Effects of prolonged ingestion of glucose or ethanol on fatty acid synthesis by mitochondria and cell sap of rat liver and adipose tissue

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ABSTRACT Effects of prolonged ingestion of glucose and ethanol on the rate of fatty acid synthesis by liver and adipose tissue have been investigated in male rats. Ethanol significantly enhanced the rate of fatty acid synthesis from malonyl-2-^{14}C CoA in liver cell sap; glucose feeding enhanced the rate of fatty acid synthesis from both malonyl-2-^{14}C and acetyl-1-^{14}C CoA.

Neither dietary supplement modified the types of fatty acid synthesized in this enzyme system. Palmitic acid was the principal product synthesized from a mixture of malonyl and acetyl CoA, whereas myristic and palmitic acids were the predominant products formed from acetyl CoA alone. Neither glucose nor ethanol affected fatty acid synthesis by adipose tissue cell sap.

Mitochondria derived from liver and adipose tissue of control, glucose-fed, and ethanol-fed animals all incorporated acetyl-1-^{14}C CoA into lipid at about the same rate, but did not utilize malonyl CoA for lipid synthesis to any significant degree. The label appeared in fatty acids, one-half of which were contained in phospholipid. Both unsaturated and saturated fatty acids synthesized by mitochondria contained isotope, most of which was present in the carboxyl groups. Ethanol and glucose feeding stimulated the labeling of monoenoic fatty acids in liver mitochondria, but only glucose did so for adipose tissue.

These findings agree with results previously obtained when lipogenesis was measured with acetate-^{14}C in vivo.

KEY WORDS ethanol · glucose · prolonged feeding · fatty acid synthesis · acetyl CoA · malonyl CoA · liver · adipose tissue · cell sap · mitochondria · rat

Part I of this series on the effects of prolonged glucose and ethanol ingestion on lipid metabolism is the preceding paper (reference 1).

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

BOTH THE ACUTE (2) AND chronic (1, 3, 4) ingestion of either ethanol or glucose enhance the in vivo incorporation of acetate-^{14}C into hepatic fatty acids. This effect may be dependent upon an increase in the reducing potential of the liver (5), since ethanol oxidation yields NADH and glucose oxidation, via the pentose pathway, generates NADPH. These reduced nucleotides are required for fatty acid anabolism (6–9), both serving as reductants for fatty acid chain elongation and unsaturation by subcellular particles, and NADPH being required for de novo fatty acid synthesis by cell sap. However, the possibility that ethanol and glucose supplements also increase the concentration or activities of the enzyme systems that synthesize fatty acids merits consideration, since there is an enhanced rate of fatty acid synthesis by liver preparations prepared from carbohydrate-fed rats (10–12). For this reason we have investigated the rate of fatty acid synthesis in rats after prolonged ingestion of ethanol or glucose, by measuring the rate at which ^{14}C-labeled precursors are incorporated into individual fatty acids in mitochondria and cell sap of liver and adipose tissue. The incubations were carried out with optimum concentrations of known activators and cofactors.

MATERIALS AND METHODS

Three groups of male, Sprague-Dawley, weanling rats were investigated. Group I was maintained on Purina Lab Chow and water ad lib. Group II received Purina Lab Chow and 15% (v/v) ethanol in lieu of drinking water ad lib. Group III was group pair-fed with Group II, but was given an isocaloric supplement of glucose instead of ethanol. As previously shown (1), the total

caloric intakes and growth rates were nearly identical in the three groups, and about 30% of the calories consumed by the ethanol group were derived from the alcohol. On the days indicated in the text, one animal from each group was sacrificed.

Mitochondria and cell sap were prepared from 10% homogenates of liver and from 25% adipose tissue homogenates obtained from the combination of perirenal and epididymal fat depots. This procedure has been described previously (1) and is based on the method of Hogeboom (13). After centrifugation, surface fat was carefully removed with a spatula and discarded. Mitochondria were first washed with 10–20 ml of 0.25 M sucrose and then resuspended with the aid of a Potter-Elvehjem homogenizer in a volume of sucrose equivalent to the original wet weight of the liver or one-tenth of the original weight of the adipose tissue. Mitochondria were frozen and thawed at least once before being used for incubation studies.

