Effect of ethionine on carbohydrate and lipid metabolism

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Abstract In ethionine-treated rats, the ATP content of adipose tissue was not decreased whereas in liver it was drastically reduced to about one-fifth of the control level. Shortly after the injection of ethionine, hepatic glycogen was depleted and the blood glucose concentration fell from 120 to 80 mg/100 ml. This was followed by a two- to threefold elevation of the plasma fatty acid level. Hepatic glucose-6-phosphate was decreased and was not elevated by administration of 2 mmoles of glucose unless ATP was partially restored to normal levels. When hepatic ATP was decreased, the disappearance of [^{14}C]glucose from the blood and its incorporation into glycogen and glyceride-glycerol and the incorporation of [3-^{14}C]pyruvate into glyceride-glycerol were reduced.

6 hr after ethionine injection the plasma triglyceride level fell but there was no significant change in the liver triglyceride concentration, but by 24 hr it had increased markedly. Lipogenesis in adipose tissue was depressed in vivo, possibly due to decreased glycerol-3-phosphate concentrations.

A marked decrease of glycerol-3-phosphate in both liver and adipose tissue was noted. Administration of glucose effectively depressed plasma free fatty acid concentration and elevated the glycerol-3-phosphate levels. Ethionine injection to fasted animals further depressed the blood glucose and elevated the plasma free fatty acid level.

Supplementary key words ATP · hepatic glycogen · glucose-6-phosphate · gluconeogenesis · blood glucose · plasma free fatty acid · glycerol-3-phosphate · liver · adipose tissue · fatty acid assimilation

It has been established by many investigators that when ethionine-treated rats are fasted there is a marked accumulation of hepatic fat (1–4). While the inhibition of lipoprotein formation has been considered as a main cause of the accumulation of hepatic fat (5–7), Farber and Popper (8) reported that accumulation of fat was blocked by glucose administration. Also, ethionine treatment results in lower blood glucose levels (4, 9, 10) and in significantly elevated plasma free fatty acid levels (2, 10, 11, 12). Hepatic ATP is drastically decreased a short time after injection of ethionine (10, 13) as a result of the decrease of the hepatic adenine pool (14, 15). We also postulated that the large decrease of hepatic ATP resulted in the suppression of gluconeogenesis and blood glucose (10), in confirmation of a suggestion of Bartels and Hohorst (9). Therefore, as a further stage in our study of metabolic effects of ethionine, we attempted to analyze the effect of glucose on fatty acid mobilization and to clarify the above interrelationships between carbohydrate and lipid metabolism in ethionine-treated rats.

The present study suggests that the increased fatty acid release from adipose tissue in ethionine-treated rats is a result of suppressed gluconeogenesis and is probably caused by a lowered rate of esterification of fatty acid in adipose tissue. It is possible that flux of lipid between liver and adipose tissue may be regulated by the blood glucose level and, especially in the fasted states, by the rate of gluconeogenesis. There may be a feedback control of fatty acid mobilization. Furthermore, the present study suggests that the rapid depletion of hepatic glycogen in ethionine-treated rats also may be a result of the drastic decrease of hepatic ATP.

METHODS AND MATERIALS

Chemicals

All radiochemicals were purchased from Radiochemical Centre, Amersham, England. Enzyme preparations and chemicals for the determination of metabolic intermediates were purchased from Boehringer Corp. Permutit was obtained from the Japan Vitamin Society.

Treatment of animals

Female rats of the Sprague-Dawley strain, weighing 200 g, were used. They were treated with ethionine as previously described (4, 10) and were then fasted for 6, 12, or 24 hr. Glucose, pyruvate, or xylitol was administered 1 or 2 hr before the rats were killed.

Utilization of [1-^{14}C]- or [U-^{14}C]glucose and [3-^{14}C]pyruvate in vivo

6 hr after administration of ethionine, 2.5 μCi of [3-^{14}C]pyruvate was injected intraperitoneally with 1 mmole of nonradioactive pyruvate; 60 min later, blood was withdrawn and collected in heparinized containers. Blood glucose was determined by the glucose oxidase method (16). In experiments with [^{14}C]glucose, 2.5 μCi of [1-^{14}C]- or [U-^{14}C]glucose/ml of distilled water was injected. [^{14}C]Glucose in the deproteinized supernatant solution was measured as described previously (4, 10). Hepatic glycogen was determined by the anthrone method (17) or weighed after purification (4).

