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THE EFFECT OF CHOLINE ON THE LIVER FAT OF RATS IN VARIOUS STATES OF NUTRITION.

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In previous communications from this department and from the School of Hygiene, the effect of choline in preventing the deposition of fat in the liver of the white rat has been discussed. In this paper the action of choline in accelerating the removal of fat from the livers of these animals will be reported. Choline is found to be effective in curing as well as preventing the condition in white rats, as it does in the diabetic dog [Best, Ferguson and Hershey, 1933]. Not only does the fat disappear from the liver but there is definite improvement in the histological appearance of the liver cells [Best and MacLean, 1934]. The significance of the presence of choline, or of substances with similar action on liver fat, in feeding experiments with rats, will be made clear.

Methods.

The white rats were of the Wistar strain which we have used previously, and were supplied from the Connaught Laboratories' colony. The fatty livers were produced by feeding the stock diet of mixed grains to which beef fat had been added. This diet was the same as that used in previous experiments. The choline and the casein free from vitamins and from fat were obtained from the British Drug Houses. In the absence of sufficient amounts of a more satisfactory choline-free carbohydrate, commercial sucrose was used. The vitamin A, B_1 and D preparations were provided by Ayerst, McKenna and Harrison, Ltd. The total choline estimations were made by acetylation of the choline after acid hydrolysis of the mixture containing it and comparison of the acetylcholine with standard acetylcholine, using the isolated intestine of the rabbit. Liver and body fat was estimated by the saponification procedure. The iodine numbers were determined by the Rosenmund-Kuhnhenn method.

Results.

Curative experiment with continued feeding of grain diet and fat. Thirty rats were placed upon the diet rich in fat. After 21 days the livers were obtained from ten, and the average fat content was 15·2 p.c. The remaining twenty were allowed to remain on the same diet, but ten of these received in addition 70 mg. of choline daily. At the end of a further 3 weeks the liver fat of the animals which had received choline had fallen to 5·3 p.c., while the livers of those that had not were as fatty (15·4 p.c.) as those obtained 3 weeks before. These results are summarized in Table I.

Table I. Fat diet. Stock diet plus fat (fat 40 p.c. of total food).

No. of rats	Length of experiment (days)	Av. change in wt. (g.)	Fat eaten daily (g.)	Fatty acids of liver (p.c.)	Iodine No.
10	21	−22	2·5	15·2	—
10	42	−25	2·3	15·4	96
*10	42	−24	2·3	5·3	119

* 70 mg. choline were given daily during the last 21 days of this experiment.

Choline therefore is effective in removing the fat from fatty livers in spite of the continuance of the fatty diet just as it is in preventing its accumulation in normal livers. The results also suggest that a maximal deposition of fat has occurred when the rats have ingested the grain diet and beef-fat ration for 3 weeks.

Curative experiment without added fat. Ninety rats were fed on the 40 p.c. beef fat diet for 35 days. That their livers were fatty was indicated by analyses made on ten animals. The remaining eighty were placed on the mixed grain diet without added fat and half of them were given choline. In the previous experiment the diet contained 40 p.c. of fat; the grain diet contained less than 1 p.c.; so the condition of the liver was not likely to be aggravated. While the curves in Fig. 1 suggest that the loss in liver fat is more rapid in animals receiving added choline (this is particularly noticeable at the twelfth day) it is significant that the animals fed on grain with no choline added also lost fat from their livers. To decide whether this was due to the small amount of choline which we know to be present in the grain, to the low fat-content of the diet, or to the low caloric intake obtaining in this experiment, we tried first of all the effects of diets containing no choline, or very small amounts.

The choline content of our stock diet of mixed grains, as determined by biological assay, is 106 mg./100 g. in this experiment, therefore each rat got about 10 mg. daily. Other substances which exert a similar effect on liver fat may be present in the grain diet. Betaine is effective [Best and Huntsman, 1932]. Betaines are widely distributed among plants

