Mechanism of Action of Choline

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IT IS obvious that an accumulation of fat such as is found in the liver of animals with choline deficiency may result from a change in the rate of a variety of metabolic processes. For instance, it may be due to an increased synthesis, a decreased oxidation in the liver, an increased mobilization from the depots to the liver or a decreased mobilization from the liver to other tissues. Accordingly, the lipotropic action of choline could be explained by a stimulation or inhibition of any one or more of such processes.

During the last twenty years a number of investigators have attempted to choose between these various interpretations by giving heavy water,[1-4] or, more recently, C^{14}-acetate[4,6] to animals with or without a deficiency of choline, and then comparing the amounts and concentrations of the newly synthesized fatty acids in the livers and depots of these two groups of animals. The results of recent experiments carried out in our laboratory on mice fed labeled acetate[6] are essentially in agreement with those previously obtained by others. In all animals the specific activities of the fatty acids were higher in the liver than in the muscle, suggesting that the labeled fatty acids which accumulate in the liver are chiefly synthesized in this tissue rather than originating in the depots. On the other hand, no significant differences were found when the specific activities of the fatty acids in the animals receiving, or not receiving, choline were compared. This might be taken as an indication that choline does not affect either the synthesis or the oxidation of fatty acids.

However, a simple calculation of the differences in the fatty acid content of the choline-deficient and non-choline-deficient livers shows that such differences could well be explained by small changes in the rate of either the synthesis or the disposal of fatty acids, and that these changes might be small enough to fall within the limits of error of the isotopic determinations.

Because of the difficulty of interpreting the results obtained on intact animals, we thought years ago that more direct information on a possible role of choline in the oxidation of fatty acids could be obtained in experiments on isolated preparations of the liver. In this type of experiment, the use of labeled fatty acids as substrates seemed quite appropriate, since, by determining the isotopic concentration in the carbon dioxide and acetoacetate produced, changes in the oxidation rate of fatty acids could be detected independently from changes in the over-all metabolism of the tissue. Moreover, because of the sensitivity of radioactive measurements, extremely small amounts of isotopic substrates can be used, and thus one may minimize the well known inhibition of oxidation which occurs when fatty acids are added to tissue preparations.

In each of our experiments,[7] isolated preparations of the liver were incubated with C^{14}-stearate, or C^{14}-palmitate. The livers of choline-deficient rats produced much less isotopic carbon dioxide and acetoacetate than similar preparations from the livers of rats which had been maintained on the same diet, but which had received choline in the diet, or by injection shortly before sacrifice. Since similar differences were found with liver slices as well as with unfractionated homogenates, or mitochondria, such differences cannot be due to a change in the permeability of the cell membranes. Moreover,

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Presented at the First Symposium of the Lipotropic Research Foundation, April 13, 1958, Philadelphia, Pennsylvania.

Most of the work covered by this report was carried out with the aid of the U. S. Atomic Energy Commission.

since most fat is eliminated in the preparation of the mitochondria, our results could not be ascribed to a greater isotopic dilution of the substrate by the neutral fat accumulating in the choline-deficient livers.

The last statement is further corroborated by the results of more recent experiments[6] in which we have studied the distribution of the isotopic carbon in the neutral fat, phospholipid and free fatty acid fractions of the incubated livers. During the incubation, part of the isotopic fatty acid is incorporated into the neutral fat and phospholipid, the amounts incorporated being almost identical in the deficient or non-deficient livers. The only differences between these two types of livers are in the C^{14} contents of the free fatty acids remaining at the end of incubation. These correspond almost exactly to the opposite differences in the $C^{14}O_2$ and C^{14}-acetoacetate produced during the incubation. In other words, it seems that the oxidation has occurred at the expense of the free fatty acid only, and that an isotopic dilution of this fraction by the neutral fat present in the tissue is unlikely.

In subsequent experiments with a similar technic,[8] we have shown that, besides the liver, other tissues of the deficient animals, especially the kidney and heart, exhibit a decreased ability to oxidize fatty acids, and that this decrease is also prevented by the administration of choline to the animals. These findings may have some interest in relation to the problem of the development of atherosclerotic lesions in the myocardium and kidney of choline-deficient animals.