Incorporation of labeled substrates into lipid

The liver was homogenized in 20 vol of chloroform–methanol 2:1, and the homogenate was filtered 3 hr later. The filtrate was purified according to the method of Folch, Lees, and Sloane Stanley (18). One portion of the extract was taken to dryness, and the lipid was dissolved in toluene and counted to determine the total ^{14}C incorporated into the lipid fraction (19). Another portion of the sample was hydrolyzed with 4 N KOH for 6 hr. After the pH was adjusted to below 1 with 6 N H_2SO_4, fatty acids were extracted with petroleum ether and the extract was dried and counted by the same method used for the total lipids (19).

Determination of ATP, glucose-6-phosphate, and glycerol-3-phosphate

For the determination of ATP, the animals were anesthetized with pentobarbital (25 mg/kg body wt) before they were killed. The concentration of ATP in adipose tissue was determined by a slight modification of the method of Lamprecht and Trautshold (20). Periovarial adipose tissues were frozen in situ using liquid nitrogen; they were excised and homogenized with 6% perchloric acid. After centrifugation to remove the fat, the supernatant solution was used for determination of ATP. Hepatic glucose-6-phosphate was determined by the method of Lamprecht and Trautshold (20) and glycerol-3-phosphate by the method of Hohorst (21).

In vitro assays for esterification of fatty acid and glucose utilization

200 mg of periovarian adipose tissue was incubated at 37°C in 5 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 0.5 μCi of [1-^{14}C]palmitate or [U-^{14}C]glucose and various concentrations of glucose. The incubation was carried out in 25-ml rubber-stoppered Erlenmeyer flasks with center wells. The gas phase was 95% O_2–5% CO_2. After various times of incubation, the reaction was stopped by addition of 0.2 ml of 6 N H_2SO_4. The $^{14}CO_2$ that was formed was absorbed with ethanolamine injected into the center well through the rubber stoppers; it was counted by the method of Jeffay and Alvarez (22). The tissues were washed with saline and then homogenized in chloroform–methanol 2:1, and the lipid was purified by the method of Folch et al. (18) and dried. Incorporation of [U-^{14}C]glucose into lipid was determined by the method described before. For determination of the incorporation of [1-^{14}C]palmitate into fat, the lipid extract was redissolved in 30 ml of chloroform and shaken mechanically with 2 g of Permutit, which absorbed more than 95% of fatty acids. The treatment with Permutit was repeated. After removal of the solvent, the purified extract was counted as described previously (19).

Determination of fatty acid, triglyceride, and cholesterol

Plasma triglyceride was determined by the method of Fletcher (23). Hepatic triglyceride was determined by the same method after treatment of the extracted lipid with Permutit. Plasma free fatty acid was determined by the method of Itaya and Ui (24). Free and total cholesterol were determined by the method of Zak et al. (25).

RESULTS

As shown in Table 1, administration of ethionine to the rats caused a marked decrease in hepatic glycogen, which was depleted 5 hr after injection. At this time the blood glucose was significantly decreased and the level of plasma free fatty acids was elevated. No marked changes were observed in the control group. Although the marked decrease in the hepatic glycogen levels may have been due to suppression of gluconeogenesis by ethionine treatment (4, 10), the results in Table 2 also suggest a depressed phosphorylation of glucose in accordance with the drastic decrease of hepatic ATP. As shown in Table 2, the disappearance of [1-^{14}C]glucose from blood was significantly retarded in ethionine-treated animals. Fig. 1*A* shows that hepatic glucose-6-phosphate was decreased to one-half of the control values after 2–3 hr. 6 hr after injection, glucose-6-phosphate was decreased to the same level as that of the control animals fasted for longer periods (e.g., 24 or 48 hr). Furthermore, as shown in Fig. 1*B*, administration of glucose to the ethionine-treated animals did not

TABLE 1. Effects of ethionine on hepatic glycogen, blood glucose, and plasma free fatty acids

Treatment	Time after Injection	Number of Animals	Liver Glycogen	Blood Glucose	Plasma Free Fatty Acids
	hr		*mg/g liver*	*mg/100 ml*	*μmoles/ml*
Ethionine[a]	0[b]	6		122 ± 14[c]	0.410 ± 0.065
	0.5	3		126 ± 12	0.384 ± 0.031
	1	3	61 ± 11	110 ± 12	0.431 ± 0.077
	2[d]	3	64 ± 5	125 ± 3	0.384 ± 0.054
	3	6	40 ± 16	118 ± 4	0.384 ± 0.031
	4	3	39 ± 27	117 ± 5	0.367 ± 0.031
	5	6	4 ± 1[e]	96 ± 3[e]	0.540 ± 0.010[e]
	6	6	4 ± 1[e]	79 ± 8[e]	0.784 ± 0.054[e]
Control[f]	6	4	48 ± 6	116 ± 10	0.382 ± 0.032

[a] Fed rats were injected with DL-ethionine (0.75 mg/g body wt).
[b] First injection (0.375 mg/g body wt).
[c] Each value is the mean of three to six animals ± SD.
[d] Second injection (0.375 mg/g body wt).
[e] Significantly different from the other values ($P < 0.05$).
[f] Fasted for 6 hr after injection of 0.85% saline.

