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Ethionine Administration in the Rat

1. EFFECTS ON THE LIVER AND PLASMA LIPIDS AND ON THE DISPOSAL OF DIETARY FAT

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Dyer (1938) showed that the administration of ethionine (α -amino- γ -ethylmercaptobutyric acid) to the rat decreased the growth rate of immature animals and caused a loss of body weight in the adult. Since then several other effects of the administration of ethionine to experimental animals have been established (see Farber, 1959). Two of these, a marked and rapid accumulation of triglyceride in the liver (Farber, Simpson & Tarver, 1950; Koch-Weser, Farber & Popper, 1951) and a fall in the concentration of the plasma lipids to very low values (Feinberg, Rubin, Hill, Entenman & Chaikoff, 1954; Furman, Norcia, Robinson & Gonzalez, 1957), suggest that ethionine may interfere with fat transport in the body. The present study was undertaken to obtain evidence on this point.

MATERIALS AND METHODS

Wistar-strain albino rats weighing 160–200 g. were fed on the laboratory stock diet, containing 4% of fat, throughout the experimental period. DL-Ethionine (25 mg./ml.) was given subcutaneously in 0.9% NaCl soln. Control animals were given corresponding injections of 0.9% NaCl soln. Chyle was collected by establishing fistulae in the thoracic ducts of normal rats which had been previously fed with 1 ml. of olive oil by stomach tube (French, Robinson & Florey, 1953). Labelled chyle was obtained by feeding rats, in which thoracic-duct fistulae had been established 24 hr. previously, with 20 μ C of glyceryl tri-[1- 14 C]palmitate dissolved in 1 ml. of olive oil. Chyle samples were collected in 3.8% (w/v) trisodium citrate soln., approx. 1 vol. of citrate being used for 9 vol. of chyle. The chyle was stored at 0° until needed. No samples were kept for more than a week before use.

Collection of blood and citrated plasma. Rats were usually bled from the abdominal aorta (French *et al.* 1953) and the blood was collected in 3.8% (w/v) trisodium citrate soln., 1 vol. of citrate being used for 9 vol. of blood. Citrated plasma was obtained by centrifuging the blood for 10 min. at 2500 *g* at 0°. In certain experiments blood samples were obtained from the cut end of the rat's tail, warmed with an infrared lamp to ensure a free flow of blood.

Intravenous injection of labelled chyle and measurement of blood radioactivity. The rats were maintained under ether anaesthesia throughout the experiment. They were injected with either 1 ml. or 0.25 ml. of labelled chyle (42 mg. of total esterified fatty acids/ml.) into an exposed femoral vein. The injections were completed in 30 sec. Blood samples (either 20.3 μ l. or 25.1 μ l.) were obtained with micro-pipettes from the cut end of the tail. The blood samples were transferred directly to aluminium planchets and mixed there with 0.1 ml. of Teepol (Shell Chemicals) diluted 1:1000 in water. The mixture was spread evenly over the planchet surface and a disk of lens tissue, the size of the planchet base, was placed on top. The sample was dried in air and the radioactivity counted to an error of $\pm 3\%$ with a thin mica end-window Geiger-Müller tube.

Measurement of tissue-lipid radioactivity. Animals injected intravenously with labelled chyle were killed by bleeding from the abdominal aorta. The liver and a sample (1 g.) of adipose tissue were removed from each animal, rinsed in 0.9% NaCl soln., dried on filter paper, weighed and stored at 0° until all the rats in a particular group had been killed. The sample of adipose tissue included perirenal fat and fat from the dorsal abdominal wall.

Each tissue was ground in a pestle and mortar with a mixture of sand, 5 ml. of water and 200 ml. of ethanol-ether (3:1, v/v). The resulting suspension was boiled, left for 48 hr. at room temperature, reboiled and filtered while hot. The residue was boiled with a further 100 ml. of ethanol-ether (3:1, v/v) and refiltered. The combined filtrates were decreased by evaporation to a suitable volume and the radioactivity was measured by plating samples directly on to aluminium planchets covered with lens paper. The samples were dried in air and the radioactivity was counted to an error of $\pm 2\%$ with a thin mica end-window Geiger-Müller tube. Self-absorption curves were drawn and all counts are corrected to zero mass.

