Stimulation of Hepatic Fatty Acid Synthesis by Ethanol

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FATTY infiltration of the liver is commonly found in alcoholic patients, but the relationship between excessive alcohol intake and fat accumulation in the liver is much debated.[1] Recently, studies were reported *in vitro*[2] suggesting that in addition to inducing nutritional deficiencies,[3,4] ethanol may have a direct metabolic effect on liver cells. The nature of this effect and particularly the relationship between ethanol and fat metabolism has been investigated in liver slices *in vitro*,[5] and the results obtained are summarized in the present article.

Liver slices of seventeen male rats taken at random were incubated *in vitro* for three hours with either 10 mM. ethanol or 10 mM. acetate. At the end of the incubation period, the total fatty acid concentration averaged 0.885 mg. palmitic acid per milligram tissue nitrogen on incubation with ethanol, and 0.805 mg. palmitic acid on incubation with acetate. In these seventeen paired determinations, the mean difference was 0.080 ± 0.029 mg. palmitic acid per milligram tissue nitrogen ($p < 0.02$). In nine instances, slices were incubated with either 10 mM. ethanol or 5 mM. glucose. At the end of the incubation period, the total fatty acid content of the slices averaged 0.901 mg. palmitic acid per milligram of tissue nitrogen on incubation with ethanol, and 0.831 mg. palmitic acid with glucose. The mean difference was 0.070 ± 0.021 mg. palmitic acid per milligram of tissue nitrogen ($p < 0.02$).

In the following experiments, an attempt was made to elucidate the mechanisms responsible for this effect of ethanol *in vitro*. Using isotopically labeled compounds, it was found that at a low substrate concentration (0.5 mM.), incorporation of ethanol-C^{14} and acetate-C^{14} into hepatic fatty acids was similar, indicating that both ethanol and acetate serve equally well as fatty acid precursors. With a substrate concentration twenty times higher, however, labeling of the fatty acids was three to twelve times higher with ethanol-C^{14} than with acetate-C^{14}, suggesting that the metabolism of ethanol in the liver may have a stimulatory effect on the incorporation of 2-carbon fragments into fatty acids. This was confirmed by the results shown in Figure 1 which indicate that unlabeled ethanol markedly stimulated incorporation of a trace amount of acetate-C^{14} into fatty acids, as compared to unlabeled glucose or acetate. In adipose tissue, on the other hand, where lack of alcohol dehydrogenase activity prevents significant oxidation of ethanol, incorporation of labeled acetate into fatty acids was not enhanced on incubation with ethanol, suggesting that the observed stimulatory effect in liver tissue is dependent on ethanol oxidation.

Oxidation of ethanol in the liver is catalyzed by alcohol dehydrogenase,[6-8] with coupled reduction of diphosphopyridine nucleotide (DPN) to DPNH (Fig. 2). *In vitro*, perfusion

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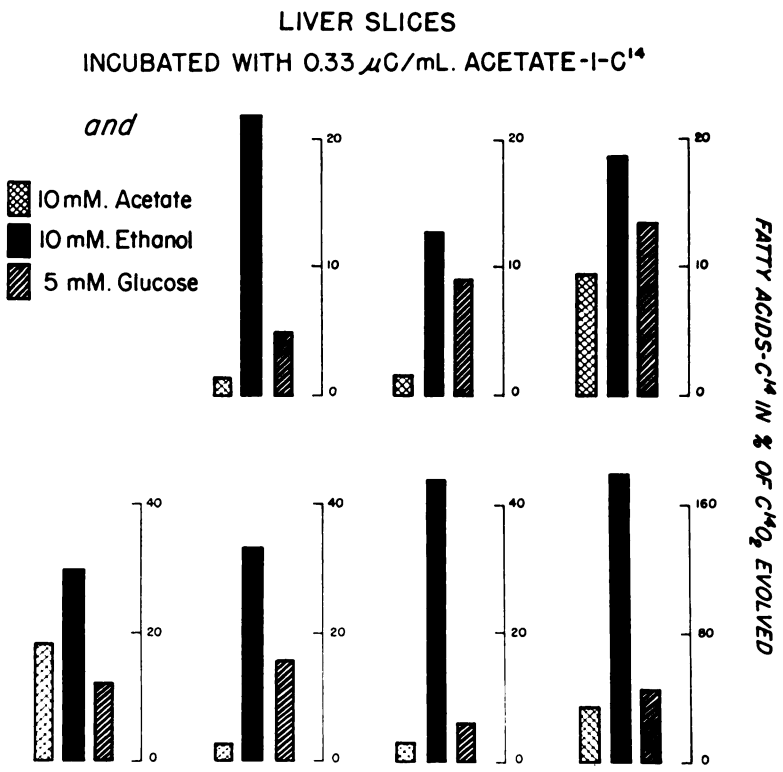

FIG. 1. Effect of unlabled acetate, ethanol and glucose on the incorporation of acetate-C^{14} into fatty acids in liver slices. From LIEBER, C. S. and SCHMID, R. *J. Clin. Invest*, 40:394, 1961.[5]

Ethanol + $DPN^+ \rightleftarrows$ Acetaldehyde + DPNH + H^+
Sorbitol + $DPN^+ \rightleftarrows$ Fructose + DPNH + H^+

FIG. 2. Oxidation of ethanol and sorbitol in the liver.

of liver with ethanol results in reduction of the DPN : DPNH ratio,[9] and *in vivo*, administration of ethanol produces a decrease in DPN and an increase in DPNH concentration in the liver.[10] Furthermore, addition of exogenous DPNH to cell free liver extracts was found to stimulate fatty acid synthesis.[11,12] These findings suggest that the stimulatory effect of ethanol on the incorporation of acetate-C^{14} into fatty acids may be due to the excess DPNH formed on ethanol oxidation.

It has not yet been determined whether DPNH acts mainly by direct stimulation of fatty acid synthesis or by reducing the activity of the tricarboxylic acid cycle (TCA), or both. Diminished TCA cycle activity is suggested by the finding that unlabeled ethanol reduced the conversion of acetate-C^{14} to $C^{14}O_2$, as compared to incubation with unlabeled acetate. Moreover, addition of DPNH to liver homogenate has been found to reduce oxidation of TCA intermediates.[13]

Supportive evidence that the observed effect of ethanol on hepatic fatty acid metabolism may be due to a shift in DPN : DPNH ratio was obtained by incubating liver slices with another DPNH-generating system. In the liver, sorbitol is oxidized to fructose with concomitant reduction of DPN to DPNH[14] (Fig. 2). Like ethanol, incubation of liver slices with sorbitol resulted in increased incorporation of trace amounts of acetate-C^{14} into fatty acids. Conversely, a hydrogen acceptor such as methylene blue partly reduced the stimulatory effect of ethanol.

These observations suggest that in the liver the excess DPNH formed on ethanol oxidation results in a shift in the relative disposition of acetyl-CoA in such a way that more acetate is incorporated into fatty acids, while less is oxidized via the TCA pathway.

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