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THE EFFECTS OF CHOLESTEROL AND CHOLINE ON LIVER FAT

By C. H. BEST and JESSIE H. RIDOUT

(*From the School of Hygiene, University of Toronto*)

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The results of the first investigation of this problem [Best and Ridout, 1933] indicated that large doses of choline or betaine inhibited the deposition of "fat" in the liver produced by feeding cholesterol. These results were confirmed and extended by Best, Channon and Ridout [1934], who noted that under certain experimental conditions the esters of glycerol were more readily affected than those of cholesterol. Channon and Wilkinson [1935] found, however, that in certain short-term experiments the rate of removal of cholesterol esters was not accelerated by choline, while a very slight effect was exerted on the glyceride fraction. These results which have been discussed by Best and Channon [1935] were due in part to the low glyceride content of the livers at the beginning of the experiment and in part to the short period of observation. Furthermore, added choline may exert relatively little effect when naturally occurring lipotropic factors are present in appreciable amounts in the diets used. Under more favourable conditions [Best and Ridout, 1935] choline accelerates the removal of the esters of both glycerol and cholesterol. Beeston, Channon and Wilkinson [1935] have recently confirmed the finding that choline inhibits the accumulation of both kinds of ester which is produced by feeding large doses of cholesterol.

This paper contains a further study of the action of choline on cholesterol esters and glyceride in the liver made fatty by cholesterol. Particular attention has been paid to the effect of choline on the rate of disappearance of cholesterol esters. The use of smaller doses of cholesterol has enabled us to demonstrate a more rapid and more extensive action of choline on these esters than previously.

Methods

The care of the rats and the methods of analysis of the tissues are similar in all essential details to those previously reported. The various components of the diets have also been described.

Experimental results and comments

I. *Experiments with continued administration of cholesterol*

(*a*) *Effect of choline when a relatively small dose of cholesterol is continued throughout experiment.* In this experiment 109 rats were placed on a diet consisting of mixed grains, bone meal (2·5 p.c.) and beef fat (20 p.c.). Fifty mg. of cholesterol were added to the daily ration of each rat. After 53 days the average liver fat in twenty of the animals was determined (Table I). All of the remaining animals were transferred to

Table I

No. of rats	Duration of exp. days	Av. change in wt. g.	Av. daily intake: Food g.	Av. daily intake: Cholesterol mg.	Av. daily intake: Choline mg.	Cholesterol: Free p.c.	Cholesterol: Ester as oleate p.c.	Glyceride as triolein p.c.	
20	53	+ 2	10	50	—	0·25	1·81	5·44	Preparatory period
14	18	−17	8	41	—	0·28	3·27	9·67	Test period
14	18	−26	7	35	87	0·30	0·65	1·27	,,
15	32	−35	7	37	—	0·28	3·16	7·15	,,
15	32	−33	6	32	81	0·28	0·31	0·94	,,
16	42	−44	7	37	—	0·29	3·44	3·63	,,
15	42	−34	6	32	81	0·28	0·34	0·54	,,

Average initial weight was 199 g.

In this and the following tables under "Glyceride" is included that portion of the total fatty acids present as simple glyceride and not as phosphorylated fat (lecithine etc.) nor as cholesterol ester. The figures for total fatty acid and for lecithine etc. are not dealt with in this paper and so are not included in the tables.

a diet low in choline (casein 11·5 p.c., egg white 3·5 p.c., beef fat 20 p.c., sucrose 58·3 p.c., salt mixture 4·8 p.c., agar 1·9 p.c., and vitamins A, D and B_1). Cholesterol feeding was continued and the various groups ingested from 31·5 to 40·5 mg. daily: slight variation in cholesterol intake does not produce a significant difference in liver fat. Choline was added to the diets of half of the animals and groups were examined after 18, 32 and 42 days. The behaviour of the glyceride fraction of the livers is very interesting in this experiment. The preliminary rise after transfer of the animals to the diet low in choline is the usual reaction of liver fat when the supply of choline and other lipotropic factors is reduced. This effect is consistently observed when animals are transferred from a diet

rich in fat and naturally occurring lipotropic factors to one free from both, *e.g.* sucrose only [Best and Huntsman, 1935]. The subsequent fall in the glyceride content of the livers of the animals which did not receive choline may be due, in part, to the appropriation of fatty acid from the glyceride by the cholesterol. While it appears unlikely, from unpublished data, that this fall in glyceride is due to depletion of body fat the possibility must also be considered in this experiment. The effect