Measurement of clearing-factor lipase activity. The amount of clearing-factor lipase which is released into the blood by the injection of heparin may be determined in several ways (Korn, 1959; Robinson & French, 1960). In the method used in this investigation the rate of clearing of a turbid triglyceride emulsion (chyle) by post-heparin plasma was measured *in vitro*. Such a method, though it does not give an absolute measure of lipase activity, is satisfactory for comparative studies when the same triglyceride substrate is used throughout.

Citrated blood samples were obtained from the tail veins of rats which had been injected with heparin. The blood was collected in ice-cold tubes and was centrifuged at 2500 *g* for 10 min. at 4°. The plasma which separated was incubated with chyle at 37° and the fall in turbidity during the first 20 min. was measured in a Hilger Spekker absorptiometer (neutral grey filter). Details of the technique have been described previously (French *et al.* 1953; Robinson & French, 1953). The heparin used was a commercial preparation (Pularin-Evans; 100 units/mg.).

Analytical determinations

Lipid extraction. Lipid extracts of liver were prepared as described for the extraction of tissue lipids in the experiments with labelled chyle. Lipid extracts of plasma were prepared by blowing samples (0.5 ml.) from a pipette into 10 ml. of ethanol-ether (3:1, v/v) so as to produce a finely divided protein suspension. This suspension was boiled, cooled to room temperature and filtered. The ppt. on the filter paper was washed with 2 ml. of ethanol-ether (3:1, v/v) and analyses were carried out on samples of the filtrate. When the plasma-lipid concentration was low, as in the animals which received ethionine, the size of the plasma sample which was extracted was increased to 1 ml.

Lipid analyses. The total esterified fatty acids present in the lipid extracts of plasma and liver were measured by the method of Stern & Shapiro (1953). Values are expressed as milligrams of esterified fatty acid, 280 being used as the fatty acid equivalent weight. Samples of the extracts were taken to dryness on a boiling-water bath and the lipids redissolved in 3 ml. of ethanol-ether (3:1, v/v). Total cholesterol was measured by the method of Pearson, Stern & McGavack (1953), applied either directly to samples (0.2 ml.) of plasma or to the residue obtained after drying suitable samples of the lipid extracts of liver *in vacuo*. Total phosphatide was measured by determining the organic phosphorus present in the lipid extracts of plasma and liver (Allen, 1940) and multiplying this value by 25. The method was modified to permit the analysis of samples containing as little as 5 μ g. of phosphorus by adjusting the quantities of reagents to allow colour development in a final volume of 10 ml. Absorption was measured at 750 μ in a Unicam spectrophotometer. The concentration of unesterified fatty acids in plasma was determined by the method of Dole (1956). A fatty acid equivalent weight of 280 has been used in expressing the analyses as milligrams of unesterified fatty acid. Precautions were taken to minimize the release of unesterified fatty acids *in vitro* by collecting the blood into ice-cold containers, by separating the plasma by centrifuging at 4° and by extracting the plasma as rapidly as possible after its separation.

Protein analysis. Samples of plasma (0.1 ml.) were blown from a micro-pipette into 4 ml. of 10% (w/v) trichloroacetic acid soln. The resulting protein suspension was centrifuged and the ppt. was washed twice with 4 ml. of 5% (w/v) trichloroacetic acid soln. Protein nitrogen was determined in the final ppt. by the micro-Kjeldahl procedure (Campbell & Hanna, 1937). The value obtained was multiplied by 6.25 to give the amount of protein. All analyses were carried out in duplicate and mean values are quoted in the results. Duplicate analyses did not differ by more than 5%.

RESULTS

Certain of the effects of ethionine administration reported by earlier workers (Farber *et al.* 1950; Jensen, Chaikoff & Tarver, 1951; Feinberg *et al.* 1954; Furman *et al.* 1957) have been confirmed under the conditions of this study. Thus in female rats the administration of ethionine results in a marked and rapid rise in the esterified fatty acid content of the liver (Tables 1 and 2). There may be a slight fall in the cholesterol content but the

Table 1. *Effect of ethionine on the total esterified fatty acids of rat plasma and liver*

Female rats were injected subcutaneously, at the times specified, with either 2 ml. of ethionine (25 mg./ml.) in 0.9 % NaCl soln. (ethionine group) or 2 ml. of 0.9 % NaCl soln. (control group). They were killed by bleeding from the abdominal aorta and the amounts of total esterified fatty acids in the plasma and liver were determined.