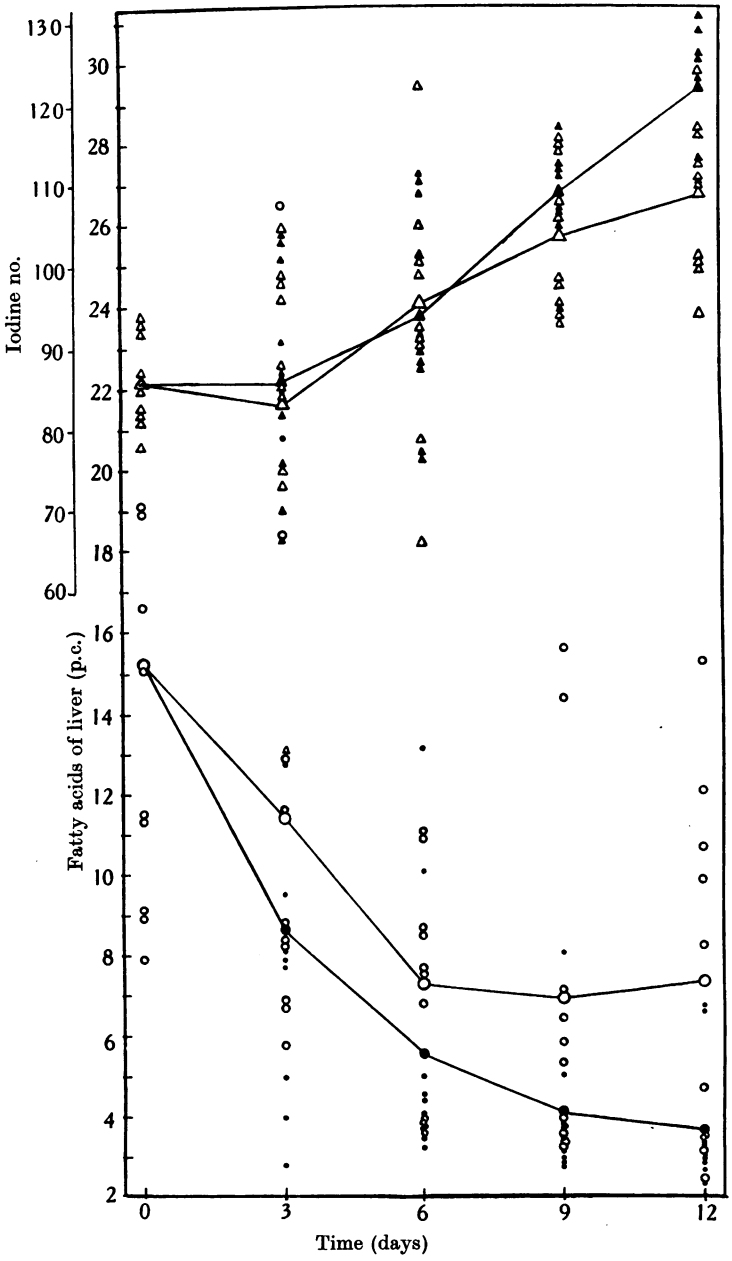


Fig. 1. Stock diet plus fat (40 p.c. of total food) for 35 days. o △ Grain diet; • ▲ grain diet plus 75 mg. choline daily.

Note: One result of 33·6 p.c. fatty acids in the control group at 0 days has been omitted.

and also occur in animal tissues, so this substance and perhaps others as yet unrecognized must be considered.

Curative experiments with a sucrose diet (choline-free). Rosenfeld [1932] has stated that a diet rich in carbohydrate prevents the development of fatty livers produced by fat feeding or by the action of certain poisons. Partly for this reason, but more particularly because sucrose contains no demonstrable amount of choline, we tried the effect of a sucrose diet; other carbohydrates which we would have preferred contain too much choline. Fatty livers were produced in rats as in the previous experiments, and the animals were then given nothing but sucrose; each animal ate about 10 g. daily. Half of them were given in addition 75 mg. of choline daily. The result (Fig. 2) was not expected. The fat in the liver increased, in some animals more than others; but this effect was clear and was confirmed in a second similar experiment (Fig. 3) in which the rats ate somewhat less. The falling off of the curve after the sixth day in this second experiment is not necessarily significant, as it is attributable entirely to three low values obtained on the ninth and twelfth days. If, as seems likely, the liver fat has been drawn from the depôts the increase could not have continued much longer, as the reserve fat of the body was being rapidly depleted. The rats were getting only 40 calories daily in the first experiment, somewhat less in the second, and were losing weight steadily. The rate at which in the second experiment they were losing fat is shown in Fig. 4, which shows also, however, that the reserves were not exhausted.

This rapid rise in liver fat is not only prevented by choline, but its amount is reduced to within normal limits in 12 days (Fig. 2) or even more promptly (Fig. 3). In the first of these experiments the average weight of the livers of the animals which received no choline was, on the twelfth day of the experiment, about 9 g. with 2·25 g. of fat, and of those that received choline was about 6 g. with 0·32 g. of fat. This is the most dramatic and easily demonstrable effect of choline on liver fat which we have encountered. While it brings to light no new feature of the action of choline, it may be useful in investigating it. Microphotographs of the liver containing 21·3 p.c. of fat from a rat which had no choline, and of a liver containing 3·3 p.c. from one that had choline are shown in Fig. 5. These amounts of fat are actually the average values found on the twelfth day in the two series respectively. In the former, in addition to the fatty infiltration, there are signs of degeneration in the liver parenchyma, which are not seen in the latter, although that one also cannot be regarded as perfectly normal.

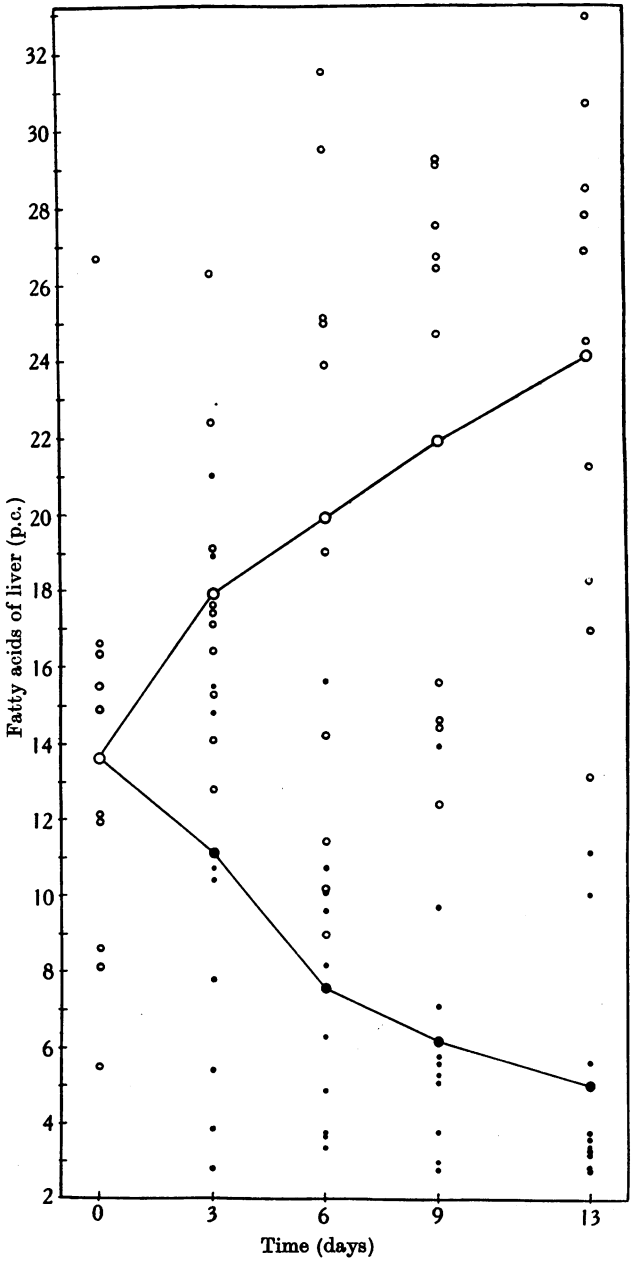


Fig. 2. Stock diet plus fat (fat 40 p.c. of total food) for 21 days. o Cane sugar; • cane sugar plus 75 mg. choline daily.

