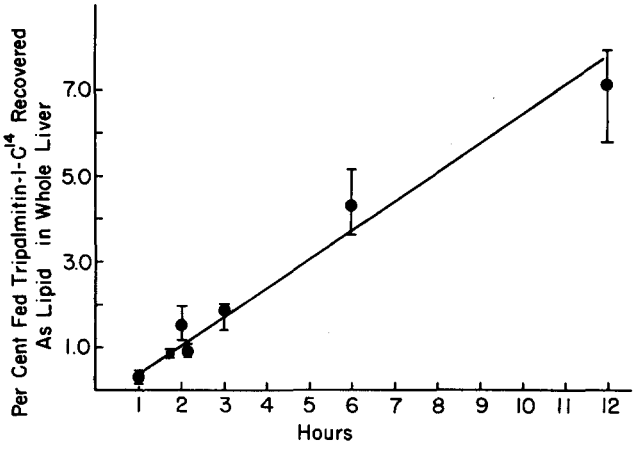

FIG. 4. Time of appearance of fed fat in the liver. Each point is the average of closely agreeing results obtained with 3 rats.

TABLE 1. EFFECT OF CORN OIL FEEDING ON INCORPORATION OF C^{14} OF ACETATE-1-C^{14} INTO FATTY ACIDS AND CHOLESTEROL BY LIVERS OF INTACT RATS

C^{14} recovered as	Hours After Start of Corn Oil Feeding		
	3	12	72
Fatty acids	−70 (−68 to −75)	−96 (−92 to −100)	−94 (−91 to −99)
Cholesterol	0 (−5 to +7)	0 (−8 to +2)	+400 (+374 to +453)

Averages and range of values obtained with 4 to 6 rats are given. They represent percentage changes from controls in the incorporation of the injected C^{14} into fatty acids and cholesterol.

The inverse effects produced by fat feeding on lipogenesis and cholesterogenesis in the liver led us to suggest earlier that cholesterogenesis, like acetoacetate formation, represents an alternate pathway for acetyl-coenzyme A when lipogenesis is depressed (6). In this case this suggestion is not borne out by the changes in cholesterogenesis and lipogenesis with time shown in Figures 1 to 4. For example, in the first experiment with liver slices, lipogenesis was depressed for more than 9 hours before a change in hepatic cholesterogenesis occurred. In the experiments with intact animals, lipogenesis was reduced for more than 12 hours before a rise in hepatic cholesterogenesis was noted.

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