Rat no.	Time of successive injections (hr.)	Total amount of ethionine injected (mg.)	Time of killing (hr.)	Total esterified fatty acids			
				Plasma (mg./ml.)		Liver (mg./whole liver)	
				Ethionine	Control	Ethionine	Control
1	0, 2, 4	150	24	0.60	1.04	1110	237
2	0, 2, 4	150	24	0.46	1.46	485	197
3	0, 2, 4	150	24	0.31	1.38	990	190
4	0, 8, 24, 32, 48	250	52	0.32	1.05	1250	233
5	0, 8, 24, 32, 48	250	52	0.32	1.32	1480	226
6	0, 8, 24, 32, 48	250	52	0.24	1.46	980	216
7	0, 8, 24, 32, 48, 56, 72	350	76	0.17	1.28	—	—
8	0, 8, 24, 32, 48, 56, 72	350	76	0.06	1.07	—	—
9	0, 8, 24, 32, 48, 56, 72	350	76	0.10	1.30	—	—

Table 2. *Effect of ethionine on the total esterified fatty acids, phosphatide and cholesterol of rat plasma and liver*

Female rats were injected subcutaneously at 0, 24, 32 and 48 hr. with either 2 ml. of ethionine (25 mg./ml.) in 0.9 % NaCl soln. (ethionine group) or 2 ml. of 0.9 % NaCl soln. (control group). At 52 hr. they were killed by bleeding from the abdominal aorta and the amounts of lipids in the plasma and liver were determined.

Group	Rat no.	Wet wt. of liver (g.)	Total esterified fatty acids		Total cholesterol		Total phosphatide	
			Plasma (mg./ml.)	Liver (mg./whole liver)	Plasma (mg./ml.)	Liver (mg./whole liver)	Plasma (mg./ml.)	Liver (mg./whole liver)
Ethionine	1	5.6	0.47	1080	0.12	14.5	0.36	218
	2	5.4	0.44	1180	0.11	14.9	0.34	242
	3	5.8	0.44	725	0.12	14.5	0.43	240
Control	1	4.9	1.48	235	0.46	15.5	0.81	268
	2	5.2	1.28	210	0.35	15.4	0.66	224
	3	5.2	1.44	220	0.39	15.3	0.79	234

phosphatide concentration is unchanged. The rise in esterified fatty acid concentration therefore is confined essentially to the liver-triglyceride fraction.

The concentrations of esterified fatty acids, phosphatide and cholesterol in the plasma of female rats fall after ethionine administration (Tables 1 and 2). In an experiment in which serial plasma-phosphatide analyses were performed on individual rats (Fig. 1) a fall in the phosphatide concentration was detected within 3 hr. of the injection of ethionine and, after 24 hr., the level was less than 50 % of normal. Similarly, the concentration of the plasma esterified fatty acids falls to 50 % of normal 24 hr. after ethionine administration and, after further injections, decreases to about 20 % of normal at 52 hr. (Table 1). The studies were not sufficiently extensive to determine relationships between the effects observed and the amount of ethionine injected or the interval between injections.

Similar effects on the concentration of esterified fatty acids in the plasma and liver were produced

Fig. 1. Effect of ethionine administration on the phosphatide content of rat plasma. Female rats were injected subcutaneously at 0, 12 and 24 hr. with either (A) 2 ml. of a soln. of ethionine (25 mg./ml.) in 0.9 % NaCl soln. (Δ, □, ○) or (B) 2 ml. of 0.9 % NaCl soln. (▲, ■, ●). Phosphatide was determined in plasma separated from samples of blood (0.5 ml.) withdrawn at intervals from the tail.

by ethionine administration in male rats (Table 3). Though the increase in the liver-triglyceride concentration was less than in the female rats it was greater than that found in previous studies with male rats (Jensen *et al.* 1951; Farber, Koch-Weser & Popper, 1951), perhaps because of differences in the dosage of ethionine or in the strain of rats used.