TABLE 2. Disappearance of [1-^{14}C]glucose from blood of ethionine-treated rats[a]

Time after Injection of [1-^{14}C]-Glucose[b]		Blood Glucose[c]	[^{14}C]Glucose[c] Remaining in Blood
hr		*mg/100 ml*	*dpm/ml plasma*
1	Control (4)	108 ± 9[d]	16,000 ± 8300
	Ethionine (5)	85 ± 10[e]	29,200 ± 4500[e]
2	Control (4)	102 ± 7	6,500 ± 1200
	Ethionine (5)	79 ± 8[e]	12,700 ± 1800[e]

[a] After treatment with ethionine, animals were fasted for 5 hr, as were the control animals.
[b] 2.5 μCi of [1-^{14}C]glucose was injected intraperitoneally, and animals of both groups were killed 1 or 2 hr later.
[c] Blood glucose and [^{14}C]glucose in blood were determined for the same samples.
[d] Each value is the mean ± SD for the number of animals shown in parentheses.
[e] Significantly different from the control ($P < 0.05$).

TABLE 3. Effect of ethionine on the incorporation of [U-^{14}C]glucose into hepatic glycogen

Treatment[a]	Hepatic Glycogen[b]	^{14}C in Glycogen from [U-^{14}C]Glucose
	mg/g liver	*cpm/g liver*
Control (5)	36 ± 6[c]	1440 ± 70
Ethionine (7)	3 ± 2[d]	240 ± 32[d]

[a] 5 hr after administration of ethionine, 2.5 μCi of [U-^{14}C]glucose/ml of distilled water was injected intraperitoneally, and 1 hr later the animals were killed.
[b] Hepatic glycogen was weighed after purification and counted as described in the text.
[c] Each value is the mean ± SD for the number of animals shown in parentheses.
[d] Significantly different from the control ($P < 0.01$).

increase hepatic glucose-6-phosphate at a time when the hepatic ATP was drastically decreased (18 ± 4 nmoles/g of liver), but there was an increase in the control animals (145 ± 19 nmoles/g of liver). To clearly demonstrate the increase of glucose-6-phosphate, rats fasted for 24 hr were used. Since the hepatic glycogen was depleted, the experimental condition could exclude the influence of glucose-6-phosphate derived from glycogen. 2 mmoles of glucose was injected intraperitoneally 6 hr after the ethionine injection. On the other hand, when the animals were fasted for 24 hr after the ethionine injection, the concentration of hepatic ATP returned to the normal range, and the administration of glucose effectively increased the glucose-6-phosphate levels. As shown in Table 3, treatment with ethionine depressed the incorporation of [U-^{14}C]glucose into hepatic glycogen. However, the specific activity of glycogen (^{14}C-labeled glycogen/nonradioactive glycogen) was not always larger in the control compared with the ethionine-treated rats. When ATP was administered simultaneously with ethionine, decrease of hepatic glycogen was partially prevented, and total ^{14}C in liver glycogen and the specific activity of glycogen were much higher compared with the results from rats treated with ethionine alone.

In agreement with previous reports (2, 9, 10, 26), plasma free fatty acids significantly increased in ethionine-treated animals, but administration of glucose readily depressed the elevated plasma free fatty acid level, as shown in Table 4. Also, the data suggested that the depressed esterification of fatty acids may be due to a decrease in the concentration of blood glucose. The amount of administered glucose was directly correlated with the decrease of plasma free fatty acids.

