REGULATION OF CHOLESTEROL SYNTHESIS IN THE LIVER: THE INFLUENCE OF DIETARY FATS*

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A variety of factors have been shown to control cholesterol synthesis in the liver. The nutritional state of the animal has a decided influence, and it has been demonstrated that fasting for a matter of 24 to 72 hours depresses, to a considerable extent, the capacity of the rat liver to incorporate acetate carbon into cholesterol (1). Restriction of caloric intake has a similar effect (1). Fat, carbohydrate, and protein appear to be equally effective in restoring hepatic cholesterogenesis in the fasted rat (1). The liver's capacity for cholesterol synthesis is also regulated by cholesterol consumption. For example, it was observed ty Tomkins *et al.* (2) that the liver of a rat fed 8 days a diet entirely devoid of cholesterol has a much greater capacity for cholesterogenesis than does the liver of a rat fed a 0.5 per cent cholesterol diet. A homeostatic regulation for hepatic cholesterol synthesis has also been reported by Gould *et al.* (3), Frantz *et al.* (4), Cox *et al.* (5), and Swell *et al.* (6). A third type of control is mediated by endocrines. Thus, the incorporation of added acetate into cholesterol is much greater in liver slices prepared from diabetic rats than in those prepared from normal rats (7, 8). Although hepatic cholesterogenesis is reduced in the liver of the hypophysectomized rat fed an adequate stock diet (9, 10), it can be made to proceed at normal rates by the feeding of a synthetic diet high in available carbohydrate (11).

Many recent reports have emphasized an influence of dietary fats upon cholesterol metabolism (12, 13), particularly upon the levels of serum cholesterol in man (14, 15). The mechanism of this action of dietary fat is not understood. Since the liver is a principal site for the formation of plasma cholesterol (16), we have studied here the effects of fats, animal as well as

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vegetable, upon the liver's capacity for synthesizing cholesterol from acetate carbon.

EXPERIMENTAL

Treatment of Animals.—Long-Evans rats were fed synthetic diets for 3 days before they were sacrificed. The control diet consisted of 50 per cent glucose, 37 per cent vitamin-free casein (Nutritional Biochemical Corp.), 4.8 per cent celluflour, 6 per cent salt mixture (17), 2 per cent defatted liver (VioBin), and an adequate vitamin B mixture (18). Changes in the fat content of other diets were made at the expense of celluflour or celluflour and casein.

Preparation of Tissues and Incubation Procedure.—Blood was withdrawn from the rats by cardiac puncture just before they were sacrificed. Their livers were removed and immersed in a chilled Krebs-Henseleit bicarbonate buffer (19). Slices approximately 0.5 mm. thick were prepared with a mechanical tissue slicer (20), and 500 ± 5 mg. portions were placed in the main compartment of a 50 ml. incubation flask containing 5.0 ml. of the bicarbonate buffer (pH 7.3–7.4) to which acetate-1-C^{14} as the sodium salt had been added. The flask was gassed with a mixture of 95 per cent O_2 and 5 per cent CO_2, and closed with a self sealing rubber cap. The tissues were incubated, with shaking, at 37.5° for 3 hours.

Analytical Procedures.—The methods used for determining $C^{14}O_2$ in respired gas and C^{14}-cholesterol of liver were described earlier (11). The procedure for measuring plasma glucose and liver glycogen have also been described elsewhere (11). Plasma and liver fatty acids were determined by the method of Bragdon (21), and plasma and liver cholesterol by a modification of the method of Zak *et al.* (22).

RESULTS

1. *Experiments with Diets Containing 15 Per Cent of Various Fats.*—

The first group of rats (1 to 8, Table I) was fed, for 3 days before they were sacrificed, a diet devoid of fat. When 500 mg. of slices prepared from their livers was incubated with acetate-1-C^{14}, approximately 1 per cent of the C^{14} was incorporated into cholesterol. The other rats (9 to 24) were fed, for the same 3 days, diets to which had been added 15 per cent of either corn oil (Mazola), Wesson oil, hydrogenated vegetable oil (Snowdrift), or lard. The calorific value per gram of these fat-containing diets was made the same as that of the fat-free diet. The average daily food intake during the 3 days of observation was about the same for each group of rats studied.

The livers of all rats that had been fed the fat-containing diets showed an augmented capacity for converting acetate carbon to cholesterol.