Data on the unesterified fatty acid and protein concentrations in the plasma of normal and ethionine-treated female rats are given in Table 4. The unesterified fatty acid concentration, unlike that of the esterified fatty acid, was raised after ethionine treatment. The plasma-protein concentration fell.

Certain additional observations were made on the ethionine-treated rats. Gross and microscopic evidence of pancreatic damage, as reported by earlier workers (Farber & Popper, 1950; Goldberg Chaikoff & Dodge, 1950; Goldberg & Chaikoff, 1951), was evident after 24 hr. in the animals which were given repeated doses of ethionine. No excess of lipid could be detected in the pancreas by chemical analysis.

Previous studies have shown that after ethionine administration the food intake is reduced and the body weight falls (Dyer, 1938; Stekol & Weiss, 1949; Levine & Fopeano, 1953). The same was true under the conditions of the present study. There was also an increase in the amount of food material retained in the stomach and intestinal tract (Table 5).

Effect of ethionine on the response to fat-feeding and to heparin injection

During fat-absorption triglyceride enters the blood from the thoracic duct and causes a rise in the concentration of the plasma esterified fatty acids. The concentration of these acids in normal and ethionine-treated rats, before and after feeding with olive oil, is shown in Table 6. The rise in esterified fatty acid concentration after fat-feeding is similar in the normal and ethionine-treated animals. However, the response to a fat meal, as expressed in these terms, shows considerable individual variation and it is evident that only

Table 3. *Effect of ethionine on the total esterified fatty acids of plasma and liver in male and female rats*

Male and female rats were injected subcutaneously at 0, 24, 48 and 72 hr. with either 2 ml. of ethionine (25 mg./ml.) in 0.9 % NaCl soln. (ethionine groups) or 2 ml. of 0.9 % NaCl soln. (control groups). At 76 hr. they were killed by bleeding from the abdominal aorta and the amounts of esterified fatty acid in the plasma and liver were determined.

Group	Rat no.	Sex	Wet wt. of liver (g.)	Total esterified fatty acid	
				Plasma (mg./ml.)	Liver (mg./whole liver)
Ethionine	1	♂	5.6	0.42	340
	2	♂	5.4	0.46	367
	3	♂	7.1	0.61	306
Control	1	♂	5.8	0.88	190
	2	♂	4.9	1.00	170
	3	♂	5.2	0.93	215
Ethionine	4	♀	5.8	0.42	460
	5	♀	5.4	0.40	700
	6	♀	5.1	0.35	500
Control	4	♀	4.5	1.10	170
	5	♀	5.2	0.94	200
	6	♀	4.8	1.00	210

Table 4. *Effect of ethionine on the concentration of protein and unesterified fatty acids in rat plasma*

Female rats were injected subcutaneously at 0, 24, 48, 54 and 72 hr. with either 2 ml. of ethionine (25 mg./ml.) in 0.9 % NaCl soln. (ethionine group) or 2 ml. of 0.9 % NaCl soln. (control group). At 80 hr. the rats were killed by bleeding from the abdominal aorta and the concentrations of protein and of esterified and unesterified fatty acids in the plasma were determined.

Group	Rat no.	Total esterified fatty acids (mg./ml. of plasma)	Total protein (mg./ml. of plasma)	Unesterified fatty acids (mg./ml. of plasma)
Ethionine	1	0.45	55	0.28
	2	0.40	49	0.29
	3	0.51	43	0.23
Control	1	0.93	62	0.11
	2	1.10	67	0.18
	3	1.20	70	0.16

Table 5. *Effect of ethionine treatment on the body weight, food intake and weight of food material retained in the stomach and intestine of the rat*

Expt. 1. Female rats were injected subcutaneously at 0, 8, 24, 32 and 48 hr. with either 2 ml. of ethionine (25 mg./ml.) in 0.9% NaCl soln. (ethionine group) or 2 ml. of 0.9% NaCl soln. (control group). At 52 hr. the animals were killed and the stomachs, including the contents, were weighed. The food and water intake and the weight of the faeces, after drying in air, were also measured over the experimental period. *Expt. 2.* As above except that the rats were injected at 0, 4, 8 and 24 hr. and killed at 28 hr. The contents of the stomach and intestine of each rat were dried for 2 hr. at 100° and weighed.