Pyruvate is rapidly incorporated into glyceride-glycerol of adipose tissue in vivo. However, as shown in Table 5, administration of pyruvate or xylitol (27–29) to

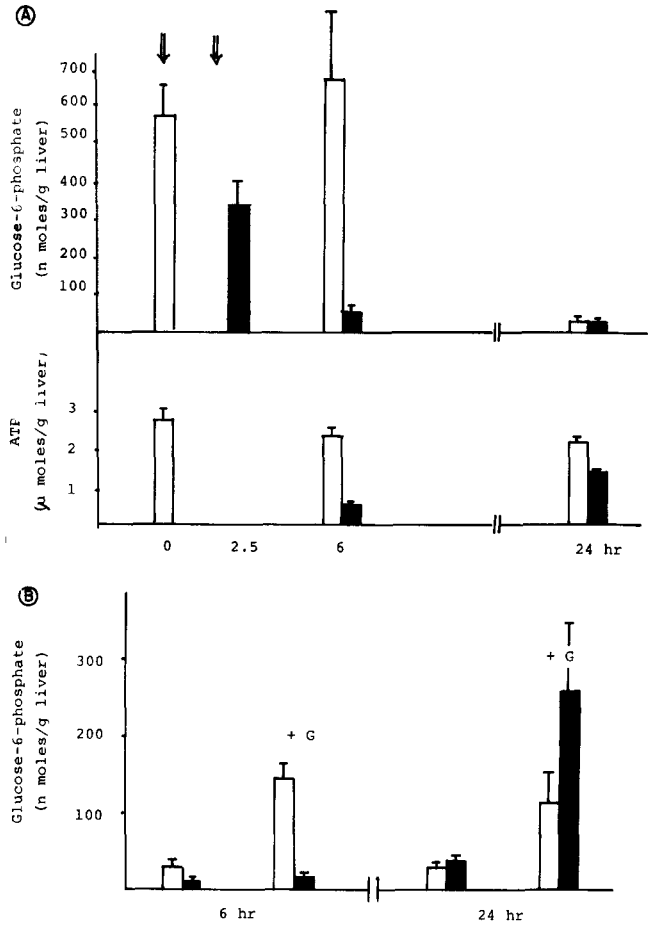

FIG. 1. Hepatic glucose-6-phosphate and ATP in ethionine treated rats. *A*, hepatic glucose-6-phosphate and ATP in ethionine-treated rats; *B*, effect of glucose administration on hepatic glucose-6-phosphate in ethionine-treated rats. Open bars, control; solid bars, ethionine-treated rats. Standard deviation of the mean of five animals is indicated by the vertical bar. In *A*, ethionine was injected at times indicated by arrows (0.375 mg/g body wt). The first was at 0 time and the second was 2 hr later. The procedure was the same with all other experiments (see Refs. 4 and 10). Abscissa, time after injection of ethionine. Animals were fasted for designated times. In *B*, *left*, animals fasted for 24 hr were injected with ethionine and 6 hr later 2 mmoles of glucose was injected; they were killed 1 hr later. In *B*, *right*, 24 hr after treatment with ethionine, 2 mmoles of glucose was injected and animals were killed 1 hr later. + G indicates glucose was administered.

TABLE 4. Effect of glucose on plasma free fatty acid levels in ethionine-treated rats

Treatment	Amount of Injected Glucose	Blood Glucose	Plasma Free Fatty Acids
	g	*mg/100 ml*[a]	*μmoles/ml*[a]
Ethionine[b]	0	79 ± 8[c]	0.784 ± 0.054[c]
	0 2	123 ± 6	0.222 ± 0.003
	0.4	126 ± 13	0.117 ± 0.013
	0.6	121 ± 10	0.089 ± 0.031
	0.8	158 ± 48	0.104 ± 0.031
Control	0	124 ± 12	0.354 ± 0.026

[a] Each value is the mean of three animals ± SD.
[b] 6 hr after injection with ethionine various amounts of glucose were injected and 60 min later animals were killed.
[c] The difference between the ethionine-treated and the non-treated groups is significant ($P < 0.01$).

TABLE 5. Effects of glucose, pyruvate, and xylitol on plasma free fatty acid levels of fasted and ethionine-treated rats

Treatment	Blood Glucose	Plasma Free Fatty Acid
	mg/100 ml	*μmoles/ml*
(A) Fasted[a]		
None (5)	66 ± 7[b]	1.09 ± 0.17
0.4 g xylitol, 1 hr[c] (5)	68 ± 7	0.79 ± 0.15
0.4 g pyruvate, 1 hr (5)	126 ± 14[d]	0.79 ± 0.10[d]
0.4 g glucose, 1 hr (4)	144 ± 7[d]	0.38 ± 0.12[d]
0.4 g xylitol, 2 hr (4)	81 ± 7	0.75 ± 0.06[d]
0.4 g pyruvate, 2 hr (4)	92 ± 13	0.93 ± 0.09
(B) Fasted,[a] ethionine-treated[e]		
0.4 g xylitol, 2 hr (4)	88 ± 13	0.44 ± 0.16[d]
0.4 g pyruvate, 1 hr (4)	96 ± 6	0.85 ± 0.02
0.4 g glucose, 1 hr (4)	126 ± 13[d]	0.12 ± 0.03[d]

