the present system and hence does not enter the synthetic pathway.

The data in Table IV and Fig. 2 show that at high concentrations of citrate, there is an inhibition of incorporation by high bicarbonate concentrations. This is in agreement with the recent findings of Majerus *et al*(19) who have shown, in agreement with Lynen(20), that the activated intermediates in fatty acid synthesis are protein bound. Moreover, the acyl groups which are bound to acyl carrier protein (ACP) are in reversible equilibrium with their products(19): Acetyl-S-ACP + malonyl-S-ACP enzyme $\rightleftarrows$ acetoacetyl-S-ACP + ACP-SH + CO_2. This clearly explains the CO_2 effect.

Summary. A preparation from rat liver containing both microsomes and soluble enzymes when supplemented with the necessary cofactors incorporates acetate-1-^{14}C preponderantly into the saponifiable fraction of the lipids. Bicarbonate has no significant stimulating effect on the synthesis whereas citrate and malonate have pronounced and similar stimulatory effects, particularly when they are preincubated with the synthesizing system. However, at high citrate concentrations bicarbonate becomes inhibitory. Malonate stimulates fatty acid synthesis without participating as a metabolite. The same is concluded with citrate, at least with concentration up to 15 mM. An interrelationship between citrate and ATP concentrations and the stimulation of fatty acid synthesis is indicated.

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Studies on Ethionine. VII. Fatty Acid Synthesis in Livers of Ethionine-Treated Rats.* (30016)

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Administration of ethionine, the S-ethyl analog of methionine, to female rats causes a marked and rapid accumulation of lipid in the liver(1,2,3). This ethionine-induced fatty liver is accompanied by a fall in triglyceride

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and a rise in free fatty acid concentration in the plasma(3). Although there is abundant evidence that the liver is the chief source of the circulating triglycerides derived from endogenous sources(4), it is not known whether the fatty acid moiety of the accumulated triglyceride in the liver of the ethionine-treated rats is mainly derived from *de novo* synthesis in the liver or from the circulating blood.

We wish to report that administration of ethionine to female rats induces a marked inhibition of fatty acid synthesis in the microsome-supernatant system of the liver described in the preceding communication(5). So the accumulation of triglyceride in the liver cannot be the result of an accelerated synthesis of fatty acids but more probably results from a reduction in rate of the transport of triglyceride from the liver.

Materials and methods. Rats of the Long-Evans strain, maintained on Wayne Lab Blox rat food and weighing from 190 to 210 g, were used in all experiments. DL-Ethionine (25 mg/ml of saline), in a total dose of 1 mg/g of body weight, was given intraperitoneally in 2 equal doses at zero and 1 hour. Control animals received an equal volume of saline. The food was withdrawn at the time of the first injection and the animals were sacrificed by decapitation at intervals noted in the text.

The combined microsome-supernatant fraction was prepared as described previously (5). In experiments with isolated microsomes and supernatant, the combined microsomal-supernatant fraction obtained by centrifugation at 10,000 $\times$ *g* was further centrifuged at 105,000 $\times$ *g* for 1 hour. The microsomes were resuspended in the corresponding volume of 0.1 *M* potassium phosphate buffer, pH 7.5, or in the 105,000 $\times$ *g* supernatant from control or ethionine-treated animals to make the microsomal concentrations equal to the original microsome-supernatant fraction.

Incubation with acetate-1-C^{14}, extraction of the labeled fatty acids, and determination of the radioactivity of the samples were carried out as described(5).