Group	Rat no.	Change in body wt. (g.)	Food eaten (g.)	Water drunk (ml.)	Wet wt. of stomach and contents (g.)	Dry wt. of contents of stomach and intestine (g.)	Dry wt. of faeces (g.)
Expt. 1							
Ethionine	1	-5	10.5	25	4.6	—	3.3
	2	-8	7.5	30	3.3	—	4.5
	3	-11	10.5	13	2.4	—	5.5
Control	1	+2	20.0	25	1.2	—	10.0
	2	+7	20.5	28	1.5	—	7.5
	3	+4	35.5	37	2.3	—	17.0
Expt. 2							
Ethionine	1	—	8.5	12	—	3.25	1.7
	2	—	7.5	21	—	1.85	1.6
	3	—	8.5	—	—	1.45	2.5
Control	1	—	14.0	21	—	1.55	3.6
	2	—	14.0	16	—	0.85	4.8
	3	—	13.0	12	—	0.55	3.9

Table 6. *Changes in the concentration of plasma esterified fatty acids after fat-feeding in normal and ethionine-treated rats*

Female rats were injected subcutaneously at 0, 8, 24, 32 and 48 hr. with either 2 ml. of ethionine (25 mg./ml.) in 0.9% NaCl soln. (ethionine group) or 2 ml. of 0.9% NaCl soln. (control group). At 48 hr., after blood samples (1 ml.) had been taken from the tail, each rat was fed with 1 ml. of olive oil by stomach tube. At 53 hr. each rat was killed by bleeding from the abdominal aorta. The esterified fatty acid contents of the plasma samples were determined.

Group	Rat no.	Plasma esterified fatty acids (mg./ml.)	
		Before fat-feeding	After fat-feeding
Ethionine	1	0.43	0.73
	2	0.37	0.67
	3	0.32	0.50
Control	1	1.24	1.67
	2	1.44	1.69
	3	1.26	1.54

major differences in response in the two groups of rats would be detected.

The clearing activity of the plasma 3 min. after the injection of heparin into normal and ethionine-treated rats is shown in Table 7. The dosage of heparin used in this experiment was in the range where the release of clearing-factor lipase into the blood is proportional to the amount of heparin injected (Robinson, Jeffries & Poole, 1955). Under these conditions no effect of ethionine treatment on clearing activity could be demonstrated.

Effect of ethionine on the removal of injected ¹⁴C-labelled chylomicra from the blood

Recent studies have shown that when chylomicra, in which the triglyceride fatty acids have been labelled with ¹⁴C, are injected intravenously into normal rats the labelled fatty acid is trans-

Table 7. *Effect of ethionine treatment on the concentration of plasma clearing-factor activity after heparin injection*

Female rats were injected by the tail vein with 0.5 ml. of heparin (1 u./ml.) in 0.9% NaCl soln. and 1.5 ml. of blood was withdrawn from the tail 3 min. later. The rats were then injected subcutaneously at 0, 8, 24, 32 and 48 hr. with either 2 ml. of ethionine (25 mg./ml.) in 0.9% NaCl soln. (ethionine group) or 2 ml. of 0.9% NaCl soln. (control group). At 52 hr. the rats were injected as before with heparin and bled from the tail 3 min. later. The clearing activity of the post-heparin plasma samples was measured as described in the Materials and Methods section.

Group	Rat no.	Decrease in <i>E</i> in 20 min.	
		Before ethionine	After ethionine
Ethionine	1	0.27	0.26
	2	0.35	0.27
	3	0.27	0.39
Control	1	Before 0.9% NaCl soln. 0.32	After 0.9% NaCl soln. 0.32
	2	0.36	0.23
	3	0.26	0.25

ferred rapidly from the blood to the tissues (French & Morris, 1957, 1958; Bragdon & Gordon, 1958). A considerable proportion of the injected fatty acid appears in the liver. Experiments were carried out to determine whether ethionine administration, which results in an accumulation of triglyceride in the liver, would alter the rate of disappearance of the ^{14}C -labelled chyle from the blood. The radioactivity was measured in serial blood samples taken from normal or ethionine-treated rats after injecting labelled chyle.