[a] Animals were fasted for 48 hr.
[b] Each value is the mean ± SD for the number of animals shown in parentheses.
[c] Pyruvate or xylitol was administered intraperitoneally 1 or 2 hr before the animals were killed.
[d] Significantly different from the control ($P < 0.05$).
[e] Ethionine was injected 6 hr before the animals were killed.

fasted rats resulted only in a slight depression of the plasma free fatty acid level. Administration of pyruvate markedly elevated the blood glucose level in the fasted control rats. Blood glucose reached a level of 200 mg/100 ml or more 30–45 min after injection of 0.4 g of pyruvate. Administration of glucose effectively depressed the plasma free fatty acid level. In the ethionine-treated animals, glucose administration effectively depressed the level of free fatty acids; pyruvate administration was less effective in increasing blood glucose and it did not significantly depress the plasma free fatty acid level. On the other hand, administration of xylitol effectively depressed the elevated plasma free fatty acid level of ethionine-treated rats. These results suggested that adipose tissues of ethionine-treated rats might be impaired metabolically under such an experimental condition.

As described before, treatment with ethionine caused marked alterations in lipid metabolism. As shown in Table 6, plasma triglyceride was markedly decreased 6 hr

TABLE 6. Effects of ethionine treatment on concentrations of free fatty acids, triglyceride, and cholesterol in plasma and liver

Time after Injection		Plasma Free Fatty Acids	Triglyceride		Cholesterol		
			Plasma	Liver	Plasma		Liver
					Total	Free	Total
hr		*μmoles/ml*	*mg/100 ml*	*mg/g*	*mg/100 ml*		*mg/g*
	Control (4)	0.42 ± 0.03[a]	52 ± 14	14 ± 3	61 ± 2	23 ± 3	3.9 ± 0.1
6	Ethionine (5)	0.70 ± 0.13[b]	29 ± 4[b]	18 ± 5	48 ± 8	16 ± 2	4.0 ± 0.6
	Ethionine + glucose[c] (5)	0.17 ± 0.06	28 ± 3[b]	8 ± 1[b]	54 ± 4	22 ± 2	3.8 ± 0.3
24	Control (3)	0.69 ± 0.03	34 ± 4	28 ± 4	64 ± 9	18 ± 1	4.4 ± 0.3
	Ethionine (4)	0.70 ± 0.14	31 ± 5	128 ± 33[b]	29 ± 6[b]	11 ± 2[b]	5.4 ± 0.9

[a] Each value is the mean ± SD for the number of animals shown in parentheses.
[b] Significantly different from the control ($P < 0.01$).
[c] 1 g of glucose was injected intraperitoneally 2 hr before the animals were killed.

after the rats were injected with ethionine. Hepatic triglyceride, on the other hand, was not significantly affected at this time, but a marked increase was noted at 24 hr. Plasma cholesteryl esters (total minus free) were significantly decreased 24 hr after ethionine injection. These results may be explained by a disturbance of lipid transport from the liver (see below). When [1-^{14}C]-glucose was administered, its incorporation into lipid was significantly depressed 6 hr after treatment with ethionine (Table 7). The incorporation of [1-^{14}C]-glucose was significantly decreased in both glyceride-glycerol and fatty acid fractions. However, in the liver the conversion of [1-^{14}C]glucose to lipid was not depressed significantly 2 hr after the injection compared with the control. This may be due to the retarded disappearance of ^{14}C-labeled glucose from the blood (Table 2) and the disturbed lipid transport from the liver. Since the [^{14}C]glucose in blood was higher in the ethionine-treated rats 2 hr after the injection, the high radioactivity in liver lipid may be explained by the experimental conditions. In adipose tissue the incorporation of radioactivity into lipid was depressed after 2 hr. Such results are also in agreement with the rapid turnover rate of hepatic triglyceride of intact animals (2, 7, 30–32). As shown in Table 8, incorporation of [3-^{14}C]-pyruvate into lipid was also depressed in both the glycerol and the fatty acid moieties in ethionine-treated rats. These results suggest a depression of both esterification and synthesis of fatty acids.