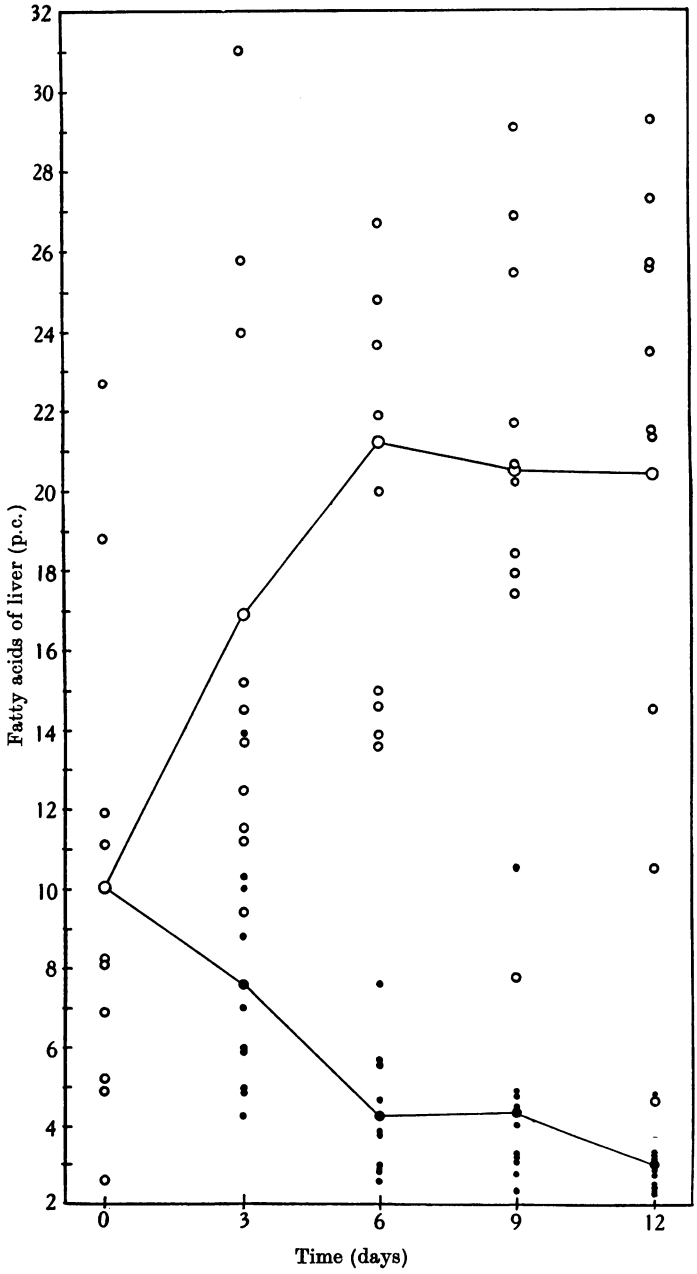


Fig. 3. Stock diet plus fat (fat 40 p.c. of total food) for 24 days. o Cane sugar; • cane sugar plus 50 mg. choline daily.

Note: One result of 37·7 p.c., sugar only, on the sixth day has been omitted.

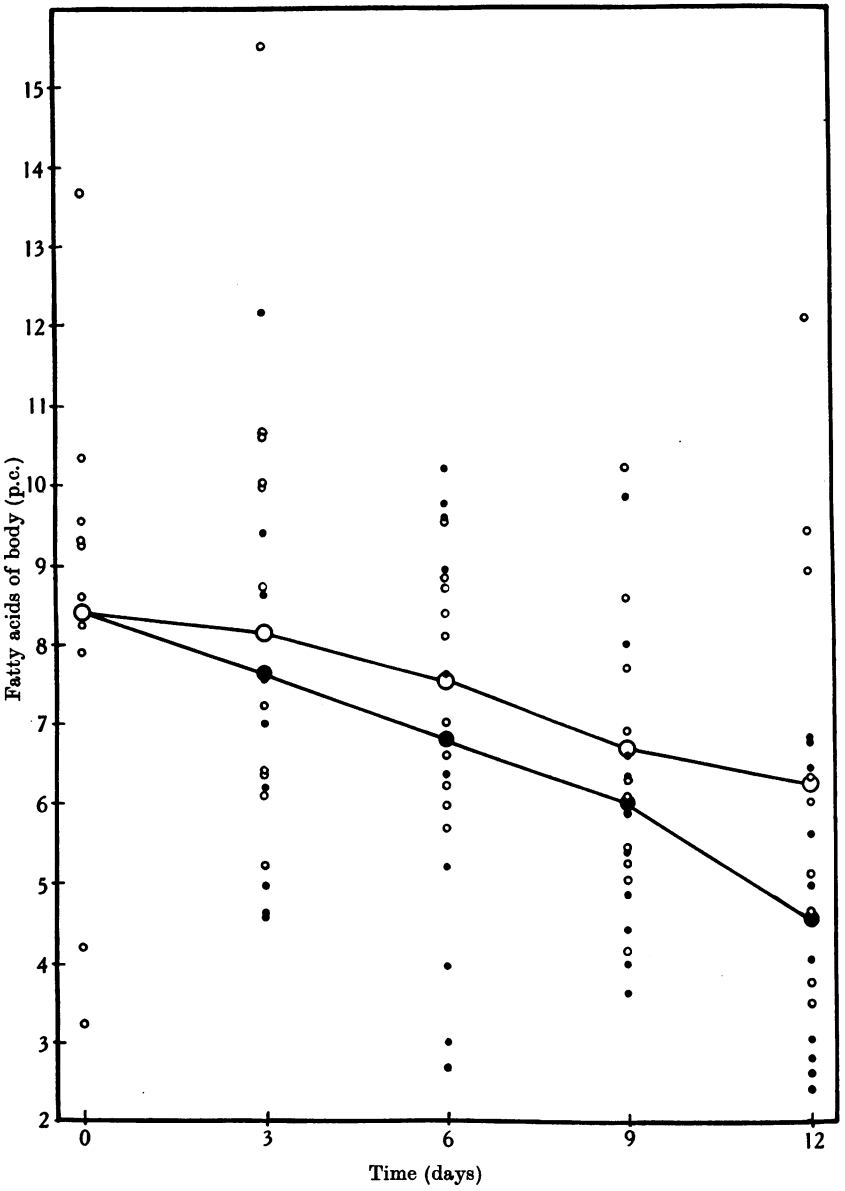
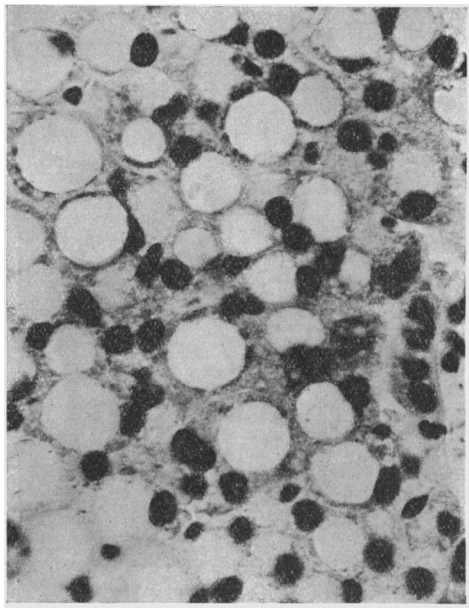
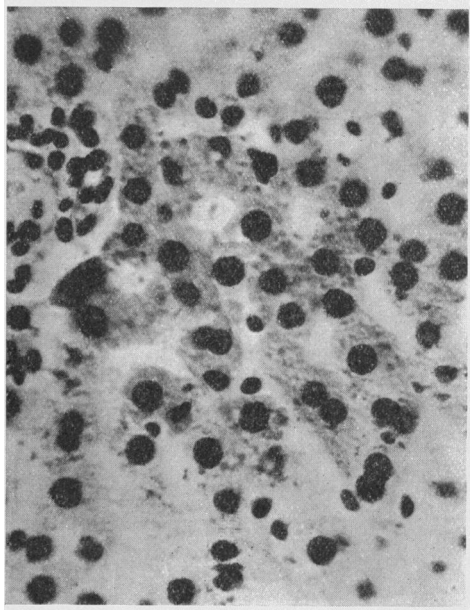