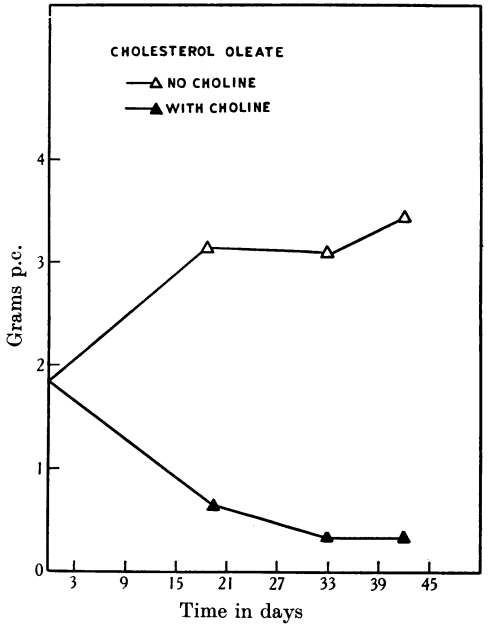

Fig. 1.

of choline on the glyceride fraction is typical. The change in cholesterol esters is very definite (Fig. 1). Without choline there is a prolonged rise while with choline a decrease is observed.

(*b*) *Effect of choline when a larger dose of cholesterol is continued throughout experiment.* In this experiment 105 rats were placed on the low choline diet and 95 mg. of cholesterol were added to the daily ration of each rat. After 24 days liver fat was determined on fifteen animals (Table II), and the remainder were kept on the same diet. Choline was given to half the animals and an attempt was made to keep the caloric intake of the two groups at the same level. It is interesting that with the amounts of choline and cholesterol used the glyceride fell at first even with no choline, while the cholesterol esters at first increased rapidly

even with it. The simplest explanation of the transient fall in glyceride without choline is the appropriation of fatty acid by the cholesterol. Body fats were certainly not seriously depleted at this stage of the experiment, since the loss in weight was slight. The rise in cholesterol esters

TABLE II

No. of rats	Duration of exp. days	Av. change in wt. g.	Av. daily intake: Food g.	Cholesterol mg.	Choline mg.	Cholesterol: Free p.c.	Ester as oleate p.c.	Glyceride as triolein p.c.	
15	24	—	9	95	—	0·31	3·10	8·24	Preparatory period
15	18	−10	8	81	—	0·34	6·92	3·12	Test period
15	18	−16	8	82	82	0·33	6·88	1·39	,,
15	27	−20	8	76	—	0·32	5·84	4·66	,,
15	27	−18	7	72	72	0·32	5·20	1·86	,,
15	58	−30	8	80	—	0·32	7·59	6·10	,,
15	58	−31	8	84	84	0·32	3·96	2·67	,,

Average initial weight was 168 g.

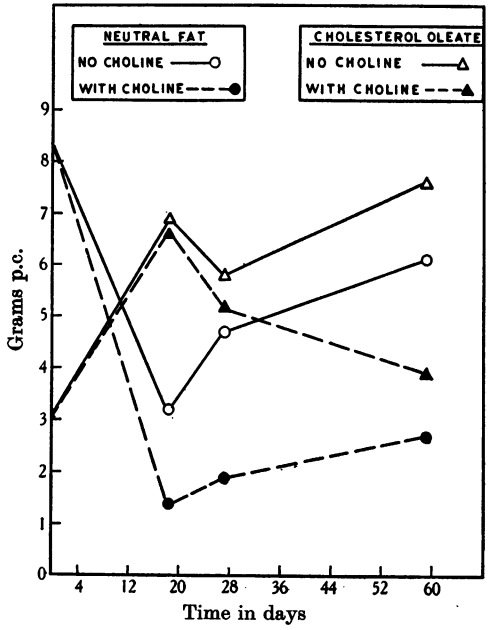

Fig. 2.

in spite of choline obviously indicates that cholesterol was in the ascendancy at this particular phase of the experiment. Later the effect of choline, which in the case of glycerides was obvious from the first, is quite well demonstrated on the cholesterol esters too (Fig. 2).

