Failure of Ethanol to Stimulate Incorporation of Acetate-1-C^{14} into Hepatic Fatty Acids *in vitro*.* (28985)

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In vivo administration of single large doses of ethanol has been shown to elevate the quantity of fat in the liver of animals(3,10). Several possible mechanisms for this process have been proposed. Horning *et al*(4), and Brodie *et al*(2) have shown that ethanol interferes with the control of fat transport by the hypothalamic-pituitary system and they suggest that this interference with fat transport is responsible for relocation of the adipose tissue fat into the liver. More recently, Lieber and coworkers(5,7) presented data which apparently support the hypothesis(5, 6,7) that ethanol stimulates the synthesis of fatty acids in the liver. The studies of Lieber *et al*, performed on rat liver slices, contrast with our previously reported work on similar tissues(11,8,9) in which we found that ethanol and other short chain aliphatic alcohols, allyl alcohol and trifluorethanol suppress the incorporation of acetate(11,8), glucose and fructose(9), into hepatic lipids, proteins, and fatty acids(8). Our findings suggested that the increase in hepatic lipid concentration induced by ethanol *in vivo* did not arise as a result of stimulation of hepatic fatty acid synthesis from acetate.

Our earlier experiments were performed under somewhat different experimental conditions than those of Lieber; *e.g.*, we employed Krebs-Ringer phosphate buffer while they employed a bicarbonate buffer. Because of the differences between our findings and those of Lieber, we have performed additional experiments with liver slices *in vitro* under incubation conditions similar to those of Lieber *et al*(5,7). In these studies, carried out under carefully controlled conditions, we have measured the incorporation of radioactive carbon derived from acetate-1-C^{14} into hepatic fatty acids in the presence and absence of added unlabeled ethanol or acetate. The results reported here confirm our previous findings that ethanol does not stimulate formation of fatty acids from acetate in rat liver slices.

Materials and methods. Wistar albino rats, weighing between 150-250 g and fed *ad libitum* up to the time of experiment were used. Liver slices (not more than 0.5 mm thick) were cut with a chilled Stadie-Riggs knife microtome, surface slices being discarded. Usually 2 slices, weighing a total of 170-200 mg wet weight were placed in Warburg manometer flasks containing 3 ml of ice cold Krebs-Ringer bicarbonate medium pH 7.4 (12), with a trace amount of sodium acetate-1-C^{14}. Control vessels contained no further additions, whereas to the experimental vessels either acetate or ethanol was added at varying concentrations (Table I). The vessels were gassed with 5% CO_2-95% O_2 for 3 minutes. After 3 hours of incubation at 37°C, trichloracetic acid (0.2 ml, 30%) was tipped from the side arm into the main compartment to stop the reaction.

The fatty acids were isolated essentially according to the procedure described by Abraham, Hirsch and Chaikoff(1). Washed slices were saponified and fatty acids were extracted from acidified residue. The washed petroleum ether extract was plated on 5 cm planchets. Two drops of 0.2 N NaOH were added to stabilize free fatty acids. After drying at room temperature, the radioactivity of fatty acids was measured in a Tracerlab low background counter.

Results and discussion. The results are given in Table I. It is apparent that both ethanol and sodium acetate decrease the radioactivity of the liver fatty acids substantially below the level found in the control experiment in which only 0.1 mM sodium

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TABLE I. Incorporation of Acetate-1-C^{14} Derived Ratioactive Carbon into Hepatic Fatty Acids *in vitro*.

(Rat liver slices, 170-200 mg wet wt; Krebs-Ringer bicarbonate medium, pH 7.4; total vol, 3 ml; 95% O_2, 5%, CO_2; sodium acetate-1-C^{14}, 176,000 ± 12,500* c.p.m./vessel, 0.1 mmolar; 3 hr; 37°)

Additions to standard incubation medium (mmolar final conc.)		Radioactivity of fatty acids (c.p.m./mg dry wt of tissue)	% Inhibition
Control	nil	258 ± 90*	—
Ethanol	2.5	177 ± 52.5	31.4
	10	118 ± 27.5	54.3
	60	105 ± 33	59.3
Sodium acetate	2.5	144 ± 45.8	44.2
	10	63 ± 16.2	75.6
	60	11.4 ± 4.9	95.6

* Means of at least 8 experiments. ± Standard deviations from the mean. Experiments were carried out in duplicate, controls in triplicate.

acetate-1-C^{14} was used. Ethanol exerts a suppressing effect at 2.5 mmolar final concentration and this effect becomes maximal at a concentration of 10 mmolar. An increase of ethanol concentration up to 60 mmolar does not appreciably change the extent of suppression. These results are similar to those reported earlier(8), which showed that suppression of C^{14}-O_2 formation from acetate-1-C^{14} reaches a maximum at approximately 5 mmolar ethanol and that further increases in concentration of ethanol had no effect on the amount of acetate-1-C^{14} converted into C^{14}-O_2.

Addition of unlabeled sodium acetate causes an even greater suppression of the radioactivity of the synthesized hepatic fatty acids than does ethanol and in contrast to the ethanol experiments the degree of suppression does not reach a maximum until 60 mmolar acetate, at which point the suppression is almost complete.