Fig. 4. Stock diet plus fat (fat 40 p.c. of total food) for 24 days. o Cane sugar; • cane sugar plus 50 mg. choline daily.



Without added choline fat content 21·3 p.c.



With added choline fat content 3·3 p.c.

Fig. 5. Rat liver—haematoxylin and cosin ×700. Rats in both groups received the stock grain diet plus fat (fat 40 p.c. of total food) for 24 days and then an exclusively carbohydrate ration (sucrose) for 13 days. During this latter period one half of the animals were given added choline.

This observation that feeding with sucrose will cause a further accumulation of fat in livers which are already loaded with fat is new and suggests that it was the choline present in the grain diet used in other experiments which caused the fat to leave the liver when the 40 p.c. of fat added to that diet was omitted.

Normal rats on a carbohydrate diet. This observation suggested also that normal rats on a similar diet might exhibit an accumulation of liver fat. That this is so, and that it is prevented by giving choline is shown in Fig. 6, where the iodine numbers of the liver fats are also given. The rate of weight loss was rapid whether choline was given or not.

In short-term experiments such as these the absence of vitamins may not be important, but it is possible that the animals would eat more food if these were supplied. No signs of vitamin deficiency were observed, though all the animals were undernourished. The normal rats ate 7·9 g. of sugar daily; those with fatty livers 10·0 g. in the first, 7·0 g. in the second experiment.

Curative experiment with a casein and sucrose diet. This experiment was similar to the preceding one, but after the livers had become fatty all the animals were placed on a diet consisting of 20 p.c. casein and 80 p.c. sugar. Each animal received not more than 0·2 mg. choline and 6 mg. ether-soluble material daily. The results are shown in Fig. 7. The livers of animals which received no additional choline lost no fat, those of animals which had 75 mg. daily lost fat rapidly. This diet also was deficient in many respects, but the animals were previously well fed and had it for only 12 days. The caloric intake was low and there was a moderate loss of body weight. The body fat content fell on the average from 13·6 to 5·7 p.c., as judged by analyses made on five animals from each group. Depôt fat was being rapidly mobilized, as in the experiments in which the diet consisted only of cane sugar. It has been found by Mottram [1909] and by Dible [1932, 1934] that in starvation when the fat reserves are rapidly drawn upon, appreciable amounts of fat may accumulate in the liver. Our own unpublished work shows that in starvation fat deposition may be prevented by giving appropriate amounts of choline.

It should be noted that with this diet the amount of fat in the liver did not increase as it did when sucrose alone was given, though it did not diminish as on the grain diet, which as we have said gave the rats 10 mg. of choline daily. Whether this was due to the fraction of a mg. of choline which the diet provided daily cannot be decided until experiments to determine the minimum effective dose of choline which are now in progress are finished. That it is small is shown later in this paper. It may