Under the conditions of the short term feeding, the addition of fat to the diet did not affect the levels of glucose, fatty acids, and cholesterol of plasma nor the glycogen, fatty acids, and cholesterol content of the liver (Table I).

2. *The Question of Protein in the Diet.*—In the construction of the diets used in the preceding experiments, isocaloric values per gram of diet were maintained by reducing the casein content from 37 per cent in the fat-free diet to 15 per cent in the diets to which fat had been added. It therefore became necessary to determine whether protein, *per se*, can affect hepatic cholesterogenesis. To test this point, rats were fed, for 3 days before they were sacrificed, fat-free diets containing 50 per cent glucose and either 37 or 15 per cent protein. The results, recorded in Table II, show that a doubling in

TABLE I

Effect of Dietary Fat upon Hepatic Cholesterogenesis

Duplicate 500 ± 5 mg. portions of liver slices were incubated at 37° for 3 hrs. in 5.0 ml. of bicarbonate buffer containing 2 μM acetate-1-C^{14}. Average values are reported.

Rat		Diet			Average daily food intake	Plasma			Liver				Percent of added acetate-C^{14} recovered as:	
No.	Weight	Glucose	Fat	Casein		Glucose	Fatty acids	Cholesterol	Weight	Glycogen	Fatty acids	Cholesterol	CO_2	Cholesterol
	gm.	*per cent*	*per cent*	*per cent*	*gm.*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*gm./100 gm.*	*per cent wet wt.*	*per cent wet wt.*	*per cent wet wt.*		
1	284	50	None	37	12	81	282	41	4.05	3.52	2.75	0.35	32	0.66
2	260	"	"	"	15	97	236	37	4.50	4.83	2.44	0.22	35	1.09
3	295	"	"	"	14	92	231	35	4.82	5.53	2.52	0.26	29	0.82
4	285	"	"	"	16	85	212	38	4.35	4.95	2.35	0.25	33	0.98
5	220	"	"	"	13				3.64	5.80	2.07	0.28	33	1.24
6	240	"	"	"	14				4.21	5.60	2.03	0.29	28	1.46
7	250	"	"	"	14				4.56	6.20	2.22	0.27	30	1.35
8	240	"	"	"	16				4.60	4.12	2.04	0.27	27	1.16
Averages						88	240	38	4.34	5.06	2.30	0.28	31	1.09
9	280	50	15 CO*	15	12	81	255	42	4.42	3.16	3.06	0.30	33	2.36
10	275	"	"	"	13	78	268	37	3.38	3.60	3.15	0.28	32	2.86
11	320	"	"	"	14	87	248	58	3.40	3.44	3.10	0.27	32	1.90
12	285	"	"	"	8	67	285	46	3.55	4.68	3.12	0.27	32	2.69
Averages						78	264	46	3.69	3.72	3.10	0.28	32	2.45
13	250	50	15 L*	15	12	79	240	41	3.60	3.18	2.62	0.24	33	3.05
14	240	"	"	"	9	84	261	38	4.10	4.40	2.98	0.30	34	3.10
15	310	"	"	"	13	88	256	45	3.62	4.62	2.94	0.25	37	2.55
16	310	"	"	"	13	94	219	51	3.95	4.45	2.78	0.28	31	5.45
Averages						86	244	44	3.82	4.13	2.83	0.27	34	3.54
17	270	50	15 VO*	15	14				3.71	3.95	2.92	0.29	33	3.53
18	280	"	"	"	13				4.61	5.75	2.61	0.29	31	3.24
19	230	"	"	"	11				3.56	6.12	2.53	0.32	32	3.62
20	230	"	"	"	13				4.25	5.21	2.96	0.30	33	3.58
Averages									4.03	5.26	2.75	0.30	32	3.51
21	250	50	15 HVO*	15					4.09	4.85	2.65	0.34	33	4.02
22	240	"	"	"					4.04	4.82	2.73	0.32	26	3.46
23	290	"	"	"					4.41	4.40	3.00	0.32	30	3.12
24	240	"	"	"					4.20	4.70	2.65	0.33	28	2.72
Averages									4.18	4.72	2.76	0.33	29	3.33

* CO, corn oil (Mazola); L, lard; VO, vegetable oil (Wesson); HVO, hydrogenated vegetable oil (Snowdrift).

the protein intake by the rat had no effect on the liver's capacity for synthesizing cholesterol from acetate. It would appear that the observed increase in hepatic cholesterogenesis shown in Table I resulted from ingested fat.