Despite a significant depression of incorporation of glucose into lipid in vivo (Table 7), the esterification of fatty acids in adipose tissue of ethionine-treated rats was not depressed in vitro compared with the control (Table 9). Esterification of [1-^{14}C]palmitate was increased when the glucose concentration in the incubation medium was increased. There was no significant difference between the ethionine-treated and control groups. Addition of ethionine to the medium also had no effect. The oxidation of [U-^{14}C]glucose to $^{14}CO_2$ and the conversion of the labeled glucose to lipid were not depressed compared with the control. Furthermore, the ATP content in adipose tissue was not decreased in treated rats.

In ethionine-treated rats the concentration of glycerol-3-phosphate in adipose tissue was markedly decreased and the plasma free fatty acid level was elevated, but administration of glucose significantly increased glycerol-

TABLE 7. Incorporation of radioactivity into lipid from [1-^{14}C]glucose in ethionine-treated rats

Time after Injection with [^{14}C]-Glucose[a]		Lipid	Liver		Adipose Tissue	
			Fatty Acid	Glycerol[b]	Fatty Acid	Glycerol[b]
hr		*mg/g liver*	*dpm/mg lipid*			
1	Control (4)	47 ± 3[c]	6.3 ± 0.3	104 ± 20	0.07 ± 0.03	1.24 ± 0.56
	Ethionine (5)	57 ± 10	3.0 ± 2.0[d]	55 ± 20[d]	0.04 ± 0.03	0.87 ± 0.32
2	Control (4)	45 ± 3	4.4 ± 0.8	62 ± 13	0.16 ± 0.12	3.22 ± 0.99
	Ethionine (5)	69 ± 5[d]	5.5 ± 4.1	65 ± 20	0.03 ± 0.01[d]	1.32 ± 0.16[d]

[a] Treatments were the same as those given in Table 2.
[b] Glyceride-glycerol was calculated as follows: radioactivity/mg lipid − radioactivity of fatty acid/mg lipid.
[c] Each value is the mean ± SD for the number of animals shown in parentheses.
[d] Significantly different from the control ($P < 0.05$).

3-phosphate in adipose tissue and depressed the plasma free fatty acid level, as shown in Table 10. Thus, a decrease in the glycerol-3-phosphate concentration in adipose tissue was accompanied by an increase of fatty acid release. Glycerol-3-phosphate was reported to decrease in livers of fasted rats (33). However, in the present study we noted no change in the hepatic concentration of the glycerol-3-phosphate in fasted rats and a marked decrease after ethionine treatment. Although the plasma free fatty acid level was higher in fasted rats, it was further elevated by injection with ethionine in accordance with a decrease of blood glucose.

TABLE 8. Incorporation of radioactivity into lipid from [3-^{14}C]pyruvate in ethionine-treated rats

Time after Injection with Ethionine		Liver		Adipose Tissue	
		Fatty Acid	Glyceride-glycerol[a]	Fatty Acid	Glyceride-glycerol[a]
hr		*dpm/mg lipid*			
6[b]	Control (3)	16.7 ± 2.5[c]	117 ± 2	4.3 ± 2.1	4.8 ± 1.5
	Ethionine (5)	9.3 ± 3.8[d]	78 ± 9[d]	1.0 ± 0.3[d]	2.4 ± 0.5[d]

[a] Calculated (see footnote *b*, Table 7).
[b] 6 hr after injection with ethionine, 2.5 μCi of [3-^{14}C]pyruvate was injected intraperitoneally with 1 mmole of nonradioactive pyruvate. The animals were killed 1 hr later.
[c] Each value is the mean ± SD for the number of animals shown in parentheses.
[d] Significantly different from the control ($P < 0.05$).

TABLE 9. Incorporation of [1-^{14}C]palmitate into triglyceride and [U-^{14}C]glucose utilization in adipose tissue in vitro

Treatment	Concentration of Glucose in Medium	Incorporation of ^{14}C from [1-^{14}C]Palmitate into Triglyceride[a]	Conversion of ^{14}C from [U-^{14}C]Glucose[c]	
			Into CO_2	Into Lipid
	mM	*cpm/100 mg tissue*	*dpm/mg protein*	
Control	0	15,200 ± 1800[c]		
	5	59,100 ± 3800	3937 ± 238	8580 ± 246
	10		2854 ± 369	3510 ± 198
	15	83,100 ± 2900		
Ethionine	5	60,000 ± 4200	4628 ± 230	8040 ± 350
	10		3525 ± 580	4170 ± 220
	15	69,400 ± 5200		

Animals were killed 6 hr after injection with saline or ethionine, and 200 mg of periovarial tissue was incubated as described in text.
[a] Incubated with 0.5 μCi of [1-^{14}C]palmitate for 60 min.
[b] Incubated with 0.5 μCi of [U-^{14}C]glucose for 120 min.
[c] Each value is the mean of three animals ± SD.