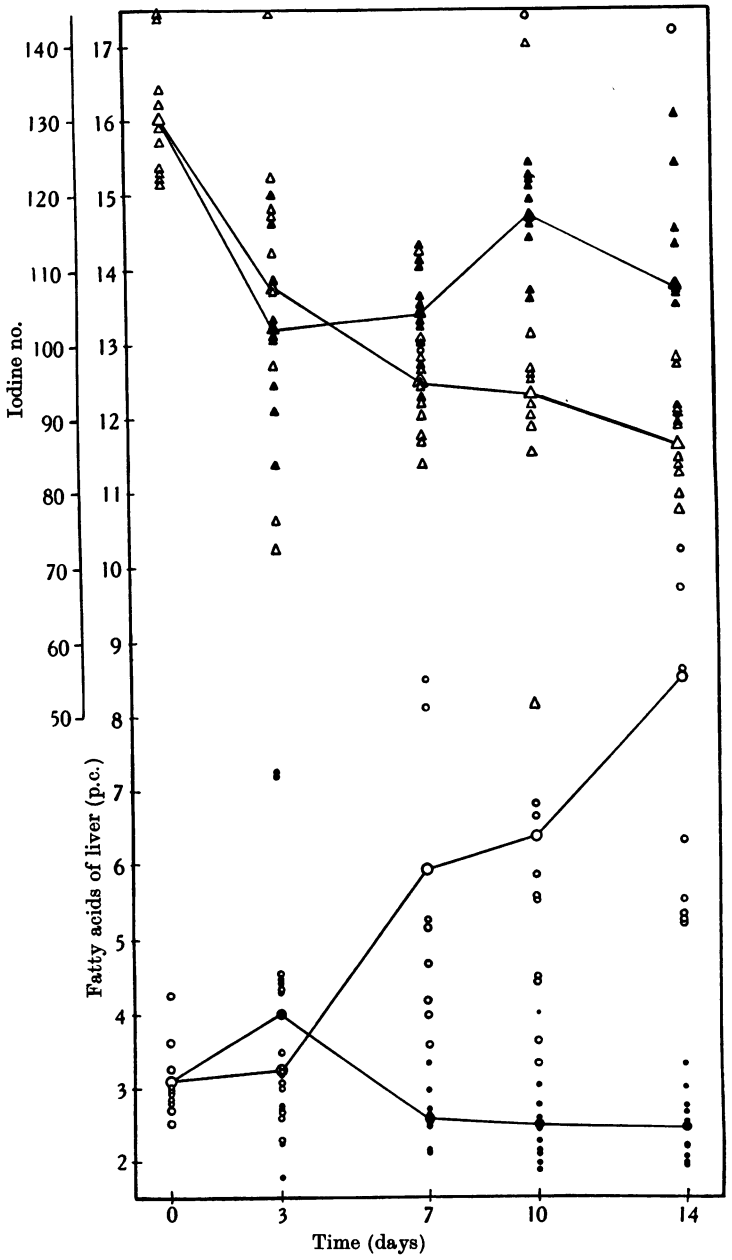


Fig. 6. Normal rats. o △ Cane sugar; • ▲ cane sugar plus 75 mg. choline daily.

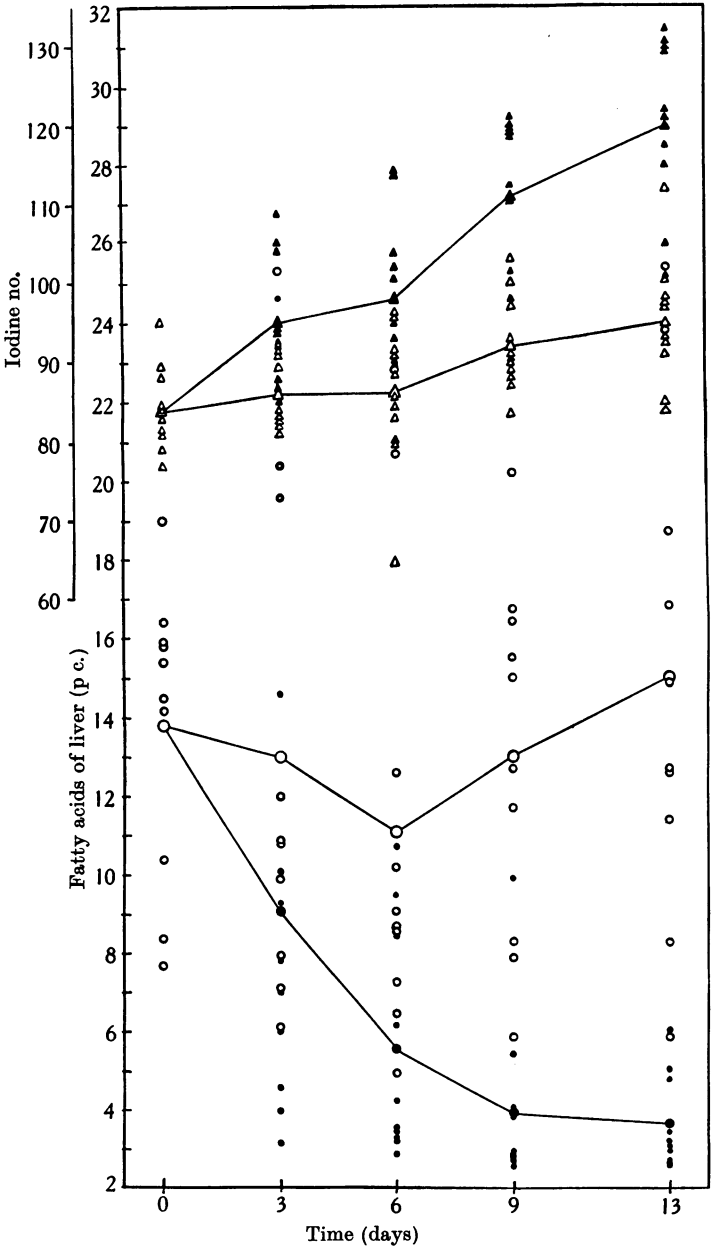


Fig. 7. Stock diet plus fat (fat 40 p.c. of total food) for 21 days. o ▵ 20 p.c. casein and 80 p.c. cane sugar; • ▲ 20 p.c. casein, 80 p.c. cane sugar plus 75 mg. choline daily.

be too that the casein provided by its metabolism betaines in effective amount; though if that is so it is curious that the metabolism of the proteins of the body should not.

Curative experiment with a more complete diet very low in choline (<0·2 mg.). Thirty rats were given the mixed grain and 40 p.c. beef dripping diet for 26 days. Livers were obtained from half of them for analysis and contained 13·2 p.c. of fat. The other half were put on the diet

TABLE II. Daily ration of diet low in choline.

Composition	Choline content based on analyses of 1 g. quantities γ
2·0 g. protein { 1·5 g. casein	75
2·0 g. protein { 0·5 g. egg white	63
0·5 g. fat (beef dripping)	3
12·0 g. sucrose	0
0·2 c.c. vitamin B_1 concentrate (Peters' method)	44
0·01 mg. vitamin A and D concentrate	0

Total food = 14·7 g. (60·5 calories). Total choline 1·25 mg./100 g.
A vitamin E concentrate very low in choline was also available but was not used in this short experiment. McCollum's salt mixture was supplied in some of the experiments.