3. *The Question of Insulin Secretion.*—It has been reported that the feeding of fat reduces the insulin content of the rat pancreas (23). This observation, in conjunction with an earlier finding that the liver of the diabetic rat has an enhanced capacity for converting acetate carbon to cholesterol (7), led us

TABLE II

The Effect of Dietary Protein upon Hepatic Cholesterogenesis

For experimental details see Table I.

Rat		Diet*		Average daily food intake	Liver				Per cent of added acetate-C^{14} recovered as	
No.	Weight	Glucose	Casein		Weight	Glycogen	Fatty acids	Cholesterol	CO_2	Cholesterol
	gm.	*per cent*	*per cent*	*gm.*	*gm./100 gm.*	*per cent wet wt.*	*per cent wet wt.*	*per cent wet wt.*		
24	230	50	37	12	5.21	4.16	3.12	0.28	32	1.63
26	250	"	"	16	4.63	4.40	2.96	0.32	28	1.58
27	240	"	"	12	4.74	5.06	2.78	0.26	31	1.94
28	235	"	"	13	4.80	4.16	3.05	0.27	26	1.82
29	265	"	"	9	5.26	3.60	2.69	0.28	33	1.18
Averages					4.90	4.28	2.98	0.28	30	1.63
30	230	50	15	15	4.30	4.70	3.28	0.28	30	1.25
31	255	"	"	9	4.62	4.20	2.48	0.30	26	1.16
32	295	"	"	12	4.90	5.10	2.76	0.33	28	1.35
33	205	"	"	14	5.20	4.70	2.90	0.27	39	1.89
34	195	"	"	13	4.70	4.05	2.88	0.29	30	1.10
Averages					4.74	4.55	2.86	0.29	31	1.35

* The diets contained no added fat.

to consider the possibility that the effect of ingested fat on hepatic cholesterogenesis in the normal rat may reflect a decreased production of insulin. The results presented in Table III, however, demonstrate that the administration of extra insulin to normal rats fed the fat-containing diets did not reduce hepatic cholesterogenesis to the levels observed in normal rats fed the fat-free diets.

4. *Studies with Intact Rats.*—The data reported so far were obtained from experiments with liver slices. The feeding of fat also increased the recoveries of C^{14}-cholesterol in the livers of normal rats injected with acetate-1-C^{14}.

TABLE III

Effect of Insulin on Hepatic Cholesterogenesis in Normal Rats Fed Fat-Free and Fat-Containing Diets

For experimental details see Table I.

Rat		Diet			Insulin	Average daily food intake	Liver				Per cent of added acetate-C^{14} recovered as:		Average blood glucose
No.	Weight	Glucose	Corn oil	Casein			Weight	Glycogen	Fatty acids	Cholesterol	CO_2	Cholesterol	
	gm.	*per cent*	*per cent*	*per cent*		*gm.*	*gm./100 gm.*	*per cent wet wt.*	*per cent wet wt.*	*per cent wet wt.*			*mg. per cent*
35	220	50	0	37	None	12	3.90	4.36	2.71	0.28	31	1.58	
36	190	"	"	"	"	14	4.10	3.22	2.58	0.33	34	1.99	86
37	205	"	"	"	"	10	3.95	4.66	2.74	0.26	33	1.76	
38	230	"	"	"	"	12	3.88	3.50	2.69	0.26	30	1.86	
Averages						12	3.90	3.93	2.68	0.28	32	1.80	
39	180	50	0	37	Yes*	14	4.21	2.08	2.50	0.30	32	1.70	
40	195	"	"	"	"	16	3.56	5.70	3.22	0.29	32	1.36	41
41	245	"	"	"	"	20	3.42	5.40	2.62	0.26	26	1.29	
42	220	"	"	"	"	16	3.95	5.92	2.68	0.28	30	1.96	
Averages						16	3.78	4.78	2.75	0.28	30	1.58	
43	210	50	15	15	None	8	4.21	3.40	4.22	0.32	36	4.25	
44	210	"	"	"	"	13	4.02	4.72	3.16	0.30	34	3.06	91
45	185	"	"	"	"	12	3.75	3.36	4.05	0.37	32	4.55	
46	220	"	"	"	"	12	3.90	4.96	3.72	0.28	25	4.15	
Averages						11	3.99	4.11	3.78	0.31	32	4.00	
47	195	50	15	15	Yes*	16	3.82	3.78	2.92	0.30	32	4.15	
48	190	"	"	"	"	20	3.68	4.56	3.18	0.28	34	3.94	36
49	215	"	"	"	"	18	4.15	4.82	3.78	0.28	33	2.96	
50	205	"	"	"	"	18	3.98	3.97	4.02	0.29	30	3.55	
Averages						18	3.90	4.27	3.47	0.29	32	3.65	

* Protamine zinc insulin 4 u. per kg. body weight. It was given in 2 equal doses each day for 3 days before the rat was sacrificed.