In normal rats, disappearance curves of a composite nature were obtained, similar to those which French & Morris (1957) resolved into two exponential components. In Fig. 2 the percentage of the injected radioactivity remaining in the blood has been plotted against time on semilogarithmic co-ordinates for the first 20 min. after the injection of chyle. During this time over 75% of the radioactivity is removed from the blood in normal rats and the contribution of the slower exponential component to this phase of the disappearance curve is minimal. As in the experiments of French

& Morris the initial removal rate was found to depend on the amount of chyle injected. When 10.5 mg. of total esterified fatty acid was injected the mean circulating half-life ($t_{\frac{1}{2}}$) was 4 min. (Fig. 2A), and when 42 mg. of total esterified fatty acid was injected it was 6.6 min. (Fig. 2B).

The disappearance of radioactivity from the blood was much slower in ethionine-treated than in normal rats (Fig. 2). Thus the mean time for the removal of 25% of the injected dose rose after ethionine treatment from 1.7 to 5.8 min. after the injection of 10.5 mg. of esterified fatty acids, and from 2.5 to 20 min. after the injection of 42 mg. of esterified fatty acids. The mean proportionate increase in removal rate with a decrease in the amount of chyle injected was also greater in the ethionine-treated rats than in the normal rats. Thus $t_{\frac{1}{2}}^{1\text{ ml.}}:t_{\frac{1}{2}}^{0.25\text{ ml.}}$ was 1.5 for normal animals and 3.4 for ethionine-treated animals.

In rats given glucose as well as ethionine the concentration of liver triglyceride is raised but less than in animals given ethionine alone (Table 8). The rate of removal of injected chyle from the blood of such animals was intermediate between that in normal animals and that in ethionine-treated animals which had not been given glucose. No significant change in the rate of removal of injected chyle was produced by giving glucose to normal animals (Table 8), in agreement with the findings of Bragdon, Havel & Gordon (1957).

Effect of ethionine on the distribution in liver and adipose tissue of injected ^{14}C -labelled chylomicron fatty acids

Since the rate of removal of labelled chylomicron triglyceride from the blood was so markedly decreased in animals given ethionine it seemed possible that there might be an effect on the distribution of the injected fatty acids in the tissues after their disappearance from the blood. Control and ethionine-treated rats were injected with ^{14}C -labelled chylomicron triglyceride and the percentage of the injected radioactivity in the liver-lipid fraction was determined. The rats were killed 90 min. after the injection, when in both groups of animals the blood radioactivity had fallen to a low value. The data in Table 9 show that at this time there were no marked differences in the percentage of labelled material deposited in the liver although, since the numbers of animals in each group were small and the individual variation was considerable, the possibility of minor changes after ethionine treatment cannot be excluded. In further experiments rats were killed 30 min. after chyle injection. In these studies radioactive material was still present in the blood of the animals given ethionine, but the percentage of the radioactivity which had left the blood which was recovered from the liver

Fig. 2. Removal of ^{14}C -labelled chylomicron fatty acids from the circulation of normal and ethionine-treated rats. Six female rats were injected subcutaneously at 0, 16, 24, 40, 48 and 64 hr. with 2 ml. of ethionine (25 mg./ml.) in 0.9% NaCl soln. (ethionine group). At corresponding times six female rats were injected with 2 ml. of 0.9% NaCl soln. (control group). Five hr. after the last subcutaneous injection each rat was injected intravenously with either 0.25 ml. (A) or 1 ml. (B) of ^{14}C -labelled chyle (42 mg. of esterified fatty acid/ml.) and the fall with time in the radioactivity in the blood was followed as described in the Materials and Methods section. The symbols indicate the blood radioactivity in individual rats in each group. Ethionine-treated rats, Δ , $\square$, $\circ$: normal rats, $\blacktriangle$, $\blacksquare$, $\bullet$.