Results and discussion. The conversion of acetate to fatty acids by the liver preparation from male and female rats is shown in Fig. 1. At 3 hours after the administration

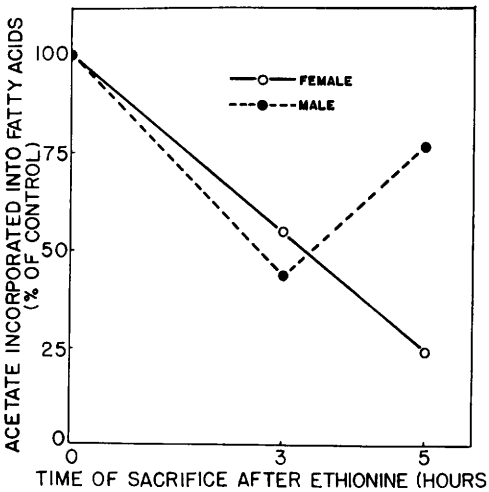

FIG. 1. Incorporation of acetate-1-C^{14} into fatty acids by the microsome-supernatant system from male and female rat-livers treated by injection of ethionine at various time intervals before sacrifice. Each point represents the mean of the values from 6 ethionine-treated rats or from 12 control animals of each sex. Actual incorporation for control animals was 11.2 mμmoles for male and 13.1 mμmoles for female animals.

of ethionine, fatty acid synthesis in the livers of both sexes was reduced to about 50% of the controls. At 5 hours, male rats showed recovery from the initial inhibition to 77% of the control, whereas the incorporation of acetate in preparations from females was further reduced to 24%. All subsequent experiments were done with female rats sacrificed at 5 hours after administration of ethionine.

Since administration of ethionine to rats has been shown to depress the levels of ATP (6), NAD(7) and various intermediates of the glycolytic pathway in the liver(8), effects of the additions *in vitro* of NADPH and CoA were tested. The addition of these compounds to the preparation from the ethionine-treated rats was without effect (Table I). In this connection, it should be noted that the concentration of ATP present in the incubation mixture was so high as to make the contribution from the endogenous ATP in the liver homogenate practically negligible. Therefore, it is improbable that the ethionine-induced inhibition of fatty acids synthesis observed *in vitro* results from a deficiency of ATP, NADPH, or CoA in the supernatant fraction from the treated animals.

Ethionine and methionine are converted to

reaction yielding malonyl-CoA(16), and that a specific interaction between microsomes and supernatant enzymes might be required for optimal carboxylation of acetyl-CoA in the supernatant-microsomal system(15). Thus it is probable that the ethionine-induced inhibition of fatty acid synthesis in female rats results primarily from inhibition of the synthesis of acetyl-CoA carboxylase in the supernatant fraction and secondarily from impairment of the microsomal function. This view is in agreement with observations that the administration of ethionine inhibits hepatic protein synthesis more effectively in female than in male rats(12,13,14). However, it does not explain why the preparations from male rats exhibit the inhibition to the extent of 50% of the control at 3 hours after ethionine administration (Fig. 1) whereas there is no appreciable inhibition of protein synthesis. The inhibition observed at 3 hours for both sexes may be due to an acute physiological, possibly hormonal, disturbance brought about by administration of ethionine which stimulates the mobilization of fat from depots to the liver where the fatty acid synthesis is inhibited. It is of interest to note, in this connection, that epinephrine stimulates hepatic fat accumulation in adrenalectomized cortisone- and ethionine-treated rats, whereas metanephrine is ineffective, and that ethionine administration inhibits the methylation *in vivo* of epinephrine to metanephrine resulting in the prolonged action of epinephrine on fat mobilization(17).

Summary. The effect of administration of ethionine upon fatty acid synthesis in a system from the livers of female and male rats has been investigated. Three hours after administration of ethionine, fatty acid synthesis in the livers of both sexes was reduced to about 50% of the control. At 5 hours, male rats recovered from the initial inhibition to 77% of the control, whereas the synthesis in females was further reduced to 24%. It was found that the ethionine effect was not merely due to a deficiency in the supply, or in availability of ATP, NADPH, or CoA. Nor was the impairment due to an accumulation of ethionine itself or S-adenosylethionine. The possibility is considered that the inhibition of fatty acid synthesis at 5 hours in the liver of female rats may result from the inhibition of the synthesis of acetyl-CoA carboxylase, which is found in the supernatant fraction, in addition to a partial impairment of microsomal function. The cause of the inhibition observed at 3 hours for both sexes remains to be determined.