Rats weighing 225 to 250 gm. were fed, for 3 days, isocaloric diets containing either no fat or 15 per cent corn oil. At the end of that time each rat was injected intraperitoneally with 0.5 ml. of 0.9 per cent sodium chloride solution containing the sodium salt of acetate-1-C^{14}. Each 0.5 ml. of this solution contained 3 to 5 $\times$ 10^6 C.P.M. of C^{14}. The rats were sacrificed 15 minutes after the C^{14} injections, and their entire livers were excised and analyzed for C^{14}-cholesterol.

The values for the rats fed the non-fat diet ranged from 0.35 to 0.42 per cent of the injected C^{14} per whole liver, and those for the fat-fed rats ranged from 0.71 to 0.77 per cent.

DISCUSSION

We showed earlier that the amount of carbohydrate ingested by the rat—or apparently the extent of glycolytic activity in the liver—influences the liver's capacity for incorporating acetate carbon into cholesterol (1, 8). For this reason, in the present study, care was taken to insure that, whether the diets contained no fat or 15 per cent fat, the proportion of glucose remained constant, namely, 50 per cent. It is therefore not surprising that the concentrations of glycogen in the livers of the rats fed the 0 and 15 per cent fat diets were essentially the same. Since the variations in protein content of the diet (necessary to keep the caloric value per unit weight of the diets constant) had no measurable effect on hepatic cholesterogenesis, our results demonstrate that the liver's capacity for incorporating acetate carbon into cholesterol was related to the quantity of fat in the diet.

Under conditions of fat feeding identical with those used here, we have observed a lowering in the liver's capacity for synthesis of fatty acids (24). Indeed, lipogenesis from acetate is extremely sensitive to fat feeding—the addition of as little as 2.5 per cent fat to the diet decreased the liver's capacity for incorporating acetate carbon into fatty acids. Thus, when fat is fed, hepatic lipogenesis and cholesterogenesis are inversely affected. A similar inverse relation between cholesterogenesis and lipogenesis has been observed in the liver of the diabetic rat (25, 7). These observations indicate that hepatic cholesterogenesis, like acetoacetate formation (26), may represent an alternate pathway for acetyl CoA when lipogenesis is impaired under certain conditions.

Previous work dealing with the effect of dietary fat on cholesterol synthesis has yielded results differing from those observed here. Alfin-Slater *et al.* (27) studied the synthesis of cholesterol in intact rats which received deuterium oxide after they had been fed diets containing either 2.2 or 30 per cent fat. According to these investigators, the amount of newly formed cholesterol present in the liver and plasma of rats prefed a low fat diet is not changed by feeding them a high fat diet. The results obtained by Brice and Okey (28) with C^{14}-acetate were too variable to enable them to state whether rats fed 5 per cent fat differed, in their ability to synthesize cholesterol, from rats that had been fed a diet containing 40 per cent fat for 3 weeks.

Recent work on man and animals suggests that saturated and unsaturated fatty acids differ in their effects upon cholesterol metabolism (12, 13, 15). In this connection it is of interest to note that one aspect of cholesterol metabolism in the rat, namely, its synthesis in liver, responded in a uniform manner

to the feeding of four fats: lard, corn oil, a vegetable oil, and a hydrogenated vegetable oil.

SUMMARY

Rats were fed, for 3 days, four synthetic diets, all of which contained the same proportion of carbohydrate (50 per cent) and were of equal caloric value per gram. These diets contained either 0 or 15 per cent fat. The fats used were lard, corn oil, Wesson oil, and a hydrogenated vegetable oil. The feeding of the fat-containing diet for 3 days increased the liver's capacity for incorporating acetate carbon to cholesterol. The fats tested were of about equal value in stimulating hepatic cholesterogenesis.

The various diets fed had no effect on the lipide or glycogen content of the liver nor on the lipide content of plasma.

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