Acetyl-1-^{14}C CoA and malonic acid-2-^{14}C were purchased from the New England Nuclear Corp., Boston, Mass. Malonyl-2-^{14}C CoA was synthesized as described by Trams and Brady (14). Acetyl CoA and all nucleotides were obtained from P-L (Pabst) Biochemicals, Milwaukee, Wis.

The assays for cell sap and mitochondrial incubations utilized conditions similar to those described by others (15–20). Mitochondrial incubations were usually performed with preparations that had been stored overnight in a frozen state, but cell sap incubations were always carried out on the day that the animals were sacrificed. Incubations were performed in screw-cap test tubes shaken in a Dubnoff incubator at 37°C.

When only the rate of fatty acid synthesis was to be measured in cell sap, the reaction was stopped by adding 0.5 ml of 2 N KOH containing 10 μmoles of carrier sodium acetate. Saponification was carried out for 10–14 hr at 90°C in a heating block. The hydrolysate was cooled and acidified to pH 1 with 36 N H_2SO_4 and the fatty acids were extracted with two or three 5-ml portions of *n*-hexane. Radioactivity of aliquots of the hexane extracts was measured in a Nuclear-Chicago liquid scintillation spectrometer after 12 ml of Liquifluor (Pilot Chemicals, Inc., Watertown, Mass.) was added. Quenching was determined by a channels ratio method (21).

When both the rate and incorporation of isotope into total lipid and fatty acids were to be determined, the reaction was stopped by extracting the total lipid with chloroform–methanol (17). An aliquot of the lipid extract was evaporated to dryness in a counting vial and the residue was counted in 12 ml of Liquifluor. A portion of the extract was concentrated under nitrogen; lipid classes were separated by TLC and their radioactivity determined (1). Fatty acid methyl esters were prepared from this lipid extract, separated by preparative GLC, and counted as previously described (1). Each sample injected into the column contained between 1500 and 6000 dpm. The methyl esters were hydrolyzed with alkali, and carboxyl-^{14}C was determined by carrying out the Schmidt reaction (22, 23). Unsaturated fatty acids were separated as mercuric acetate adducts by TLC (24).

Protein concentrations were determined (25) with crystalline bovine serum albumin as a standard. All enzyme assays were carried out in duplicate and usually agreed within 5%. Radioactivity extracted by lipid solvents from incubation mixtures containing boiled enzyme preparations was subtracted from the unboiled enzyme preparation to correct for nonenzymatic utilization of ^{14}C-substrate. Values are expressed as the mean ± SEM. The data were analyzed statistically by the paired "*t*" test (26). In comparing the glucose and ethanol groups with the control group *P* values less than 0.05 were considered significant.

RESULTS

Fatty Acid Synthesis by Cell Sap

The rate of fatty acid synthesis from acetyl CoA by liver cell sap was linear for at least 40 min (Fig. 1), a