(Table II) and were all killed on the 12th day by which time, as we have shown, the curative effect of choline is manifest. The livers contained not less but slightly more fat than the controls (Table III). For comparison

TABLE III. Stock diet plus fat (fat 40 p.c. of total food) for 27 days. Low choline diet for 12 days.

Rat No.	Food intake (cal. per day)	Change in wt. (g.)	Total fatty acids of liver (g.)	Fatty acids of liver (p.c.)	Iodine No.
1	25·3	− 9	1·493	20·4	79·0
2	31·6	− 3	1·361	17·2	79·2
3	24·2	−18	0·270	5·5	107·5
4	38·3	+ 5	0·843	12·3	80·0
5	36·4	− 3	1·056	12·6	80·7
6	41·5	− 3	0·739	11·0	82·0
7	41·7	0	0·881	12·0	82·0
8	31·2	−11	1·896	22·2	88·9
10	33·7	− 8	1·572	20·7	78·1
11	32·5	+ 4	1·488	19·6	72·0
12	31·2	−17	1·310	15·7	108·5
13	34·5	− 5	2·153	21·5	82·2
14	42·8	−16	2·478	27·2	84·0
15	40·7	−31	1·288	20·2	95·9
Average	34·7	− 8·2	1·345	17·0	85·8

with these results those given in Table IV are from rats which had the same diet but in addition 5 mg. of choline. They show that 5 mg. of choline daily is an effective dose and that the choline content of the grain

TABLE IV. Stock diet plus fat (fat 40 p.c. of total food) for 27 days. Low choline diet plus 5 mg. choline daily for 12 days.

Rat No.	Food intake (cal. per day)	Change in wt. (g.)	Total fatty acids of liver (g.)	Fatty acids of liver (p.c.)	Iodine No.
31	28·4	− 1	0·248	4·45	85·0
32	30·6	− 4	0·959	16·00	68·2
33	38·1	+ 8	0·265	3·90	89·5
35	26·4	−14	2·271	25·8	79·0
36	36·3	− 2	0·269	3·92	90·8
37	36·2	−13	0·201	3·35	86·1
38	29·1	− 4	0·206	3·53	69·0
39	32·2	− 5	0·205	3·67	86·2
40	29·5	− 8	0·246	4·20	70·7
41	31·9	− 6	0·198	3·22	84·1
42	35·2	− 2	0·235	3·96	69·5
43	34·0	+ 4	0·253	3·18	87·0
44	39·8	− 7	0·355	4·79	74·0
45	34·0	−15	0·216	3·53	81·1
Average	33·0	− 5·0	0·438	6·25	80·0

diet was the chief factor effective in reducing the amount of liver fat in the rats to which Fig. 1 refers. The only two high values observed in this series (Table IV) were from livers which to the naked eye obviously contained more fat than the rest, some parts being extremely fatty though others were comparatively free from fat. We suggest that the fatty changes in parts of these livers had, before choline was supplied, proceeded so far that they could not then be affected. Possibly the choline could not reach these particular parts of the liver. This phenomenon, which has been observed repeatedly in curative experiments, demands histological investigation.

The effect of low caloric intake on the disappearance of fat from the liver. In another experiment after the period on the beef fat the animals were given only the equivalent of 30 calories, half the amount given to those reported in Table III; but unfortunately the latter did not eat more than the equivalent of 35 calories on an average, some of them less than others on the lower ration. However, if we select five animals from each experiment the average daily caloric intake of one group (from Table III) is 41·0, and of the other group 25·6 calories. The composition of the diet was the same in the two cases. The figures (Table V) do not prove that there is any definite effect due to the different caloric intakes but favour the view that a lower intake produces a decreased rate of deposition of fat. An extensive investigation of the effect of caloric intake on the disappearance of liver fat has been planned, but it appears unlikely that moderate undernutrition will prove a very important factor. The effects of this variable are apparently overshadowed by the action of choline at the

TABLE V. Stock diet plus fat (fat 40 p.c. of total food) for 27 days. Low choline diet for 12 days.

Rat No.	Food intake (cal. per day)	Wt. of rat (g.)	Total fatty acids of liver (g.)	Fatty acids of liver (p.c.)
17	24·3	140	1·156	16·4
19	24·8	134	0·631	10·2
20	25·9	164	0·725	11·5
28	27·5	157	0·755	8·3
29	25·4	181	0·820	10·5
Average	25·6	155	0·817	11·4
4	38·3	141	0·843	12·3
6	41·5	148	0·739	11·0
7	41·7	161	0·881	12·0
14	42·8	165	2·478	27·2
15	40·7	167	1·288	20·2
Average	41·0	156	1·246	16·5

levels of caloric intake which we have thus far studied. Complete starvation, however, presents a different picture, and very low caloric intakes might give somewhat similar results.

Effect of starvation on the disappearance of fat from the liver. Seventy rats were fed on the diet rich in fat as before. Livers were obtained from ten for analysis and the remainder were fasted. One-half of the animals received 75 mg. of choline dissolved in 20 c.c. of drinking water. Additional water was supplied when that containing the choline had been consumed. The other half received water freely. The results, which are shown in Fig. 8, indicate that there is no increase in the fat content of fatty livers after 48 hours of fasting, at which time there is usually a well-marked infiltration of fat in the livers of fasted normal animals. Instead there is an abrupt decrease in fat content which proceeds to a very low level. There is no definite indication that this reduction in liver fat is hastened by choline, although the figures obtained on the fourth day suggest to us that this may prove to be the case. Body fat estimations on the same animals indicate that the decrease in fat content of the liver and of the rest of the body run a parallel course. The results of this experiment obviously show that the ingestion of choline is not essential for the disappearance of fat from the liver. Further starvation experiments in which the animals have at the beginning of the fast different levels of body and of liver fat will answer some of the questions which these results raise.