(c) *Low choline diet with* 10 *p.c. protein.* In this experiment seventy-five rats were placed on the diet low in choline, but the protein content was reduced from 15 to 10 p.c. and the carbohydrate correspondingly increased. Each animal had 100 mg. of cholesterol. After 25 days the liver fat was determined on the pooled livers of eighteen rats. The remaining animals were divided into four groups. The animals in two of the groups received choline. There was no significant difference in the cholesterol (70 mg.) intake of the four groups during the test period.

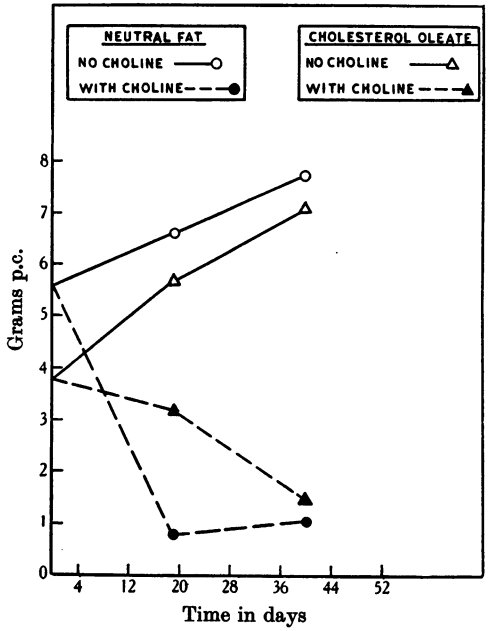

Fig. 3.

The livers from two groups of animals were analysed after 18 days and the others after 40 days. The effects of choline on glyceride and cholesterol esters are clearly demonstrated (Fig. 3).

II. *Experiments with cholesterol administration discontinued*

Three experiments of this type will be described briefly.

(a) In the first the animals were placed on the low choline diet, with 167 mg. of cholesterol added, for 14 days. The test period, with cholesterol discontinued, was 12 days. Two groups received sucrose only, while two were given sucrose (80 p.c.) and fat (20 p.c.). One of each of the two groups was supplied with choline. The results are shown in Table III.

TABLE III

No. of rats	Duration of exp. days	Av. change in wt. g.	Av. daily intake: Food g.	Cholesterol mg.	Choline mg.	Cholesterol: Free p.c.	Ester as oleate p.c.	Glyceride as triolein p.c.	
20	14	+ 4	10	167	—	0·24	2·58	6·09	Preparatory period
15	12	−32	10	—	—	0·24	3·83	6·81	Test period
14	12	−31	9	—	90	0·27	3·00	1·44	,,
13	12	−33	9	—	—	0·28	3·42	6·94	,,
14	12	−25	9	—	103	0·26	3·13	2·10	,,

Average initial weight was 190 g.

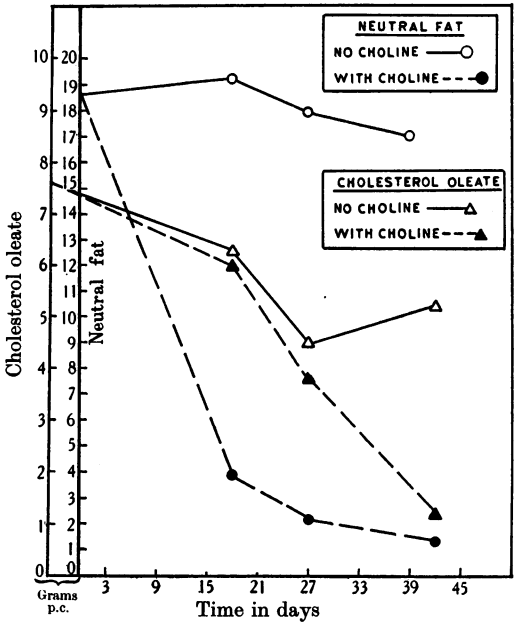

Fig. 4.