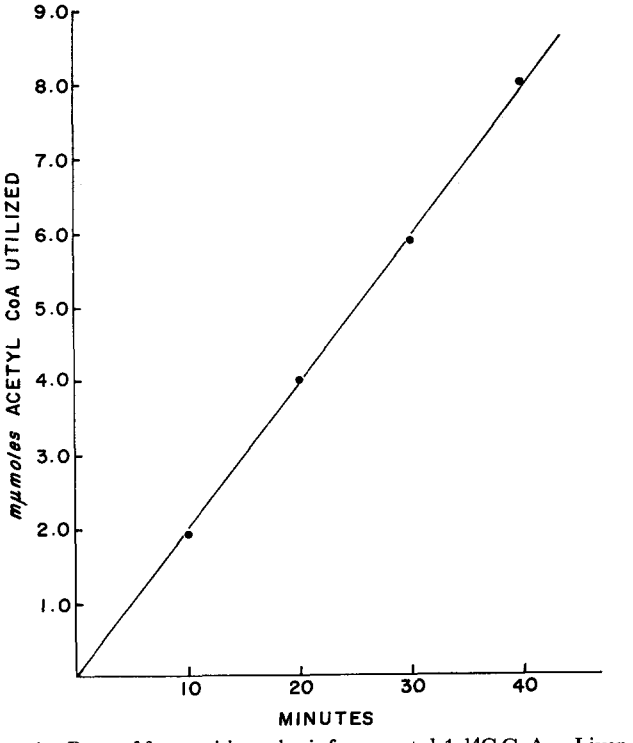

FIG. 1 Rate of fatty acid synthesis from acetyl-1-^{14}C CoA. Liver cell sap from a control rat containing 0.5 mg of protein was incubated for different periods under conditions shown in Table 1. The units for the ordinate are millimicromoles of acetyl CoA incorporated into fatty acids per milligram of protein.

TABLE 1 RATE OF FATTY ACID SYNTHESIS FROM ACETYL-1-^{14}C CoA BY CELL SAP OF LIVER AND ADIPOSE TISSUE

Dietary Regimen	Liver Cell Sap	Adipose Cell Sap
	mμmoles incorporated per mg protein	
Control	4.5 ± 0.8	1.9 ± 0.4
Glucose	10.2 ± 1.7 ($P < 0.01$)	3.1 ± 1.3 (NS)
Ethanol	7.6 ± 1.8 (NS)	1.9 ± 0.4 (NS)

Fatty acid synthesis by cell sap from acetyl-1-^{14}C CoA was measured in a 0.8 ml mixture containing 0.5–1.0 mg of cell sap protein and the following (amounts in micromoles): potassium phosphate buffer, pH 7.4, 60; sodium citrate, 10.0; mercaptoethanol, 3.0. This was "preincubated" for 20 min to ensure maximum activity of acetyl CoA carboxylase (15), and was then incubated for 30 min with 200 μl of a mixture containing the following (amounts in micromoles): potassium phosphate buffer, pH 7.4, 30.0; ATP (neutralized with KOH), 3.0; $KHCO_3$, 10.0; $MgCl_2$, 10.0; NADPH, 1.2; malonic acid, 7.5; $MnCl_2$, 1.5; and acetyl-1-^{14}C CoA (1–5 × 10^5 dpm), 0.1.

The data for the liver cell sap are mean values obtained from eight rats in each group which had been on their diets for 84, 101, 136, 155, 175, 199, 227, and 241 days. Mean values for adipose tissue cell sap were from five rats in each group which had been fed for 70, 155, 175, 199, and 227 days.

finding in agreement with that reported by others (18, 27). In addition, it was found that in the range of 0.5–1.0 mg of cell sap protein, isotope incorporation from acetyl-1-^{14}C CoA was directly related to the amount of added cell sap. Similar linear relationships were obtained with malonyl-2-^{14}C CoA. However, this substrate was utilized 4–10 times more efficiently than acetyl CoA for fatty acid synthesis even though the cell sap that was incubated with ^{14}C-acetyl CoA was first preincubated with citrate, which is known to increase acetyl CoA carboxylase activity 3-fold (15). Cell sap derived from adipose tissue behaved like that obtained from liver with respect to fatty acid synthesis from both acetyl and malonyl CoA.

The rate of fatty acid synthesis from acetyl CoA in liver cell sap was increased to more than twice that in control animals when large amounts of glucose were added to the stock diet (Table 1). Ethanol feeding increased acetyl CoA utilization by more than 50%, but the difference was not statistically significant ($0.1 > P > 0.05$), possibly because too few animals were assayed. Cell sap derived from adipose tissue utilized acetyl CoA for fatty acid synthesis less effectively than that derived from liver, and neither ethanol nor glucose feeding altered significantly the rate of fatty acid synthesis. Results obtained at different time periods in each dietary group were pooled because the activities of liver and adipose tissue remained relatively constant during the time periods studied.