We interpret the suppressive effect of added unlabeled acetate on the incorporation of radioactivity into the hepatic fatty acids as being due to isotopic dilution. Thus, in our experiments, where the original concentration of acetate (0.1 mM labeled acetate) was increased to 2.5 mM, 10 mM and 60 mM by addition of unlabeled acetate, there is an immediate 25-, 100-, and 600-fold dilution of acetate-1-C^{14} respectively. This dilution accounts for the lower radioactivity of fatty acids synthesized under these conditions. In the ethanol experiments, the specific activity of acetate at start of the experiment is essentially equal to that of the original trace amount of acetate-1-C^{14} and it is independent of the ethanol concentration. However, during the course of the experiment as the added ethanol is oxidized to acetate or acetyl-CoA, the initial trace amount of acetate-1-C^{14} also became diluted and the specific activity decreased to an unknown extent. Thus, the radioactivity of fatty acids synthesized in the presence of added acetate must be lower than that of fatty acids synthesized in the control experiments or in the presence of added ethanol.

Our results also suggest that the enzyme concerned with oxidation of ethanol (*i.e.*, alcohol dehydrogenase) is saturated at relatively low concentrations. Such a conclusion would be compatible with the finding that 60 mmolar ethanol does not suppress significantly more than 10 mmolar.

These observations lead to the conclusion that the suppressing effect of ethanol on the incorporation of labeled acetate into the liver fatty acids can be partly accounted for by isotopic dilution of labeled acetate-1-C^{14}. An additional true inhibitory effect of ethanol on the metabolism of acetate described by us previously(8) may also be partly responsible for the suppressing effect of ethanol in these experiments.

The conclusions deduced from our results differ from those of Lieber, Davidson *et al* (5,6,7) who performed experiments on liver slices in which a trace amount of acetate-1-C^{14} was incubated either with 10 mmolar unlabeled acetate or with 10 mmolar ethanol. Control experiments using only trace acetate-1-C^{14} were not performed. They found that the radioactivity of the hepatic fatty acids in the vessels containing unlabeled acetate was significantly lower than that found in the presence of added ethanol. From these data, they interpreted their findings to mean that ethanol stimulates incorporation of acetate into fatty acid, apparently in agreement with the concept that the synthesis of fatty

acids in a reductive process requiring the participation of NADH and that such reductive conditions occur when ethanol is metabolized in the liver(5,6,7).

Were it not for our control experiments, our data could be interpreted similarly. We, too, have found higher activity in fatty acids of liver slices incubated with trace quantities of acetate-1-C^{14} and ethanol than in those incubated with acetate-1-C^{14} and unlabeled acetate (Table I). However, both ethanol and unlabeled acetate diminish the radioactivity of hepatic fatty acids below that found in the control experiments and at equimolar concentrations acetate suppresses more than does ethanol. Consequently, in interpreting both our results and theirs, necessary recognition must be given to the isotopic dilution of the labeled acetate by unlabeled added acetate or by unlabeled acetate derived from the added ethanol. Both of these reduce the specific activity of the trace quantity of acetate-1-C^{14} and, consequently, reduce the radioactivity of the synthesized hepatic fatty acids.

Viewed in this light, it should be apparent that a valid conclusion concerning the nature of the ethanol effect may be drawn only when both ethanol experiments and acetate experiments are compared against a proper control experiment. The mere comparison of the results obtained in the presence of added ethanol with those with added unlabeled acetate may lead to spurious deductions; *e.g.*, it may be calculated from the results of Lieber and Schmidt(7, Table I) that at 10 mM ethanol, the mean stimulation of apparent synthesis of fatty acids amounts to 509% with a range from 1307% stimulation to 59% inhibition for individual results. If similar reasoning is applied to our results, then there would be 23%, 87% and 821% stimulation at 2.5 mM, 10mM and 60 mM ethanol respectively (Table I). Thus, the conclusion of Lieber *et al* (5,6,7) that ethanol stimulates the incorporation of acetate into fatty acids, rests upon the interpretation of inadequately controlled experiments.

Our results offer no evidence upon the mechanisms by which ethanol administration might result in production of fatty livers. Thus, they are in no way incompatible with the hypothesis set forth by Horning and Brodie(2,4) regarding the effects of ethanol upon lipid transport from adipose tissue into the liver. Other mechanisms by which ethanol may stimulate the synthesis and storage of hepatic fats have yet to be demonstrated *in vivo* and *in vitro*.

Summary. Experiments conducted to determine the effects of ethanol on fatty acid synthesis in liver slices indicate that ethanol, at all concentrations tested, suppresses the incorporation of labeled carbon derived from acetate-1-C^{14} into hepatic fatty acids *in vitro*. This suppression is explained as being mostly due to isotopic dilution of labeled acetyl-CoA derived from acetate-1-C^{14} by unlabeled acetyl-CoA derived from ethanol, although the inhibitory effect of ethanol on the metabolism of acetate in liver slices may also be partly responsible for this suppression.

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