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TABLE I. Effect of Various Cofactors and Ethionine-Related Compounds on Acetate Incorporation into Fatty Acids by the Microsome-Supernatant Fractions from Livers of Control and Ethionine-Treated Female Rats.

Addition		Incorporation of acetate μmoles/g protein/2 hr	
Compound	Amt	Control	Ethionine-treated*
	μmoles/flask		
None		9.9	2.3
NADPH	2.5	9.6	2.2
CoA	.25	12.7	2.3
L-ethionine	12	9.0	
S-adenosyl-*L*-ethionine	1.3	9.1	
L-methionine	15	8.8	
S-adenosyl-*L*-methionine	5.9	9.9	

* Female rats sacrificed 5 hr after the first dose of ethionine.

their respective S-adenosyl derivatives by the methionine-activating enzyme of rat liver to approximately the same extent(9), and after ethionine, S-adenosylethionine accumulates because it is only slowly transethylated to acceptors(10,11). Therefore, the possible inhibitory effects of the *in vitro* addition of ethionine and S-adenosylethionine were tested. As shown by the data of Table I, no significant inhibition was observed with ethionine and its S-adenosyl derivative. Likewise, the addition of methionine or S-adenosylmethionine had no effect.

The data presented in Table II provide further evidence that the ethionine-induced inhibition of fatty acid synthesis cannot be due to accumulation of ethionine or its metabolite in the liver. The combined homogenate fractions from the control and the ethionine-treated animals incorporated about 80% of the calculated additive amount of acetate into fatty acids. If the accumulation of some inhibitors were the main cause of the ethionine-inhibition, the combined fractions would

TABLE II. Fatty Acid Synthesis from Acetate by Homogenate-Fractions from Livers of Control and Ethionine-Treated Female Rats.

Homogenate fraction	Incorporation of acetate μmoles/g protein/2 hr	
	Exp 1	Exp 2
Control	10.4	10.7
Ethionine	2.3	1.9
½ control plus ½ ethionine	5.0	5.3

be expected to have given a much lower value. In view of the relatively low rate of hepatic protein synthesis which prevails in female rats following ethionine administration(12,13,14), it seems likely that the presently observed effect of ethionine results from an interference with the synthesis, rather than with the function of some enzymes (or coenzymes) directly or indirectly involved in fatty acid synthesis.

Since both the particle-free supernatant and microsomal fractions are necessary for optimal synthesis of fatty acid(5,15), an attempt was made to evaluate the relative extent of the defects produced by ethionine in these two fractions. It is apparent from the results presented in Table III that each fraction alone synthesizes a very small amount of fatty acid under the present experimental conditions and the supernatant fraction is affected to a greater degree by ethionine administration than the microsomal fraction. It is also noteworthy that the inhibition in the reconstituted ethionine-treated system (76%) is almost equal to the sum of the inhibitions observed in the supernatant (45%) and the microsomal (26%) fractions. This finding should be contrasted with the result of the *in vitro* protein synthesis system in which the ethionine-induced inhibition was shown to be localized in the microsomes and not in the supernatant fraction(13).

TABLE III. Fatty Acid Synthesis from Acetate by Reconstituted Homogenate-Fractions from Livers of Control and Ethionine-Treated Female Rats.

Origin of fractions*	Incorporation of acetate μmoles/g protein/2 hr	Difference from control (%)
MC + SE	16.7	
MC + SE	9.2	−45
ME + SC	12.4	−26
ME + SE	4.0	−76
MC	.3	−98
SC	.6	−96

* MC, microsomes from control rats; ME, microsomes from ethionine-treated rats; SC, 105,000 × *g* supernatant from control rats; SE, 105,000 × *g* supernatant from ethionine-treated rats.

It has been suggested that the rate-limiting step in the synthesis of fatty acids from acetate or acetyl-CoA by particle-free supernatant fraction of rat liver is a carboxylation