Hepatic Adenosine Triphosphate Levels During Acute Choline Deficiency in the Rat[1] (36507)

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Previous studies carried out in this laboratory have indicated that during the rapid induction of choline deficiency in young rats there is a slowdown in the intracellular transport of albumin (1, 2). This defect has been attributed (2, 3) to alterations in the structure (4) and composition (2, 5, 6) of the endoplasmic reticulum and of the Golgi apparatus of hepatocytes which occurs in these animals. It is indeed known (7–9) that the processes of intracellular transport and secretion of proteins synthesized for export are a function of the membranous organelles of cells. However, it has been shown recently that certain phases of the processes of intracellular transport and secretion of proteins, at least in pancreas, are energy dependent (10–12), the energy being supplied by ATP. The question therefore arises of whether the slowdown in intracellular transport in choline-deficient rats may be the result of or related to a reduced level of hepatic ATP.

The effect of choline deficiency on the level of hepatic ATP has been studied previously by other investigators. Simon, Scheig and Klatskin (13) reported no difference between choline-deficient and choline-supplemented rats fed the respective diet for 5 days. On the other hand, Chalvardjian (14) and Dianzani (15) observed decreased levels of hepatic ATP in rats fed a choline-deficient diet for 2 days to 6 weeks. However, Chalvardjian (14) noted that restoration of hepatic ATP to control values, following administration of adenine sulfate, did not alter the development of the fatty liver induced by choline deficiency.

In view of this conflicting evidence, and of the fact that results previously obtained in this laboratory were obtained mostly in rats fed a choline-deficient diet for only 1 day, it was decided to reinvestigate whether choline deficiency affects the level of hepatic ATP. Since the animals are fed the choline-deficient and choline-supplemented diets after an overnight fast, the effect of fasting was also investigated.

White male rats of the Sprague-Dawley strain (Sprague-Dawley, Inc., Madison, WI) were prepared for the experiments as previously reported (2, 16). Animals weighing 95 to 105 g were divided into groups of 5 animals each. Two experiments were performed. In each experiment, one group of rats was fed laboratory chow and a second group was fasted overnight. In the first experiment, a third group of rats was fasted overnight and then refed laboratory chow for 15 hr. In both experiments, the other groups of rats were fasted overnight and then fed the choline-supplemented or the choline-deficient diet for either 15 or 24 hr. The latter two diets were prepared according to the basal B diet of Young *et al.* (17).

Liver ATP was determined as previously reported (18). The animals were anesthetized with Penthrane (Abbott Laboratories, North

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TABLE I. Effect of Choline Deficiency and of Fasting on the ATP Level of Rat Liver.

Nutritional state	Liver wt (g[a])	Liver ATP[a] (μmoles)	
		per g wet wt	per 100 g body wt
Lab. chow fed	5.51 ± 0.07	1.87 ± 0.16	10.90 ± 0.69
Fasted overnight	3.88 ± 0.04	2.27 ± 0.11	8.26 ± 0.31
Refed Lab. chow, 15 hr	6.48 ± 0.12	1.75 ± 0.07	11.33 ± 0.28
Refed CS[b] diet, 15 hr	7.56 ± 0.21	1.48 ± 0.10	11.12 ± 0.58
Refed CD[b] diet, 15 hr	7.17 ± 0.22	1.56 ± 0.13	11.23 ± 0.28
Lab. chow fed	4.85 ± 0.11	2.33 ± 0.11	10.55 ± 0.39
Fasted overnight	3.43 ± 0.07	2.17 ± 0.09	8.00 ± 0.48
Refed CS diet, 24 hr	5.76 ± 0.26	1.31 ± 0.20	7.80 ± 1.0
Refed CD diet, 24 hr	5.10 ± 0.23	1.46 ± 0.21	7.90 ± 1.35

[a] Mean ± SEM, 5 animals/group.

[b] CS, choline supplemented; CD, choline deficient.

Chicago, IL) and immediately exposed to 100% oxygen. After laparotomy, a segment of liver, approximately 500 mg, was frozen between the faces of metal tongs precooled in liquid nitrogen (19). The frozen aliquot was then extracted with ice-cold perchloric acid, 3.14% (w/v). ATP was determined on the neutralized extract by the luciferin–luciferase reaction (20).

The results are presented in Table I. Overnight fasting did not alter significantly the concentration (μmoles/g wet wt) of hepatic ATP but reduced the level (μmoles/100 g body wt) significantly ($p < .01$, Student's t test) in both experiments. This reduction can apparently be accounted for by a parallel decrease in liver weight. In rats refed laboratory chow, the choline-supplemented or the choline-deficient diet for 15 hr, the level of hepatic ATP was essentially the same as that in the nonfasted group fed laboratory chow. On the other hand the concentration of ATP tended to be lower the higher the liver weight. However, the mean concentration in the 3 groups was not significantly different from that of the nonfasted group, but was so ($p > .0005$) when compared to the mean of the fasted animals. In rats fed the choline-supplemented or the choline-deficient diet for 24 hr the level of ATP was the same as that in the fasted group but significantly ($p < .05$) lower than that in the nonfasted group, while the concentration was significantly ($p < .025$) lower than that in both the fasted and nonfasted group. Again there was no difference in either the concentration or the level of ATP between rats fed the choline-supplemented or the choline-deficient diet.

The results, therefore, indicate that fasting overnight decreases the level of hepatic ATP of rats along with their liver weight. After 15 hr of refeeding, when the animals most likely are in an active absorptive state, the level of ATP is the same as that in nonfasted animals, while after 24 hr, with the animals probably in a postabsorptive state, the level is the same as that after overnight fasting. On the other hand, the concentration of ATP seems to be related more to the weight of the liver than to the nutritional state of the animals.

In agreement with the findings of Simon, Scheig and Klatskin (13), acute choline deficiency was found not to affect hepatic ATP. Indeed in rats fed the choline-deficient diet for 15 to 24 hr both the concentration and the level of hepatic ATP were the same as those of the proper controls fed the choline-supplemented diet. Thus, assuming that also a redistribution of ATP among the various cellular compartments does not occur, it appears that the slowdown in the intracellular transport of albumin occurring in choline-deficient rats is not attributable to a lack of ATP. It has been shown, recently, that albu-

min is synthesized by membrane-bound ribosomes (21, 22), and once transferred into the cysternae of the endoplasmic reticulum moves to the Golgi apparatus (8, 23) of the liver cell before being secreted into the circulation. Acute choline deficiency causes structural and compositional changes of the endoplasmic reticulum and Golgi apparatus of hepatocytes (2, 4, 6). It appears as more likely that these changes are responsible for the slowdown in the intracellular transport of albumin caused by choline deficiency.

Summary. The effect of feeding a choline-deficient diet on the level of hepatic ATP was studied. Groups of rats were fed a choline-deficient or a choline-supplemented diet for 15 and 24 hr, and the level of hepatic ATP was found to be similar in rats fed the two regimens. A deficit of ATP does not appear to be a factor in the delayed intracellular transport of albumin observed in rats fed a choline-deficient diet.

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