Table 8. *Effect of nutritional state on the distribution of radioactivity in rat liver and adipose tissue after the injection of labelled chylomicra*

Two groups of female rats (A and B, three animals/group) were injected subcutaneously with 2 ml. of ethionine (25 mg./ml.) in 0.9 % NaCl soln. at 0, 6, 12, 24, 30 and 48 hr. Two control groups (C and D) were injected with 0.9 % NaCl soln. at corresponding times. From 36 hr. onwards groups B and D were given 10 % (w/v) glucose soln. to drink instead of water. Five hr. after the last subcutaneous injection of ethionine each rat was injected intravenously with 1 ml. of labelled chyle (37 mg. of esterified fatty acid/ml.). The fall in the blood radioactivity was followed. After 40 min., the rats were killed and the livers and samples of adipose tissue were removed for analysis and for the determination of tissue radioactivity as described in the Materials and Methods section.

Rat no.	Treatment	Glucose taken in 12 hr. (g.)	Total esterified fatty acids in liver (mg.)	Percentage of injected activity removed from blood at		Percentage of activity disappearing from blood present in	
				20 min.	40 min.	Whole liver	Adipose tissue/g. wet wt.
A 1	Ethionine	—	1390	40	60	24	1.2
A 2		—	680	20	30	31	1.4
A 3		—	830	40	65	25	0.9
B 1	Ethionine + glucose	5	340	55	85	15	2.0
B 2		4.5	300	60	90	16	3.6
B 3		6	320	35	60	20	2.5
C 1	Control	—	240	75	90	33	0.7
C 2		—	250	80	95	32	0.4
C 3		—	190	75	95	25	0.3
D 1	Control + glucose	10	230	65	85	24	1.3
D 2		13	200	75	90	31	1.0
D 3		10	190	75	90	25	0.8

Table 9. *Recovery of ^{14}C -labelled fatty acids from the liver of ethionine-treated and control rats 90 min. after the injection of 1 ml. of ^{14}C -labelled chyle intravenously*

Female rats were injected as described in the legend to Fig. 2 and were bled 90 min. after the injection. The liver radioactivity was determined as described in the Materials and Methods section.

Group	Rat no.	Percentage of injected activity removed from the blood	Percentage of injected activity removed from the blood present in the liver
Ethionine	1	81	20
	2	89	32
	3	94	20
Control	1	98	30
	2	99	21
	3	99	24

was similar in the normal and ethionine-treated animals.

In the normal rat the distribution of injected ^{14}C -labelled chylomicron fatty acids in the tissues after their removal from the blood depends upon the nutritional state of the animal (Bragdon & Gordon, 1958). In the fasting animal, a large proportion appears in the liver and very little in the fat depots. In animals fed with carbohydrate in excess of their nutritional requirements, a high proportion is recovered from the fat depots and the

amount in the liver is much less. An experiment was carried out to determine whether the same was true for ethionine-treated rats.

Two groups of rats were fed with their normal diet and were given ethionine: two control groups were fed with their normal diet alone. One of the test groups and one of the control groups had access to glucose in their drinking water during the final 12 hr. of the experiment. The amount of glucose taken during this period was measured for each rat and was itself in excess of that needed to provide for the caloric requirements (Table 8).

^{14}C -Labelled chylomicra were injected intravenously into each rat 5 hr. after the final ethionine injection and 40 min. later the animals were killed. The percentage of the radioactivity which had disappeared from the blood and which was recovered in the liver and adipose tissue is shown in Table 8.

In both ethionine-treated and control rats the feeding of glucose increased the percentage of the radioactivity lost from the blood, which was recovered in 1 g. of adipose tissue. The percentage recovered in the liver fell after glucose feeding in the ethionine-treated animals: in the control animals the results with respect to the liver recovery were inconclusive.

The amounts of radioactivity per gram of adipose tissue cannot be compared in the ethionine-treated and control groups since after ethionine

administration the weight of adipose tissue in the body probably falls with the fall in the body weight (see Discussion).