Malonyl CoA was a better substrate (Table 2) for fatty acid synthesis than acetyl CoA, especially with cell sap derived from adipose tissue. Both ethanol and glucose feeding more than doubled the rate of fatty acid synthesis by liver cell sap, but neither ethanol nor glucose significantly altered the rate of fatty acid synthesis in adipose tissue. Mean values were again obtained by pooling the results from animals fed for varying times because the enzymatic activity in each group remained relatively constant.

In liver and adipose tissue cell sap from all three groups incubated with malonyl-2-^{14}C CoA, palmitic acid contained most of the label, while fatty acids with chain lengths of less than 16 contained < 11% of the label found in fatty acids (Table 3). Little synthesis of unsaturated fatty acids was observed. The fatty acids from only one animal in each group were analyzed by GLC because it had previously been shown by others that

TABLE 2 RATE OF FATTY ACID SYNTHESIS FROM MALONYL-2-^{14}C CoA BY CELL SAP OF LIVER AND ADIPOSE TISSUE

Dietary Regimen	Liver Cell Sap	Adipose Cell Sap
	mμmoles incorporated per mg protein	
Control	7.3 ± 1.5	8.6 ± 4.8
Glucose	16.9 ± 1.4 ($P < 0.01$)	10.5 ± 1.8 (NS)
Ethanol	16.2 ± 2.9 ($P < 0.05$)	5.3 ± 1.0 (NS)

Fatty acid synthesis by cell sap from malonyl-2-^{14}C CoA required no "preincubation," and was measured in a 1.0 ml mixture containing 0.5–1.0 mg of cell sap protein and the following (amounts in micromoles): potassium phosphate buffer, pH 6.5, 90.0; NADPH, 1.2; mercaptoethanol, 5.0; acetyl CoA, 0.1; and malonyl-2-^{14}C CoA (1–5 × 10^5 dpm), 0.1. The mixture was incubated for 15 min. The data are means from five rats in each group that had been on their diets for 136, 175, 199, 227, and 239 days.

TABLE 3 INCORPORATION BY CELL SAP OF LABEL FROM MALONYL-2-^{14}C CoA INTO FATTY ACIDS

Cell Sap of	Dietary Regimen	Percentage of Isotope		
		<16*	16:0	All Others†
Liver	Control	10	76	14
	Glucose	10	76	14
	Ethanol	11	75	14
Adipose tissue	Control	8	76	16
	Glucose	4	87	9
	Ethanol	4	85	11

Data were obtained from one animal in each group sacrificed on day 136.

* All fatty acids of chain length <16.

† All unsaturated C_{16}–C_{22} fatty acids and all saturated C_{17}–C_{22} fatty acids.

TABLE 4 INCORPORATION BY CELL SAP OF LABEL FROM ACETYL-1-^{14}C CoA INTO FATTY ACIDS

Cell Sap of	Dietary Regimen	Percentage Distribution of Isotope						
		<16*	16:0	16un†	18:0	18un	20:0	20un and 22un‡
Liver	Control	54	20	7	2	8	1	8
	Glucose	52	21	5	3	6	3	10
	Ethanol	44	19	6	2	6	7	15
Adipose tissue	Control	34	40	6	3	8	2	7
	Glucose	53	31	6	1	3	1	5
	Ethanol	43	33	6	3	5	3	7

Each value represents the mean of determinations of four animals in each group sacrificed on days 29, 101, 136, and 175.
* <16 refers to all fatty acids with chain length <16.
† un, unsaturated.
‡ Isotope incorporated into 22:0 was <1% of total and is not included.

palmitate is the predominant product from malonyl CoA (6–8).