(*b*) In the second of these experiments 112 rats were placed on the low choline diet with 200 mg. of cholesterol added for a period of 23 days. At this time the cholesterol ester in the pooled livers of fifteen rats had reached a value of 7·51 p.c. and the glyceride 18·89 p.c. Cholesterol feeding was discontinued and choline (100 mg. daily) was given to half the animals. A preliminary account of this experiment was reported previously [Best and Ridout, 1935]; the results are illustrated in Fig. 4.

(*c*) In the third experiment an attempt was made to keep the glyceride at a low level during the preparatory period by supplying a small

TABLE IV

No. of rats	Duration of exp. days	Av. change in wt. g.	Av. daily intake: Food g.	Cholesterol mg.	Choline mg.	Cholesterol: Free p.c.	Ester as oleate p.c.	Glyceride as triolein p.c.	
20	45	—	9	46	23	0·26	1·02	4·43	Preparatory period
16	18	−10	9	—	—	0·23	0·38	6·74	Test period
16	18	−24	8	—	83	0·23	0·02	0·58	,,
17	27	−17	9	—	—	0·24	0·20	4·77	,,
17	27	−24	8	—	83	0·24	0·06	0·61	,,

Average initial weight was 182 g.

amount of choline (Table IV). During this time the animals received the stock grain diet, beef fat (20 p.c.) and 23 mg. of choline daily, together with 46 mg. of cholesterol. In the test period the diet low in choline was provided and cholesterol feeding was discontinued; the choline for half the animals was increased and eliminated from the diet of the remainder. In both groups the cholesterol esters of the liver decreased. The rate of fall was slightly but definitely greater in the animals receiving choline. The glyceride content in the rats without choline after a slight increase was at approximately the same level on the 27th day as at the beginning of the test period. These results provide further evidence that even in the absence of dietary choline the cholesterol esters decrease rapidly when cholesterol feeding is discontinued. There is, of course, a certain amount of non-choline lipotropic factor present in this diet, but since it has not caused a decrease in glyceride there is no reason to believe that it is exerting any effect on the cholesterol esters. All available data indicate that more choline is required to affect cholesterol esters than glyceride.

DISCUSSION

The finding that choline inhibited the deposition of cholesterol esters made it probable that the base would also accelerate the disappearance of these substances under appropriate conditions. The results described above show that this is the case. Appropriate conditions for the demonstration of this action are provided when a relatively small dose of cholesterol is given during the test period to animals which are receiving a diet low in lipotropic factors. It is then found that the cholesterol esters in the livers of the control series increase in amount while a rather prompt decrease is observed in the choline-fed group.

The rapid decrease in the glyceride content of the "cholesterol" fatty liver and the much slower fall in cholesterol esters when choline is

supplied appears to support the suggestion [Best, Channon and Ridout, 1934; Best and Ridout, 1935] that the primary effect of this substance is on the glyceride fraction. In the short experiments referred to above (Exp. II *a* and Table III), choline produced no effect on cholesterol esters while the glyceride fraction was very definitely affected. Furthermore, it has been consistently observed that the dose of choline required to influence deposition of cholesterol esters is appreciably greater than in the case of the glyceride. All the results reported here may be interpreted on the basis that an effect on the neutral fat precedes that on the cholesterol ester fraction, but the inference from this suggestion that the glyceride must be reduced to very low levels before an effect on the cholesterol esters is observed is not justified. These latter have shown a definite fall in several cases while an abundance of glyceride is present —and presumably available. The possibility that choline affects both cholesterol esters and glyceride directly has, therefore, not been eliminated.

The fall in glyceride in certain of the experiments (Exps. I *b* and II *a*) while the cholesterol esters are increasing is independent of the action of choline and suggests, in our opinion, an appropriation of fatty acids from neutral fat by the cholesterol. It will not be easy to determine whether the fatty acid which is appropriated is taken from the liver stores or is diverted from the fat which presumably is being steadily delivered to the liver under the conditions of our experiments. It will be noted that this phenomenon, which we have tentatively termed appropriation, is not consistently observed. It is not apparent, for example, in the short experiments (Exp. II *a* and Table III). There is a suggestion that it may only be observed when there is a rapid deposition of cholesterol esters (Exp. I *b* and Fig. 2). The problem is being investigated more fully.