TABLE 10. Effects of ethionine treatment and glucose administration on plasma free fatty acids and on glycerol-3-phosphate levels in liver and adipose tissue

Treatment[a]	Blood Glucose	Plasma Free Fatty Acids	Glycerol-3-phosphate	
			Liver	Adipose Tissue
	mg/100 ml	*μmoles/ml*	*μmoles/g*	
(A) Fed				
Control (7)	104 ± 7[b]	0.213 ± 0.032	0.667 ± 0.105	0.034 ± 0.003
Ethionine (7)	80 ± 9[c]	0.764 ± 0.048[c]	0.451 ± 0.148[c]	0.024 ± 0.002[c]
Ethionine + glucose[d] (5)	160 ± 48	0.130 ± 0.040	0.794 ± 0.077	0.047 ± 0.008
(B) Fasted				
Control (7)	72 ± 9	1.076 ± 0.247	0.759 ± 0.110	0.014 ± 0.009
Ethionine (7)	53 ± 7[c]	1.532 ± 0.155[c]	0.144 ± 0.027[c]	0.005 ± 0.003

[a] Fed animals and animals fasted for 24 hr were injected with ethionine and killed 6 hr later.
[b] Each value is the mean ± SD for the number of animals shown in parentheses.
[c] Significantly different from the control ($P < 0.05$).
[d] 1 g of glucose was injected 1 hr before the rats were killed.

DISCUSSION

The results of this study clearly show the following marked metabolic changes caused by treatment with ethionine: *1*) a rapid and drastic decrease of hepatic glycogen; *2*) an increase of plasma free fatty acids and a suppression of gluconeogenesis; and *3*) a marked decrease of glycerol-3-phosphate in liver and adipose tissue. However, almost all these phenomena may be explained by the drastic decrease of hepatic ATP, which is one of the most marked changes noted in ethionine-treated rats (10, 13). In this context, some regulatory mechanism between carbohydrate and lipid metabolism will be discussed.

The rapid depletion of hepatic glycogen as a result of ethionine administration probably results from depression of glucose-6-phosphate formation and the decrease of glycogen synthesis via glucose-6-phosphate. Under the condition of a low hepatic ATP level, the marked decrease of glucose-6-phosphate (Fig. 1) is probably due to two types of glucokinase depression: *1*) drastic decrease of hepatic ATP (K_m value of glucokinase for ATP is 5×10^{-4} M) and *2*) decrease of enzyme activity (34). As suggested previously (10), when hepatic ATP is markedly decreased as observed in rats treated with ethionine, glucose formation from pyruvate is markedly suppressed and the flow of intermediates to the reversed direction (the conversion of glucose-6-phosphate to pyruvate) overcomes the former. Most of the pyruvate is converted to lactate or flows into the tricarboxylic acid cycle.