A different pattern of fatty acid labeling was seen when acetyl-1-^{14}C CoA served as substrate (Table 4). In all three groups liver cell sap incorporated approximately half the label into fatty acids with chain lengths of less than 16, and only 20% into palmitic acid. More than 80% of the label in fatty acids containing less than 16 carbon atoms was in myristic acid. However, in the ethanol group the fraction of label incorporated into fatty acids with chain lengths of less than 16 was significantly smaller ($P<0.01$). This change appeared in the animals which had been on the dietary regimens for 29 days and persisted in the subsequent time periods of 101, 136, and 175 days.

In control animals, adipose tissue cell sap incorporated 40% of acetyl CoA into palmitic acid and 34% into shorter-chain fatty acids (Table 4). Although both ethanol and glucose feeding appeared to reduce the proportion of the label incorporated into palmitate and increase the proportion incorporated into shorter-chain fatty acids, these differences were not statistically significant.

Cell sap of adipose tissue and of liver incorporated a significant fraction of acetyl CoA into unsaturated fatty acids (Table 4). This was probably due to contamination of cell sap with mitochondria and microsomes, since such particles are known to form unsaturated fatty acids (6).

TABLE 5 RATE OF INCORPORATION OF ACETYL CoA INTO LIPID BY MITOCHONDRIA OF LIVER AND ADIPOSE TISSUE

Dietary Regimen	Liver Mitochondria	Adipose Mitochondria
	mμmoles incorporated per mg protein	
Control	7.9 ± 4.0	10.5 ± 2.3
Glucose	9.4 ± 3.3 (NS)	14.8 ± 2.2 (NS)
Ethanol	7.0 ± 3.3 (NS)	10.9 ± 1.8 (NS)

Mitochondria (0.5–1.0 mg of protein) were incubated for 30 min in a total volume of 0.5 or 0.7 ml containing the following in micromole amounts: potassium phosphate buffer, pH 6.5, 60.0; ATP, 7.5; NADPH, 2.0; NADH, 2.0; potassium DL-isocitrate, 20.0; acetyl-1-^{14}C CoA (5×10^5 dpm), 0.1.

The data for the liver mitochondria were obtained from four animals in each group that had been on the designated diets for 84, 101, 136, and 155 days respectively. Adipose tissue mitochondria were from these same animals and from three additional animals in each group that had been on the diets for 199, 227, and 239 days, respectively.

Fatty Acid Synthesis by Mitochondria

As previously reported by others in the case of liver particles (8, 16), mitochondria isolated from both liver and adipose tissue utilized acetyl CoA for lipid synthesis at a linear rate for 30 min. Within the range of 0.5–1.0 mg of protein in the incubation mixture, the rate was dependent on enzyme concentration.

From the data in Table 5 it can be seen that neither ethanol nor glucose feeding affected the rate of acetyl CoA incorporation into lipid by mitochondria from liver or adipose tissue. Of interest is the fact that mitochondria derived from adipose tissue utilized (per milligram of protein) 33% more acetyl CoA for lipid synthesis than those derived from liver tissue. Other results not in Table 5 showed that in none of the three groups of animals studied did avidin inhibit acetyl CoA utilization by mitochondria. Malonyl CoA was only 1/20th as good a substrate as acetyl CoA for mitochondrial fatty acid synthesis.

In control animals, unsaturated fatty acids accounted (Table 6) for more than 60% of the isotope incorporated into fatty acids when mitochondria from liver or adipose tissue were incubated with acetyl-1-^{14}C CoA. Less than 7% of the label appeared in palmitic acid and only 15–21% in stearic acid. Both glucose and ethanol feeding increased the fraction of the label incorporated in C_{18} unsaturated fatty acids by liver mitochondria, but only glucose significantly increased the synthesis of these fatty acids by mitochondria from adipose tissue. It should be

TABLE 6 INCORPORATION BY MITOCHONDRIA OF LABEL FROM ACETYL-1-^{14}C CoA INTO FATTY ACIDS

Mitochondria of	Dietary Regimen	Percentage Distribution of Isotope*							
		16:0	16un	18:0	18un	20:0	20un	22:0	22un
Liver	Control	7	4	21	13	5	31	4	15
	Glucose	9	5	19	18†	5	25	4	15
	Ethanol	6	4	18	18†	3	37	2	12
Adipose tissue	Control	4	3	15	15	6	39	1	17
	Glucose	4	2	13	26‡	4	35	2	14
	Ethanol	4	3	18	17	4	39	1	14

Each value presented represents the mean of determinations in six animals in each group killed on days 29, 101, 136, 175, 227, and 260, respectively.