While oxidation of fatty acids in the deficient livers was enhanced by administration of choline *in vivo*, addition *in vitro* of choline or simple choline derivatives, such as betaine aldehyde, betaine or phosphorylcholine, was totally ineffective. These results suggest that the active factor is not free choline, but some compound which is formed from choline *in vivo*, not *in vitro*, and which is firmly retained in the granular structures of the cell, even after repeated washings as in the preparation of mitochondria. The most obvious possibility was that choline enhanced oxidation of fatty acids by promoting the formation of tissue lecithins.

Actually, a comparative analysis of the data obtained from the animals receiving, or not receiving, choline indicated a rough parallelism between the level of lecithins in the liver and the ability of the isolated tissue to oxidize fatty acids added *in vitro*. Furthermore, when a single large dose of choline was injected into choline-deficient animals and these animals were killed at various intervals up to three hours after injection, both the level of lecithin and the rate of oxidation of fatty acids *in vitro* increased progressively.[6]

In several other series of experiments,[6,9] we also investigated whether or not, after administration of substances which directly or indirectly might interfere with the synthesis of choline or of choline-containing phospholipids, the amounts of lecithins and the ability of the liver to oxidize fatty acids would show changes in the same direction. This was the case when the diet was supplemented with triethylcholine, the effects of this compound being similar to those observed when choline was given. However, administration of diethanolamine, an analogue of ethanolamine, was followed by a decrease in the liver lecithins, whereas the isolated tissue still oxidized fatty acids at a high speed. After rats were given ethionine, a methionine analogue, the *in vitro* oxidation of fatty acids was greatly inhibited, whereas lecithin levels were as high as in the control animals. It seems, therefore, that a correlation between the levels of lecithins in the liver and the ability of the tissue to oxidize fatty acids is apparent in some but not in all conditions, and that livers with a low content of lecithins, as in the animals fed diethanolamine, are still quite competent to carry out the *in vitro* oxidation of fatty acids.

Recent observations from this and other laboratories have introduced some further complications in the interpretation of the results of our earlier experiments. The low casein diets which we used in our experiments were deficient not only in choline, but also in sulfur-containing amino acids. When we supplemented these diets with either methionine or cystine,[10] the oxidation of fatty acids in the isolated liver was raised to about the same degree as after supplementation with choline. Addition of tocopherol to the deficient diet was also effective. On the

other hand, when instead of a 5 or an 8 per cent diet, we used a diet containing 15 per cent of alpha protein from soybeans, the effects of choline administration on the oxidation of fatty acids *in vitro* became less marked and less consistent. Similar results have recently been published by other investigators who also used relatively high levels of proteins with a very low content of methionine.[4,11] It would seem that a considerable decrease in the ability of the isolated liver to oxidize fatty acids occurs only when the choline deficiency is combined with a severe protein deficiency. Such a decrease can be prevented to a large extent by supplementing the diet with choline, cystine or tocopherol, or just by raising the protein content of the diet without substantially increasing the supply of methionine or cystine.

CONCLUSIONS

The present evidence for a role of choline in the oxidation of fatty acids remains restricted to the results of *in vitro* experiments on the liver of animals previously maintained on low protein diets. We do not know whether or not this evidence can be extended to the conditions of the intact animal. The lipotropic action of choline probably cannot be explained only by an increased oxidation of fatty acids in the liver, and therefore the hypothesis that choline stimulates the mobilization of fatty acids from the liver is still a likely possibility. Actually, the two interpretations are not mutually exclusive. For instance, one might postulate that a deficiency of choline impairs the formation of liver lipoproteins, containing lecithins as their lipid moiety. These lipoproteins could be a transport form for fatty acids in blood plasma, and could also be important for the functional integrity of the mitochondria of the liver, perhaps by maintaining the spatial configuration of the enzymes involved in the oxidation of fatty acids.

Aside from any speculations, it is obvious that an explanation of the mechanism of the action of choline will not be fully acceptable until we can express this mechanism in chemical terms and at the molecular level. For the moment we have to confess that very little is known about this topic, and I can only express the hope that, by finding a newer approach and more appropriate tools, some younger investigators will be finally able to solve this interesting problem.

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