Effect of Cold-Exposure and Fasting on Pathways of Hepatic Glucose Oxidation.* (24709)

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Our earlier studies(1-3) have revealed that fasting rats for 24 hrs. at 0-2°C (cold-fasted rat) causes marked alterations in hepatic fatty acid metabolism. It is of great interest to know whether nutritional and environmental stress affects only fatty acid metabolism or other phases of hepatic metabolism as well. The present communication reports a study of carbohydrate metabolism in liver slices from normal and cold-fasted rats.

Methods. Adult male rats of Wistar strain were fed *ad lib.* for 10 days a diet composed of 25 g casein, 10 g fat, 51 g dextrose, 3 g salt mixture(4), 5 g cellulose, 6 g brewer's yeast, 0.04 g cod liver oil concentrate and 0.01 g α-tocopherol. After dietary stabilization, the rats were either fed the same diet *ad lib.* at room temperature (control rats) or fasted at 0-2°C for 24 hrs (cold-fasted rats). Following respective dietary and environmental treatments, the rats were killed by blow on head and the livers rapidly excised and placed in ice-cold Krebs-Henseleit bicarbonate buffer solution(5). Liver slices were prepared by a McIlwain-Buddle tissue chopper(6). The slices were collected in Petri dish containing ice-cold buffer. They were then blotted free of excess solution on filter paper, and aliquots of approximately 250 mg of tissue were weighed and transferred to incubation flasks(7). All incubations were carried out in duplicate. The main compartment of incubation flasks contained 250 mg of slices, 4.5 ml of Krebs-Henseleit bicarbonate buffer, and 0.5 ml of solution containing labeled substrate in trace amounts. The slices were incubated 3 hours at 37.5° with continuous shaking. $C^{14}O_2$ formed during incubation was recovered by the method of Chernick *et al.*(8) and the C^{14} content was determined by the $BaCO_3$ method of Entenman *et al.*(9). The C^{14} content of labeled substrate was determined by $BaCO_3$ method after oxidizing the compounds to CO_2 by the method of Katz *et al.*(10).

Results. Observations on oxidation of glucose-1-C^{14} and glucose-6-C^{14} to $C^{14}O_2$ by liver slices from control and cold-fasted rats are seen in Table I. It is not possible from these findings to calculate the precise quantitative differences in absolute amount of glucose being utilized by the 2 groups, because the large difference in hepatic glycogen content of these groups(1) makes it likely that pool sizes of the various hexose intermediates are of different orders of magnitude. However, it is clear from the data that there is a qualitative difference between livers of control and cold-fasted rats, namely that carbon 1 of glucose is forming $C^{14}O_2$ in relation to carbon 6 at a considerably faster rate in cold-fasted liver slice than in the control liver slice.

This observation might have been due only to the fact that the cold-fasted rat was accumulating the carbon 6 of glucose in an enlarged lactate pool. That this was not the case was shown by experiments with lactate-1-C^{14} and lactate-2-C^{14} (Table II) which showed that the "cold-fasted" liver was utilizing exogenous labeled lactate as rapidly as the "control" liver slice, a finding that makes it unlikely that carbon 6 is being trapped in an enlarged lactate pool of the "cold-fasted" liver. The data, therefore, indicate that relatively more metabolized glucose is being utilized *via* the hexose monophosphate pathway in the case of "cold-fasted" liver than in the "control" liver.

Discussion. Metabolism of fatty acids by liver slices from cold-fasted rats is quite different from that of liver slices from control rats(1-3). Short-chain fatty acids are oxi-

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TABLE I. Glucose Oxidation in Liver Slices from Cold-Fasted and Control Rats.*

Environment and nutrition	% glucose-1-C^{14} recovered in CO_2	% glucose-6-C^{14} recovered in CO_2	$\frac{C^{14}O_2 \text{ from glucose-6-}C^{14}}{C^{14}O_2 \text{ from glucose-1-}C^{14}}$
Fed at 25°C	3.0 ± .35†	2.0 ± .37	.64 ± .076
Fasted at 0-2°C	3.9 ± .58	1.3 ± .24	.33 ± .016

* Values represent avg for 5 rats, 4 aliquots of liver slices incubated from each rat, 2 with glucose-1-C^{14} and 2 with glucose-6-C^{14}. The ratio $\frac{C^{14}O_2 \text{ from glucose-6-}C^{14}}{C^{14}O_2 \text{ from glucose-1-}C^{14}}$ was determined for each rat and avg ratio reported for each group of rats.

† Stand. dev. of mean.

dized at a much slower rate in liver slices of the cold-fasted rat, a defect that can be repaired by adding glucose to the incubation medium. Long-chain fatty acids are oxidized at normal rates by the "cold-fasted" liver but on addition of glucose to the incubation medium the rate of long-chain fatty acid oxidation becomes much faster than that of normal liver. These results point to the important role of carbohydrate metabolism in relation to fat oxidation.

In the present report it is shown that the combined stress of cold-exposure and fasting not only affects fatty acid metabolism but carbohydrate metabolism as well. As stated above, the evidence suggests that carbohydrates are diverted towards the hexose monophosphate pathway in the liver of cold-fasted rat. This is a very surprising finding when one considers that fatty acid synthesis is almost non-existent in these livers(1,2). Tepperman and Tepperman(11) reviewed the literature and presented new evidence that implicates TPNH production *via* the hexose monophosphate pathway as a strong stimulator of fatty acid synthesis. However, no lipogenesis ocurs in the "cold-fasted" liver even under conditions where an abundance of carbohydrate is available and when, according to our report, a large part of carbohydrate metabolism is occurring *via* the hexose monophosphate pathway. There must, then, be a dearth of other factors necessary for lipogenesis in the "cold-fasted" liver.

TABLE II. Lactate Oxidation in Liver Slices from Cold-Fasted and Control Rats.

Environment and nutrition	% lactate-1-C^{14} recovered in CO_2	% lactate-2-C^{14} recovered in CO_2
Fed at 25°C	56	22
Idem	56	27
"	51	24
"	56	26
Fasted at 0-2°C	66	25
Idem	61	23

Summary. Metabolism of glucose-1-C^{14} and glucose-6-C^{14} was studied in liver slices from control rats (fed at 25°C) and cold-fasted rats (fasted 24 hours at 0-2°C). Evidence is presented that suggests that carbohydrate metabolism is diverted towards the hexose monophosphate pathway in the liver of cold-fasted rats. The depressed hepatic fatty acid synthesis in the cold-fasted rat is discussed in the light of this new evidence on carbohydrate metabolism.

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