Comparison of effects of two diets both containing 40 *p.c. fat.* In another experiment two groups of rats with fatty livers (fifteen in each group) were allowed to remain on a diet rich in fat. One group continued to receive the grain diet while in the other the diet low in choline was sub-

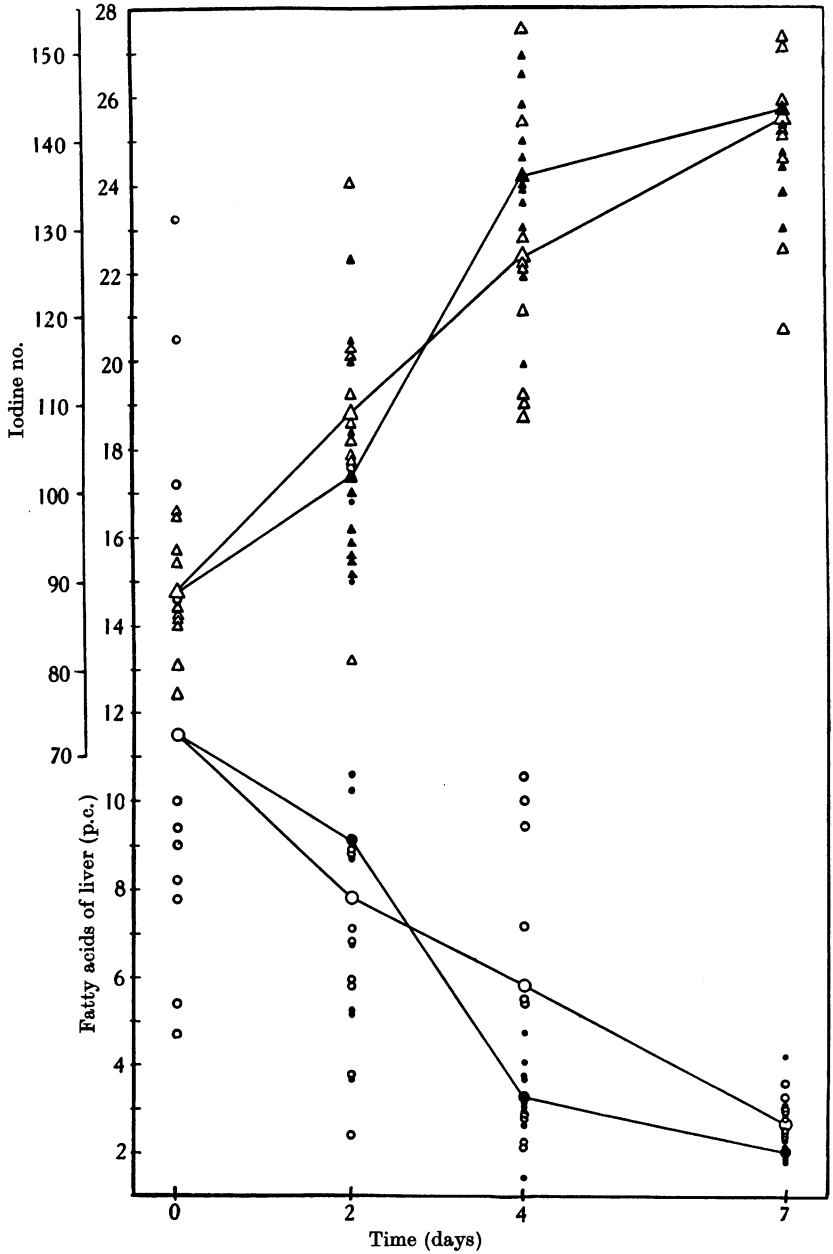


Fig. 8. Stock diet plus fat (fat 40 p.c. of total food) for 21 days. o △ Starvation; • ▲ starvation plus approximately 75 mg. choline daily.

Note: One iodine value of 183, 7th day, in the group receiving choline has been omitted.

stituted for the grain. At the end of 12 days the liver fats were determined (Table VI).

Evidently (Table VI) the choline, or some substance like it present in the grain diet, was preventing further deposition of fat. With the diet low

TABLE VI. Stock diet plus fat (fat 40 p.c. of total food) for 27 days. Diet indicated for 12 days.

Diet	No. of rats	Food intake (cal. per day)	Change in wt. (g.)	Total fatty acids of liver (g.)	Fatty acids of liver (p.c.)	Iodine No.
	15	—	—	1·00	13·2	83·3
Low choline diet with added fat (40 p.c.)	14	42·2	−1	1·93	21·1	73·2
Grain diet with added fat (40 p.c.)	14	46·2	−1	0·88	12·4	73·2

in choline further deposition took place. This result also supports the view that the amount of choline present in stock diets is capable of exerting a definite effect. On the grain fat diet the factors tending to increase liver fat had come into equilibrium with those tending to decrease it, so that a level of from 13·2 to 12·4 p.c. was maintained. When the low choline diet was substituted the liver fat rose over a period of 12 days to 21·1 p.c. The daily caloric intake of the fifteen rats on the grain diet plus fat was 46·2 calories and on the diet low in choline plus fat 42·2 calories. It will be interesting to determine whether or not the further increase in liver fat which one would expect when the rats are kept on a diet of sucrose plus beef fat will be greater than that which we have already obtained with sucrose alone or with the low choline diet plus beef fat.

DISCUSSION.

Curative effect of choline. The experiments reported leave no doubt that choline accelerates the rate of removal of fat from the liver under a variety of dietetic conditions. When the diet rich in fat is continued, the addition of choline causes a rapid decrease in the amount of liver fat. When nothing but sucrose is provided in curative experiments a maximal effect of choline may be secured in from 9 to 12 days. The addition of even 5 mg. of choline to the daily diet of rats is sufficient to cause a definite decrease in liver fat under certain conditions, so that the disappearance of fat from fatty livers with a mixed grain diet is apparently due in large part to the choline contained in the grains; for when the feeding of fat is continued, and a diet containing little choline is substituted for the grain, a

further increase in liver fat takes place, whereas in the grain diet there is sufficient choline to prevent the further increase in liver fat.

Is choline an essential dietary factor? It would appear therefore that a diet from which all choline and related substances with the same action on liver fat had been removed could not be considered adequate, although all the other known essentials were supplied. This hypothesis is supported by (1) the results of the experiments in which the grain diet exerted curative effects, (2) by the finding that 5 mg. of choline daily, that is, half the amount present in the usual ration of grain is an effective dose, (3) equally forcibly by the observation that the liver fat of animals with moderately fatty livers rises to extremely high levels when a diet low in choline but adequate in most of the known essentials is substituted for the grain, and (4) by the finding to be referred to in the next section, that normal rats on a choline-free diet develop fatty livers. An experiment bearing on this is in progress, with Dr E. W. McHenry, in which rats are being studied through several generations. Diets adequate in other respects but low in choline and with three different levels of fat intake are being used. Choline is being supplied to controls.