* Fatty acids are designated by carbon chain length and un refers to unsaturated fatty acids.

† $P < 0.05$ when glucose- and ethanol-fed animals are compared with controls.

‡ $P < 0.001$ when glucose-fed animals are compared with controls.

pointed out that, among the C_{20} and C_{22} unsaturated fatty acids, mass peaks were observed in the gas chromatograms only for 20:4 and 22:6 fatty acids in liver mitochondria, and 20:4 in adipose tissue mitochondria. However, radioactivity was observed in the effluent at retention times when fatty acids containing fewer double bonds (e.g., 20:1) were expected. Separation of the mercuric acetate adducts by TLC confirmed the results obtained by GLC, indicating that monounsaturated fatty acids accounted for 90% of the label in the C_{18} unsaturated and about 50% in the C_{20} unsaturated fatty acids.

Analysis by TLC of total lipid extracts from incubations of either liver or adipose tissue mitochondria with acetyl-1-^{14}C CoA revealed that about 55% of the label was in the phospholipid fraction, 25% in unesterified fatty acids, and the remainder in unidentified areas. No radioactivity was found in cholesterol or triglycerides, and, after hydrolysis of the total lipid extract, all the label was recovered in fatty acids. Decarboxylation of these fatty acids showed that about 70% of the radiocarbon was in carboxyl groups. When the Schmidt degradation was carried out on 20:1 fatty acids isolated from liver and adipose tissue mitochondria, about two-thirds of the radiocarbon was found in carboxyl groups. However, the carboxyl groups of 18:1 fatty acids contained one-half the radioactivity in the case of mitochondria derived from liver, and all of it in the case of mitochondria derived from adipose tissue.

DISCUSSION

De novo synthesis by cell sap of long-chain saturated fatty acids from acetyl CoA occurs in the following manner (6–8):

1. $$n\mathrm{CH_3CO{-}SCoA} + n\mathrm{CO_2} + n\mathrm{ATP} \underset{\text{Biotin}}{\overset{\mathrm{Mn^{++}}}{\rightleftharpoons}} n\mathrm{HOOCCH_2CO{-}SCoA} + n\mathrm{ADP} + n\mathrm{P_i}$$

2. $$\mathrm{CH_3CO{-}SCoA} + n\mathrm{HOOCCH_2CO{-}SCoA} + 2n\mathrm{NADPH} + 2n\mathrm{H^+} \longrightarrow (n+1)\mathrm{CoA{-}SH} + \mathrm{CH_3(CH_2)_{2n}COOH} + n\mathrm{CO_2} + 2n\mathrm{NADP^+} + (n-1)\mathrm{H_2O}$$

Acetyl CoA carboxylase (EC 6.4.1.2) catalyzes reaction *1*, and apparently controls the over-all rate of fatty acid synthesis (18, 27, 28). In the present experiments, cell sap was preincubated with citrate to ensure maximum activity of this enzyme (15, 29). Reaction *2* is catalyzed by a group of enzymes that are referred to as the "fatty acid synthetase complex" (7). The primary radioactive product when malonyl-^{14}C CoA and acetyl CoA are incubated together (reaction *2*) is palmitic acid ($n = 7$), but some stearate and myristate can be detected. However, when acetyl-^{14}C CoA is used as the substrate in the absence of added malonyl CoA, we have observed, as have others (19), that fatty acids containing less than 16 carbon atoms are the principal product. The reason for this difference is unknown but is apparently dependent on whether malonyl CoA is added or is generated from acetyl CoA. Liver mitochondria incorporate acetyl-1-^{14}C CoA into phospholipids and free fatty acids, and this appears to be primarily an elongation reaction (16, 20) since most of the isotope appears in carboxyl groups. Thus, unsaturated as well as saturated fatty acids become labeled. We have found that mitochondria of adipose tissue are slightly more active than those of liver tissue in utilizing acetyl CoA for lipid synthesis, but both types of mitochondria synthesize the same kind of lipid. In vivo, however, adipose tissue mitochondria may not be of quantitative importance in the synthesis of fatty acids, since 10 g of adipose tissue yields only 2–4 mg of mitochondrial protein whereas 10 g of liver yields 300–400 mg. It is also interesting that the yield of cell sap protein per 10 g of liver and adipose tissue is 700–900 mg and 50–60 mg respectively.