When cholesterol feeding is discontinued the cholesterol ester content of the liver tends to fall, whether choline is given or not (Exp. II *b* and *c*), while the glyceride content, if choline is not given, is maintained at a high level until the supply of body fat is reduced to extremely low levels when it falls rapidly [Best and Mawson]. These results obviously indicate that for the maintenance of the high levels of cholesterol esters and neutral fat a continued supply of the constituents is necessary. If the food contains no fat, fat may come from the depots, but this is not so with cholesterol; when very little of this is supplied by the food the amount found in ester form in the liver falls. We may assume that the accumulation of cholesterol esters is limited by the supply of cholesterol;

fatty acids in these experiments would not be lacking; they are even necessary for the absorption of the cholesterol from the gut, and could also have been supplied from the depots.

Histological evidence indicates that when livers become extremely fatty the blood supply to some areas may be seriously disturbed. This disturbance might well interfere with the movement of cholesteryl or glyceryl esters into or out of the liver. But we do not suggest that such a result contributed to the phenomena reported here.

No explanation is at present available for the fact that with certain doses of cholesterol and choline the effect of the former predominates in the early part of the test period while later the action of choline is well demonstrated. The duration of the test period is thus a very important factor in experiments of this type. It will also be appreciated that the amount of fat available from the depots and the ability of the liver cells to take up fat from the blood and to "metabolize" it may change as the experiment progresses. The effect of a diet on liver fat may therefore vary according to its duration, a fact which must be borne in mind in the study of the effects on liver fat of amounts of choline and cholesterol which are present in various food materials.

The effect of choline on the deposition of cholesterol esters in tissues other than the liver has not been extensively studied. Unpublished results obtained when Prof. Channon was working in this department with us suggested that choline exerted relatively little effect on these other tissues. More recent results have indicated the desirability of further study of the kidney in this connection. An investigation of the action of choline on the deposition of cholesterol esters in the arteries of the rabbit would be interesting, but is not for the present being undertaken.

Summary and conclusions

1. When a relatively small daily dose of cholesterol is given to rats, in which a fatty liver has been produced by cholesterol, the addition of choline to the diet causes a very definite fall in both glyceride and cholesterol ester content of liver tissue.

2. When larger amounts of cholesterol are provided there may be an increase in cholesterol esters even, during the early part of the experiment, in animals receiving choline. Later, the effect of choline may be clearly shown.

3. Under these conditions (2) the glyceride level of the livers may fall while the cholesterol esters are increasing. In the control series of

animals this effect is not attributable to choline and is perhaps due to an appropriation by the cholesterol of fatty acids from neutral fat.

4. When cholesterol feeding is discontinued during the test period, choline accelerates the fall in cholesterol esters. The decrease, in the absence of choline, is probably due to cessation of cholesterol supply.

5. While the effect of choline on the glyceride fraction apparently always precedes that on the cholesterol esters, large quantities of neutral fat may still be present in the liver when the action on the cholesterol esters is well demonstrated. The possibility that choline affects these esters directly cannot be eliminated.

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REFERENCES

Beeston, A. W., Channon, H. J. and Wilkinson, H. (1935). *Biochem. J.* **29**, 2659.
Best, C. H. and Channon, H. J. (1935). *Ibid.* **29**, 2651.
Best, C. H., Channon, H. J. and Ridout, J. H. (1934). *J. Physiol.* **81**, 409.
Best, C. H. and Huntsman, M. E. (1935). *Ibid.* **83**, 255.
Best, C. H. and Mawson, M. E. Unpublished.
Best, C. H. and Ridout, J. H. (1933). *J. Physiol.* **78**, 415.
Best, C. H. and Ridout, J. H. (1935). *Ibid.* **84**, 7*P*.
Channon, H. J. and Wilkinson, H. (1935). *Biochem. J.* **29**, 350.