Although a decrease of blood glucose and an elevation of plasma free fatty acids in ethionine-treated rats have been established by many investigators (2, 10–12, 26), the present results (Tables 1, 4, 5, and 10) indicate that the decrease of blood glucose and the increase of fatty acid release are related. Whenever ethionine was injected, blood glucose significantly decreased. The plasma free fatty acids were increased after the depression of blood glucose level. Glucose administration to the treated animals readily depressed the plasma free fatty acid concentration (Tables 4, 5, and 10) and the hepatic triglyceride content (Table 6) (8). Although the factors which regulate fatty acid mobilization are complicated (35–39), the increase of plasma free fatty acids by ethionine-treatment may be induced by the suppression of gluconeogenesis. The elevation of plasma free fatty acid levels might be induced by an activation of lipase in adipose tissue by an increased secretion of catecholamines[1] (40, 38) or by an insufficiency of insulin (35); however, this may be discounted as follows. In ethionine-treated animals such an increase of plasma free fatty acids as observed in animals treated with epinephrine was not induced until blood glucose was decreased, as shown in Table 1. Secondly, as described in Results (Table 9), marked metabolic impairments were not observed in adipose tissue taken from ethionine-treated animals despite a significant depression of the incorporation of [1-^{14}C]glucose into glyceride-glycerol in vivo (Table 7). These results agree with a previous description of adipose tissue in which the fatty acid release in vitro was not altered (5). Finally, administration of glucose to the ethionine-treated rats effectively depressed plasma free fatty acid levels. From these observations, the increase of plasma free fatty acids induced by ethionine-treatment may not have been due to an activated lipase in adipose tissue or an insufficiency of insulin. As suggested previously (5, 41), although the lesion of the pancreas also may be induced, it is not important for the early metabolic changes in ethionine-induced fatty liver. To confirm the physiological role of blood glucose for assimilation of fatty acid in adipose tissue, the metabolism of pyruvate and xylitol (27–29) was examined. Although the administration of pyruvate or xylitol also suppressed the plasma free fatty acid level in fasted rats, in ethionine-treated rats pyruvate administration caused neither an increase of blood glucose nor a decrease of plasma free fatty acids (Table 5). A lack of increase in blood glucose by pyruvate administration is probably due to suppressed gluconeogenesis (10). However, xylitol administration effectively depressed the plasma free fatty acid level in the ethionine-treated rats without any marked increase of blood glucose. Presumably the effect of xylitol on a suppression of plasma free fatty acid levels would be related to its utilization for the synthesis of fatty acid in adipose tissue. As suggested previously, the conversion of 3-carbon units to glyceride-glycerol is possible in adipose tissue (42–44) despite a lack of glycerokinase (45, 46). However, the present results (Tables 4 and 5) suggest that the effect on reesterification of fatty acid would be relatively small and physiologically such a markedly increased supply of 3-carbon units would not be induced in adipose tissue. Therefore, as considered generally, the rise and fall of blood glucose is related to the fatty acid release. Thus, the present observations confirm the previous suggestion that hypoglycemia in ethionine-treated rats might be a basis for the accumulation of hepatic fat (47).

Despite the low K_m value for ATP of hepatic glycerokinase (48) (the hepatic AMP was also decreased in ethionine-treated rats[2]), the concentration of glycerol-3-phosphate in the liver of ethionine-treated rats was markedly decreased. From a comparison of the ethionine-treated animals with control animals, the decrease in

[1] Tani, H., and T. Itatsu. Unpublished data.

[2] Ogata, K., and H. Tani. Unpublished data.

glycerol-3-phosphate in the former may be attributed to suppression of gluconeogenesis (Table 10). Since most of the fatty acids accumulated as triglyceride even in ethionine-treated rats, the assimilation of fatty acids appears not to have been inhibited, as described previously (5). However, as suggested previously, the amount of triglyceride formation in the liver agrees with the increase of plasma free fatty acids (31, 32, 39, 49, 50). As suggested previously, glycerol-3-phosphate in the liver regulates the assimilation of fatty acids (33). However, according to Olivecrona's calculation (26) the increase in the amount of plasma free fatty acid taken up by the liver of ethionine-treated rats was small despite a marked increase of free fatty acids in both plasma and liver. Therefore, perhaps the esterification of fatty acids in the liver of ethionine-treated rats may be depressed by the marked decrease of glycerol-3-phosphate in the liver (Table 10). Glycerol-3-phosphate was increased by administration of glucose but triglyceride in the liver and plasma was not increased. Therefore, with very low levels of hepatic ATP, the primary effect of an increase in blood glucose is prevention of fatty acid mobilization. As shown in Tables 7 and 8, the incorporation of [1-^{14}C]-glucose or [3-^{14}C]pyruvate into glyceride-glycerol and fatty acids was depressed in the liver, and no marked increase of liver triglyceride was observed (Table 6) when ATP and glycerol-3-phosphate in the liver were markedly decreased. These results support the above speculation of the depressed assimilation of fatty acids and suggest a depressed synthesis of fatty acids. Thus, the observations in ethionine-treated rats support the hypothesis that maintenance of the glycerol-3-phosphate level is important for lipogenesis in both liver and adipose tissue. The suppression of gluconeogenesis affects the concentration of glycerol-3-phosphate in adipose tissue by depressing the level of blood glucose.

Not only does increased fatty acid release stimulate gluconeogenesis (51–54), but the increased formation of glucose may prevent the excessive release of fatty acids. The marked hepatic fat accumulation in ethionine-treated rats therefore may involve increased release of fatty acids from adipose tissue in the absence of regulation by increased glucose production as well as by disturbed transport of hepatic triglyceride (5–7). Such a possible feedback control of fatty acid mobilization appears to be physiologically important, as is a rapid output of triglyceride from the liver (4, 7, 30) from the viewpoint of a reasonable utilization of energy.

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