Chronic feeding of large amounts of glucose stimulates the synthesis of fatty acids from acetyl and malonyl CoA

in liver sap, thus indicating increases in activities of both carboxylase and the synthetase complex. This finding agrees with previous reports by others (10–12). Ethanol feeding significantly stimulates fatty acid synthesis from malonyl CoA and possibly from acetyl CoA. These enhanced enzyme activities probably reflect increases in the concentration of the enzymes, since adequate amounts of known activators and cofactors, including citrate, pyridine nucleotides, and lipogenin (30, 31), were used in the assays. The concentration of lipogenin is depressed by fasting, but this was avoided in our experiments by feeding the animals up to the time of sacrifice. Other possible explanations for the observed increase in enzyme activities, including conversion of inactive to active enzymes and alterations in the concentration of acyl carrier protein that serves as a cofactor in the synthesis of fatty acids (32, 33), cannot be excluded, but appear less likely than an increase in enzyme concentration.

Another feasible explanation for the increased rate of fatty acid synthesis from acetyl CoA in the livers of glucose-fed, and possibly ethanol-fed, animals is that these dietary supplements reduce the hepatic concentration of long-chain fatty acyl CoA compounds, which are known to inhibit acetyl CoA carboxylase (29, 34, 35, 36). The concentration of these metabolites in the cell sap of a 10% liver homogenate from rats fed lab chow would be about $2\text{–}4 \times 10^{-6}$ M (35, 36). Inasmuch as 10^{-4} M acetyl CoA, which is the concentration in the incubation mixture, would be expected to reverse any inhibition by these materials (29), it appears that the observed enhanced rate of fatty acid synthesis after glucose feeding is not due to reduction in the endogenous concentration of long-chain fatty acyl CoA metabolites. In any event, the accelerated rate of fatty acid synthesis in liver cell sap obtained from animals after prolonged ethanol or glucose feeding is in agreement with the observation that these animals also show an increase in acetate incorporation into fatty acids in vivo, and that palmitate is the predominant fatty acid synthesized (1).

It has been reported that adding ethanol to liver slices in vitro enhances the incorporation of acetate into fatty acids (37). However, a similar study that included both glucose-treated and untreated control slices led to the conclusion that ethanol has no effect upon, or may even suppress, acetate utilization (38). Moreover, these in vitro studies may not be pertinent with respect to the fatty infiltration of the liver seen in rats after a single large dose of ethanol, since prolonged feeding of ethanol or glucose accelerates the rate of fatty acid synthesis in liver cell sap without producing a fatty liver. Thus it seems that the increase in fatty acid synthesis that occurs after a single large dose of ethanol cannot be the cause of the fatty liver that develops, and that some other alteration in fatty acid metabolism must be responsible.

