

## THE EFFECT OF CHOLINE ON THE FATTY LIVER OF CARBON TETRACHLORIDE POISONING

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IN a previous communication [Best, MacLean & Ridout, 1935] it was shown that when rats maintained on a diet low in lipotropic factors were poisoned with phosphorus, addition of choline to the diet had no effect on the degree of fatty infiltration or damage to the hepatic cells. On the other hand, the addition of choline increased the rate of removal of excess fat which was present in the livers of the poisoned animals.

Since it is well known that diets low in choline result in the accumulation of fat in the liver, it was not possible to differentiate between the liver fat which was present due to the lack of choline and that which was caused by phosphorus poisoning. Maintenance of animals on a diet with added choline, sufficient partially to counterbalance the effect of the low-choline diet, should result in a more marked difference in the amounts of fat present in the livers of the poisoned and control animals. This should make it possible to determine if the addition of choline exerts an effect on the fat which has accumulated as a result of the poisoning. This procedure was therefore adopted in the present investigation.

Carbon tetrachloride was used in the experiment to be described, since this material produces very fatty livers and the degree of poisoning is more easily controlled than with phosphorus.

### EXPERIMENTAL PROCEDURE

One hundred and twenty-four male rats with an average weight of 228 g. were divided into three groups. All animals were maintained on the following basal diet which was low in choline and other lipotropic

factors: meat powder 10%, beef fat 20%, sucrose 63%, agar 2%, salt mixture 5%, vitamins A, B<sub>1</sub> and D.

Five mg. choline for each 10 g. food were given to the animals in groups I and II. Previous experiments had shown that this amount of choline prevents, to a considerable extent, the accumulation of fat due to a low-choline diet. The rats in group II received the same amount of

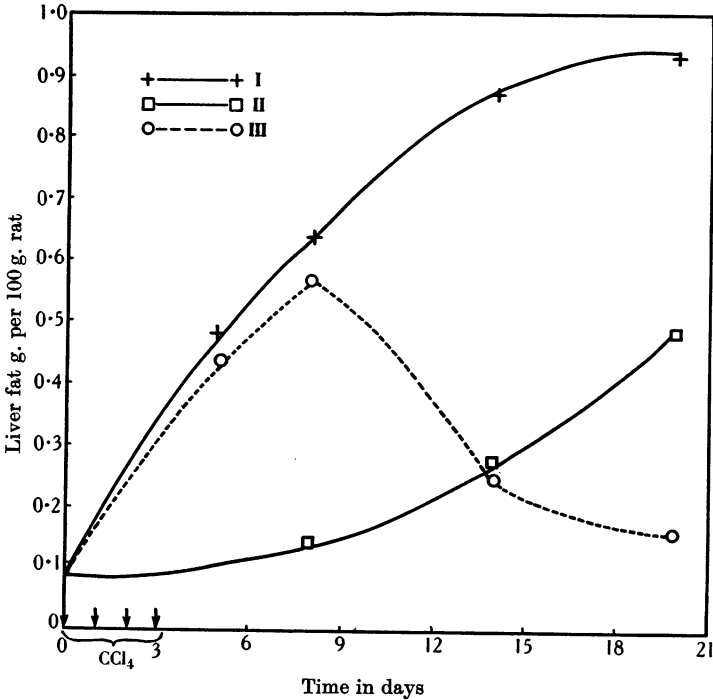


Fig. 1.

food as those in group I. One hundred mg. choline for each 10 g. food were given to the animals in group III. Groups I and III were given four subcutaneous injections of 0.8 c.c. CCl<sub>4</sub> on the first four days of the experiment. Animals were killed on the 5th, 8th, 14th and 20th days after the initial injection and the amount of fat present in individual livers was estimated by Liebermann's saponification method as modified by Leathes & Raper [1925]. The experimental results are shown in Fig. 1. Each point on the curve represents the average amount of fat obtained from the livers of twelve to fifteen animals.

## DISCUSSION

There is no significant difference in the amount of fat present in the livers of the two groups of animals poisoned with  $\text{CCl}_4$  until the 8th day. Addition of a large excess of choline to the diet had no effect on the accumulation of fat due to the poisoning. On the 14th day the amount of fat present in the livers of the poisoned animals receiving 5 mg. choline for each 10 g. food is much greater than that present on the 8th day. On the other hand, in the group of animals which received 100 mg. choline for each 10 g. food, a large decrease in liver fat occurred during this interval. At 20 days the fat content of the livers of the poisoned animals receiving 100 mg. choline is almost normal, whereas the livers from a comparable group given only 5 mg. choline contain a large amount of fat. Five mg. choline for each 10 g. food were obviously insufficient to maintain the liver fat of unpoisoned animals at a normal level. The marked difference in the amount of fat present in the livers of groups I and II at all periods of the experiment shows that a large part of the excess fat present in the livers of group I was brought about by the treatment with  $\text{CCl}_4$ . Addition of an excess of choline to the diet has resulted in the removal of almost all this excess fat within a period of 20 days.

The results of the examination of histological sections of liver taken from all groups of animals at 5, 8, 14 and 20 days were in general agreement with the chemical findings. There was no significant difference at the 5 and 8-day periods between the livers of groups I and III. At 14 days the livers from group I (receiving 5 mg. of choline) showed decidedly greater damage than those from group III which had received 100 mg. of choline. This difference was much more marked at 20 days, at which time the livers from group III presented a nearly normal appearance, whereas those from group I showed the presence of an excessive amount of fat.

The livers from group II appeared almost normal at 8 days, and at 14 days showed only a moderate accumulation of fat; there was, however, a wide variation in the appearance of individual livers. At 20 days livers from this group were very fatty; in fact it was not possible to distinguish between sections from groups I and II, although the chemical estimations showed that livers from group I contained about twice as much fat as those taken from group II. In this connexion it should be pointed out that when a liver contains a certain level of fat, it is not possible to determine by histological means either the presence of additional fat or the degree of degeneration of liver cells since these changes are masked by the vacuoles or fat globules which are present.

## SUMMARY

Rats maintained on a diet low in lipotropic factors with an amount of added choline sufficient partially to prevent the increase in liver fat due to a low-choline diet develop very fatty livers during the 20-day period

following the administration of  $\text{CCl}_4$ . Animals poisoned with  $\text{CCl}_4$  and fed the same diet, with the addition of excess choline, have almost normal livers at the end of the same period. The results of this experiment strongly suggest that choline or other lipotropic factors are essential for the removal of the excess fat which accumulates in the livers of rats as a result of  $\text{CCl}_4$  poisoning.

## REFERENCES

- Best, C. H., MacLean, D. L. & Ridout, J. H. [1935]. *J. Physiol.* **83**, 275.  
Leathes, J. B. & Raper, H. S. [1925]. *The Fats*, 2nd ed. London: Longmans, Green and Co.