Diets low in choline. In animals with fatty livers sucrose alone leads to a greater deposition of liver fat than when the casein plus sugar or the more adequate diet low in choline is provided. The difference cannot be attributed to variation in caloric intake. The effect of the very small amount of choline contained in the other diets must be determined. The rapid and pronounced effect of 5 mg. daily makes this necessary. But it is possible that there may be a "specific" effect of sucrose. That it may have been converted into fat appears to us unlikely since the animals were losing fat from their stores. The absorbed carbohydrate is probably oxidized directly. The idea that the commercial sucrose might contain some toxic substance will be tested. But the action of choline in the sucrose experiments is quite different from its effect, for example, in phosphorus poisoning. Miss Ridout, D. L. MacLean and one of us (C.H.B. 1934) have found no effect of choline on the rate of deposition of fat in the liver in this condition, but a very definite acceleration of its rate of disappearance during the recovery phase, so it is unlikely that the sucrose owes any part of its effect to a contaminant.

The influence of the caloric intake changes on the amount of fat in the liver must be borne in mind. Many hold that the transient increase in liver fat observed in normal rats when fasted is due to mobilization of depôt fat to the liver. It might equally well be caused by a decreased "metabolism" of fat in the liver, and be due in part to lack of choline. We

have shown that large amounts of choline prevent this phenomenon. But when the liver is fatty to start with our results show (1) that fasting causes a prompt and rapid decrease in liver fat, the rate of which is affected slightly if at all, by choline, (2) that a certain amount of variation in the caloric intake above the level of 25 calories per rat per day produces very little effect on the rate of deposition. Rats with fatty livers have ingested no choline in the sucrose experiments when the fat content of the livers increased rapidly, and in the fasting experiments when the liver fat decreased abruptly, the initial amount of fat in the rats being about 8 p.c. In the sucrose experiments even when the caloric intake was very low, and the same is true of experiments with the more adequate diet, there was no tendency for the liver fat to fall. There must be a "critical point" of daily caloric intake between 0 and 25 calories in experiments of this kind and this duration above which liver fat rises and below which it falls, probably related to the amount of depôt fat which it helps to maintain. In the fasting experiment the amount of depôt fat was appreciably greater than that of the "élément constant" when the decrease in liver fat began. The determination of this "critical point" is to be attempted and may be facilitated by the use of "earmarked" fats, which incidentally may also help to decide whether any significant amount of the liver fat is formed from ingested carbohydrate when this is the only food supplied.

Diets low in choline increase the amount of fat in fatty livers, and may lead to fatty livers in normal animals. This is the case when sucrose only is provided, and Miss Ridout has obtained results, as yet unpublished, which prove that the same holds true for diets adequate in most other respects but low in choline. In order to produce fatty livers in rats or in dogs as quickly as possible a choline-free diet should be used. This new information is being applied in the work with our colony of diabetic dogs as well as in experiments with normal dogs and rats.

The livers of some of the animals which have been kept on the choline-free diet may weigh more than twice as much as a normal liver, and they often extend from an inch to an inch and a half further caudally than the normal liver of rats of the same weight. When choline is supplied the liver shrinks rapidly in size. This point is mentioned because of the possible clinical interest.

Other substances with effects similar to choline. We have as yet no method for determining the amounts of substances other than choline which exert similar effects on liver fat. We are planning experiments for this purpose but at present would merely refer to two points. First, if, as

Engeland [1909] suggested, betaines arise by the methylation of amino acids and are formed during the synthesis or breakdown of proteins, the deposition of liver fat might thus be linked to protein metabolism. Second, since lecithine is a component of every cell, extensive breakdown of cells might conceivably liberate appreciable amounts of this substance and its constituent choline. We have no new evidence on either of these points, and have at present no reason to suppose that they play a significant rôle in the interpretation of our results.

While the researches described above have yielded many new facts an exceptionally large number of problems demanding further study have appeared. Some of these are at present under investigation.

Summary and conclusions.

1. When white rats were fed on a diet consisting of mixed grains and beef fat, the fat content of the liver tended to become maximal after 3 weeks. At the end of a further 12 days on the same diet the fat content was not found to be significantly different. If, at the end of the 3 weeks, however, choline was added to the diet, a rapid decrease in the fat content of the liver took place. If, instead of adding choline, a diet low in choline but adequate in most other respects was substituted for the choline-containing grain diet, a further increase in liver fat occurred during the following 12 days.

2. If, however, the grain diet was continued but the beef fat omitted, a decrease in liver fat occurred. The rate of this decrease was accelerated by choline. If, after removal of the fat, a diet, otherwise adequate for a short term experiment but low in choline, was substituted for the grain, no decrease in liver fat took place. There was, on the other hand, a slight increase. When animals with fatty livers were placed on a choline-free diet composed exclusively of sucrose, a rapid and pronounced further increase in liver fat occurred. Not only was this prevented by choline but the liver fat was reduced to normal levels within from 9 to 12 days. When rats with fatty livers were supplied daily with a diet low in choline and an added amount of choline appreciably less than that contained in one day's ration of grain, a rapid decrease in liver fat was observed.

3. When rats with fatty livers were fasted there was a rapid decrease in liver fat, the rate of which was affected only slightly, if at all, by choline. Other more preliminary results have also suggested that the low caloric intake obtaining in some of the experiments does not favour the further deposition of fat in the liver.

4. When normal rats were fed on sucrose exclusively moderately fatty livers were rapidly produced. This change was also prevented by choline.

5. The results of these experiments not only demonstrate that added choline greatly accelerates the rate of removal of fat from the rat's liver under a variety of dietetic conditions but indicate that choline naturally occurring in a diet is an important factor in determining the level of liver fat produced by the diet. The evidence also justifies the suggestion that choline, or other substance or substances with the same action on liver fat, may prove to be a significant and perhaps an essential dietary factor, the complete absence of which from a diet may lead to the accumulation of an excess of liver fat.

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