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References

1. Scheig, R., N. M. Alexander, and G. Klatskin. *J. Lipid Res.* **7:** 188, 1966.
2. Rebouças, G., and K. J. Isselbacher. *J. Clin. Invest.* **40:** 1355, 1961.
3. Scheig, R., N. M. Alexander, and G. Klatskin. *Gastroenterology* **48:** 500, 1965.
4. Scheig, R., N. M. Alexander, and G. Klatskin. *Federation Proc.* **24:** 291, 1965.
5. Isselbacher, K. J., and N. J. Greenberger. *New England J. Med.* **270:** 351, 1964.
6. Vagelos, P. R. *Ann. Rev. Biochem.* **33:** 139, 1964.
7. Lynen, F. *Federation Proc.* **20:** 941, 1961.
8. Wakil, S. J. *J. Lipid Res.* **2:** 1, 1961.
9. Dils, R., and G. Popjak. *Biochem. J.* **83:** 41, 1962.
10. Lyon, I., M. S. Masri, and I. L. Chaikoff. *J. Biol. Chem.* **196:** 25, 1952.
11. Tepperman, H. M., and J. Tepperman. *Diabetes* **7:** 478, 1958.
12. Allmann, D. W., D. D. Hubbard, and D. M. Gibson. *J. Lipid Res.* **6:** 63, 1965.
13. Hogeboom, G. H. *Methods Enzymol.* **1:** 16, 1955.
14. Trams, E. G., and R. O. Brady. *J. Am. Chem. Soc.* **82:** 2972, 1960.
15. Vagelos, P. R., A. W. Alberts, and D. B. Martin. *J. Biol. Chem.* **238:** 533, 1963.
16. Harlan, W. R., Jr., and S. J. Wakil. *J. Biol. Chem.* **238:** 3216, 1963.
17. Folch, J., M. Lees, and G. H. Sloane Stanley. *J. Biol. Chem.* **226:** 497, 1957.
18. Numa, S., M. Matsuhashi, and F. Lynen. *Biochem. Z.* **334:** 203, 1961.
19. Lorch, E., S. Abraham, and I. L. Chaikoff. *Biochim. Biophys. Acta* **70:** 627, 1963.
20. Harlan, W. R., and S. J. Wakil. *Biochem. Biophys. Res. Commun.* **8:** 131, 1962.
21. Bush, E. T. *Anal. Chem.* **33:** 1024, 1963.
22. Phares, E. F. *Arch. Biochem. Biophys.* **33:** 173, 1951.
23. Brady, R. O., R. M. Bradley, and E. G. Trams. *J. Biol. Chem.* **235:** 3093, 1960.
24. Mangold, H. K. *J. Am. Oil Chemists' Soc.* **38:** 708, 1961.
25. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. *J. Biol. Chem.* **193:** 265, 1951.
26. Mainland, D. *Elementary Medical Statistics.* W. B. Saunders Co., Philadelphia, 1963, p. 204.
27. Martin, D. B., and P. R. Vagelos. *J. Biol. Chem.* **227:** 1787, 1962.
28. Ganguly, J. *Biochim. Biophys. Acta* **40:** 110, 1960.
29. Lynen, F., M. Matsuhashi, and S. Numa. In *The Control of Lipid Metabolism*, edited by J. K. Grant. Academic Press, New York, 1964, pp. 43–55.
30. Catravas, G. N. *Biochim. Biophys. Acta* **70:** 331, 1963.
31. Catravas, G. N., and H. S. Anker. *J. Biol. Chem.* **232:** 669, 1958.

32. Majerus, P. W., A. W. Alberts, and P. R. Vagelos. *Proc. Natl. Acad. Sci.* **51:** 1231, 1964.
33. Wakil, S. J., E. L. Pugh, and F. Sauer. *Proc. Natl. Acad. Sci.* **52:** 106, 1964.
34. Bortz, W., and F. Lynen. *Biochem. Z.* **337:** 505, 1963.
35. Bortz, W., and F. Lynen. *Biochem. Z.* **339:** 77, 1963.
36. Tubbs, P. K., and P. B. Garland. *Biochem. J.* **93:** 550, 1964.
37. Lieber, C. S., and R. Schmid. *J. Clin. Invest.* **40:** 394, 1961.
38. Majchrowicz, E. *Proc. Soc. Exptl. Biol. Med.* **115:** 615, 1964.