

THE EFFECTS OF CHOLESTEROL AND CHOLINE ON DEPOSITION OF LIVER FAT.

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THE finding that the accumulation of fatty acids in the liver of the white rat which consistently results from the ingestion of food containing approximately 40 p.c. beef fat can be prevented by the inclusion in the diet of choline or betaine [Best and Huntsman, 1932], suggested that an investigation of the effect of these substances on fatty changes in the liver produced by other means might be profitable. The fatty degeneration found in phosphorus or chloroform poisoning is so intense that a very prolonged study will probably be required to determine the effect, if any, of choline. In a consideration of fatty changes in conditions which might be of more physiological importance, several possibilities presented themselves. Two of these have been investigated—pregnancy in rabbits and cholesterol feeding in rats. It has been stated that the fatty acid content of the rabbit's liver is appreciably increased during the late stages of pregnancy. With the intention of determining the effect of choline on this change the total fatty acids have been estimated in the liver of pregnant rabbits at or near term. The results of this study, which was carried out in large part by our colleague Dr D. L. MacLean, failed to demonstrate a consistent increase in the amount of liver fat even when the pregnant animals received a diet high in fat. We have been more fortunate, however, in producing fatty changes in experiments in which cholesterol has been added to a diet which alone did not produce an appreciable increase in the liver fat of white rats.

The fact that the addition of small amounts of cholesterol to the diet of rats produces an increase in liver fat has been established by Yuasa [1928], who studied the liver by histological and chemical methods in several species but did not investigate chemically the fatty acid content of the organ in this species. W. M. Sperry has very kindly informed us that he has noted fatty livers in cholesterol-fed rats. We have had no difficulty in confirming these results and are reporting in this communica-

tion the action of choline and betaine, both of which prevent the deposition of liver fat attributable to ingested cholesterol under the conditions of our experiments.

EXPERIMENTAL PROCEDURE.

White rats of the Wistar strain weighing between 150 and 250 g. were placed in individual cages and given a mixed grain diet consisting of equal parts of whole cracked wheat, rolled oats, corn meal and 2.5 p.c. bone meal. The cholesterol was dissolved in an hydrogenated vegetable oil (Crisco), which had been previously melted over a water bath, and was added with thorough mixing to the grain diet. The hydrogenated oil, which has an iodine number of approximately 63, formed 20 p.c. of the diet. The various groups into which the rats were divided were as similar as possible. The amount of food consumed daily by each rat was carefully determined and from this the amount of cholesterol ingested was calculated. When choline or betaine was given it was added to the diet in aqueous solution and thoroughly mixed. The amounts of cholesterol and of choline ingested are given in Table I. After approximately 1 month on

TABLE I. Summary.

No. of rats	Length of exp. days	Av. change in wt. g.	Av. fat eaten per diem g.	Av. fat excreted per diem g.	Iodine No. of fat in faeces	Av. cholesterol eaten per diem g.	Av. choline* eaten per diem g.	Fatty acid in liver p.c.	Iodine No.
14	31	-23	1.57	0.10	41	—	—	5.46	107
9	33	-11	1.60	0.11	43	—	0.07	5.44	116
23	27	-20	1.97	—	—	0.19	—	8.79	—
15	31	-19	1.68	0.20	45	0.13	—	10.56	95
14	28	-5	1.97	0.18	48	0.09	—	10.75	95
17	28	-7	1.97	0.19	41	0.19	—	11.60†	91
13	31	-11	1.76	0.19	46	0.14	0.07	8.21	95
13	31	-19	1.98	0.13	42	0.10	0.07	7.51	107
29	30	-12	1.98	0.23	49	0.19	0.10	6.44	108
15	28	-21	1.99	0.17	47	0.19	0.19	4.50†	103
19	27	-24	1.97	—	—	0.19	0.19	3.99	—
Betaine									
14	27	-22	2.00	0.16	—	0.20	0.33	3.36	—

* Choline hydrochloride B.D.H. was used and the dosage is expressed in terms of the base.

† The individual values in these two series are listed in Table II.

these diets the rats were killed and the livers removed immediately for estimation of total fatty acids, using the Leathes and Raper modification of Liebermann's saponification method. The iodine number was determined by the Rosenmund-Kuhnenn procedure [1923].

RESULTS AND DISCUSSION.

The significant results are summarized in Table I. Rats fed on the basal diet have from 3 to 5 p.c. liver fat. In a series of fourteen rats the basal diet plus the hydrogenated oil produced a very slight increase in liver fat. Choline added to the basal diet plus the hydrogenated oil did not appreciably change the amount. In the four series of animals which received cholesterol and no choline the average fatty acid content was just over 10 p.c. It is interesting that 70 mg. of choline daily were inadequate completely to prevent the deposition of fat attributable to cholesterol, but would certainly, on the basis of previous results, have been sufficient to control the fatty changes produced by a diet containing 40 p.c. beef fat. However, when 190 mg. of choline were provided the average liver fat did not exceed the value found in the control group. Betaine also prevented, in a very satisfactory manner, the effect of cholesterol on liver fat. The figures in Table I also show that the fat excretion was approximately the same in the experiments with and without choline.

Since the average figure for liver fat might be misleading to other investigators the fatty acid values of the individual livers for a cholesterol and a cholesterol-choline series are given in Table II.

TABLE II. Estimations of fat in liver (fatty acids p.c., and Iodine No.) in individual rats receiving:

A. Cholesterol <i>without</i> choline				B. Cholesterol <i>with</i> choline			
Fatty acids p.c.	Iodine No.	Fatty acids p.c.	Iodine No.	Fatty acids p.c.	Iodine No.	Fatty acids p.c.	Iodine No.
7.02	97	11.41	90	4.02	110	4.87	104
17.00	92	8.65	95	4.05	99	4.06	108
11.58	90	16.10	88	3.81	101	4.69	100
16.81	91	4.93	101	3.81	—	6.68	96
6.30	—	16.34	85	8.15	86	2.80	—
10.58	92	14.12	88	4.82	102	4.06	111
6.84	96	10.20	96	4.36	101	2.94	99
13.05	81	17.51	84	4.42	120		
8.69	94						

Choline or betaine produced no appreciable change in the fatty acid content of the kidney or stomach tissue of the cholesterol-fed animals.

It appears to be a foregone conclusion that orally administered lecithine would prevent the accumulation of liver fat produced by cholesterol in the same way that choline has been shown to do. This finding should provide clearer evidence for a "cholesterol-lecithine antagonism" than several of the frequently quoted results [Degkwitz, 1931].

Although choline prevents deposition of liver fat in cholesterol-fed animals there is no evidence as yet that this base is concerned in cholesterol metabolism, and in this series of experiments cholesterol was chosen merely because of its effect on deposition of liver fat. While the results thus far obtained suggest that the unsaponifiable fraction of liver fat may be less in the animals receiving choline than in the group on cholesterol alone, further experiments are required to settle the point. The results of this preliminary study suggest that investigation of the effect of choline on the cholesterol content of tissues under various conditions, as well as further work on liver fat in cholesterol-fed animals, may be profitable.

SUMMARY.

The deposition of fat in the rat's liver produced by feeding cholesterol can be prevented by adding choline or betaine to the diet.

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