EFFECT OF ETHANOL ON FATTY ACID METABOLISM IN LIVER SLICES

Charles S. Lieber*, Leonore M. DeCarli and Rudi Schmid

Thorndike Memorial Laboratory and
Second and Fourth (Harvard) Medical Services,
Boston City Hospital;
Department of Medicine,
Harvard Medical School
Boston, Massachusetts

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It has been demonstrated that in rats, administration of ethanol increases the fat content of the liver (Mallov and Hloch, 1956), but the mechanism of this effect is unknown.

In the present study, liver slices and adipose tissue were incubated with various labeled substrates and the effect of ethanol on the incorporation of the label into fatty acids was studied.

MATERIAL AND METHODS

Male Sprague-Dawley rats weighing from 250 to 350 gm. were given over a 24-hour period prior to the experiment 36.5 gm./kg. glucose and 7.5 gm./kg. ethanol or (Table IB) 50 gm./kg. fructose administered by 5 equal gastric tube feedings. The animals were killed by decapitation, and randomized liver slices (approximately 0.5 gm.) or adipose tissue (epididymal fat pad) were incubated with the C^{14}-labeled substrate for 3 hours at 37° C. in an atmosphere of 95% O_2 and 5% CO_2 in 5 ml. Krebs-Ringer bicarbonate buffer, pH 7.4. At the end of the incubation, CO_2 evolved was trapped in hyamine, fatty acids were extracted and the radioactivity of both was estimated by liquid scintillation counting

Abbreviations: DPN, DPNH: diphosphopyridine nucleotide and reduced diphosphopyridine nucleotide.

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Table I

Incubation of rat liver slices with 0.33 mM acetate-1-C^{14} (1 mC/mM)

and

	Rat #	ACETATE 10 mM			ETHANOL 10 mM		
		$C^{14}O_2$	fatty acids-C^{14}		$C^{14}O_2$	fatty acids-C^{14}	
		d.p.m./mg N	d.p.m./mg N	in % of $C^{14}O_2$	d.p.m./mg N	d.p.m./mg N	in % of $C^{14}O_2$
A.	1	5,030	1,640	32.7	12,740	23,070	181.1
	2	32,090	970	3.0	11,730	5,270	44.9
	3	20,470	250	1.2	15,580	3,400	21.9
	4	25,560	400	1.6	21,550	2,740	12.7
	5	34,070	1,700	5.0	18,920	6,290	33.2
	6	19,840	3,660	18.4	20,700	6,190	29.9
		FRUCTOSE 60 mM			SORBITOL 60 mM		
B.	7	58,180	28,920	49.7	55,660	69,640	125.1
	8	69,280	19,120	27.5	73,280	70,860	96.7
	9	62,300	20,670	33.2	72,890	53,380	73.2
	10	62,930	32,070	51.0	71,090	81,960	115.2
	11	33,840	11,810	34.9	33,180	49,920	150.0
	12	37,590	7,320	19.5	49,670	46,920	94.5

(Siperstein and Fagan, 1958). The results were expressed in d.p.m. (disintegrations per minute) per mg. protein nitrogen. Incorporation of C^{14} into fatty acids was also calculated in % of the $C^{14}O_2$ evolved to permit comparison of the results obtained with different substrates and substrate concentrations. In separate experiments, liver slices were incubated with the same substrates in the Warburg apparatus and oxygen uptake was determined. The protein nitrogen content of the tissues was measured by the micro-Kjeldahl method.

RESULTS

In preliminary studies, it was noted that in liver slices, at a substrate concentration of 0.5 mM, acetate-1,2-C^{14} and ethanol-1,2-C^{14} were incorporated into fatty acids to a similar extent. With 20 times higher substrate concentration, however, incorporation of the label from ethanol-1,2-C^{14} was 7 times higher than with acetate-1,2-C^{14}. A similar effect of ethanol on the incorporation of labeled acetate into fatty acids was also observed. The data of Table IA indicate that in the presence of 10 mM unlabeled ethanol, incorporation of 0.33 mM acetate-1-C^{14} into fatty acids was significantly higher than with 10 mM unlabeled acetate. In similar experiments, 10 mM unlabeled ethanol was found to be 3 times more effective than 5 mM unlabeled glucose. In the presence of unlabeled sorbitol (Table IB) incorporation of 0.33 mM acetate-1-C^{14} was 2 to 5 times higher than with unlabeled fructose.

No significant difference in oxidation of palmitate-1-C^{14} to $C^{14}O_2$ was observed in the presence of 10 mM unlabeled ethanol or acetate or 5 mM glucose.

In contrast to liver slices, incubation of adipose tissue with ethanol-1,2-C^{14} (0.5 mM) produced negligible amounts of $C^{14}O_2$ as compared to acetate-1,2-C^{14} and glucose-U-C^{14}. Moreover, 10 mM unlabeled ethanol did not stimulate incorporation of 0.33 mM acetate-1-C^{14} into fatty acids as compared to 10 mM acetate or 5 mM glucose.

In comparison with glucose and acetate, ethanol in the concentra-

tion used (0.5 mM and 10 mM) did not alter respiration of the incubated tissues, as determined by oxygen uptake.

DISCUSSION

In liver slices ethanol is not a better precursor than acetate for fatty acid synthesis, although it stimulates incorporation of acetate into fatty acids. In the epididymal fat pad, where ethanol metabolism is minimal, no such stimulatory effect was observed, indicating that the effect of ethanol on fatty acid metabolism is dependant on ethanol oxidation.

In the liver oxidation of ethanol with alcohol dehydrogenase results in reduction of DPN (Theorell and Bonnichsen, 1951) and it has also been demonstrated that in the rat ethanol administration reduces the DPN/DPNH ratio in the liver (Smith and Newman, 1959). Decreased concentration of DPNH in the liver is associated with reduced fatty acid synthesis (Helmreich et al., 1954), while addition of exogenous DPNH is known to stimulate fatty acid synthesis in vitro (Porter et al., 1957). Therefore, it appears likely that the enhanced incorporation of acetate-C^{14} into fatty acids with ethanol is mediated through increased formation of DPNH on ethanol oxidation. This concept is supported by the results obtained on incubation with sorbitol and fructose (Table IB). Like ethanol oxidation of sorbitol in the liver is coupled with reduction of DPN (Blakley, 1951) and stimulates incorporation of acetate into fatty acids.

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