It is assumed in setting out the results of these experiments that the amount of radioactivity contributed by blood in the tissues is negligible. This assumption is valid for normal rats and for the ethionine-treated rats which were given glucose, since in these groups 40 min. after the chyle injection the blood radioactivity was low. In the ethionine-treated rats which were not given glucose, injected chyle was removed from the blood more slowly and from 35 % to 70 % of the injected dose remained in the blood at 40 min.; although the animals were bled before the organs were removed, blood radioactivity may have contributed to the figure for tissue radioactivity in this group. Thus the effect of glucose feeding in raising the amount of radioactivity recovered in the adipose tissue of the ethionine-treated animals may be greater than indicated in Table 8.

The changes in tissue distribution are most simply interpreted as being due to differences in the sites at which the injected fat is removed from the blood. The possibility that they arise as a result of differences in the extent of oxidation or of redistribution of the fatty acids after their initial entry into the tissues cannot be entirely excluded since the rate of removal of the injected fat from the blood varied with the treatment given to the animals; the time available for such oxidation or redistribution therefore was not constant in the different groups.

DISCUSSION

The subcutaneous injection of ethionine in adult female rats causes a pronounced and rapid rise in the concentration of liver triglycerides and, at the same time, a marked fall in the concentration of plasma cholesterol, phosphatide and esterified fatty acids. These findings agree with those of earlier workers although a reduction in the serum-lipid concentration has not been reported previously in the rat. A discussion of the possible mechanisms whereby ethionine initiates these changes is given in the following paper (Robinson & Harris, 1961).

Previous work has shown that ethionine administration causes loss of body weight, decrease in food intake (Dyer, 1938; Stekol & Weiss, 1949) and damage to the submaxillary and gastric mucosal glands (Loring & Hartley, 1955) and to the pancreatic acinar cells (Farber & Popper, 1950; Goldberg *et al.* 1950). In the present study it was found that ethionine-treated animals ate less and retained more food material in the alimentary tract. These findings raise the possibility that the ethionine-treated animals are receiving insufficient

food for their caloric needs and that this may be a factor in the development of the fatty liver. In this connexion it is of interest that the concentration of unesterified fatty acids in the plasma, which rises in the fasting state (cf. Robinson & French, 1960), is elevated after ethionine administration (Table 4) and that the administration of glucose, together with ethionine, decreases the amount of triglyceride in the liver (Farber *et al.* 1950 and Table 8).

In the present study the administration of ethionine was shown to lead to a marked decrease in the rate of removal of injected chylomicron triglyceride from the bloodstream. It seems possible that the presence of excess of triglyceride in the liver may be at least partly responsible for this fall in removal rate after ethionine treatment since, in both normal and ethionine-treated rats, a considerable proportion of the injected fatty acids appears in the liver after leaving the blood. This view, that the presence of excess of triglyceride in the liver may limit its rate of uptake of additional injected chylomicron triglyceride, is supported by the observation that the increase in removal rate with decrease in dose was most marked in the ethionine-treated animals. Moreover, when the liver-triglyceride concentration was lowered by the administration of glucose, the rate of removal of injected chylomicron triglyceride from the blood increased. However, the increased uptake of injected chylomicron fatty acids by adipose tissue might contribute towards the enhanced removal rate under these conditions.

The failure to detect any differences between the ethionine-treated and normal rats in their ability to dispose of ingested triglyceride is probably due to the relatively slow rate of entry of dietary fat into the blood. Bergström, Borgström & Rottenberg (1952) found that the output of chylomicron triglyceride from the thoracic duct during fat absorption in the rat was only 1 mg./min. In the present study injected chylomicron triglyceride was removed from the blood more slowly in ethionine-treated than in normal rats but the difference between the groups was less marked when the amount of chylomicron fatty acid injected was decreased from 42 mg. to 10.5 mg. It is possible therefore that although the rate of uptake of chylomicron triglyceride by the liver is decreased after ethionine treatment it may still exceed the rate of entry of dietary fat into the blood. The further possibility that changes in the rate of absorption of a fatty meal may occur after ethionine treatment has not been studied.

In the experiments in which labelled chylomicron triglyceride was injected into rats on their normal diet no change in the percentage of the radioactivity lost from the blood which was

recovered from the liver could be detected after ethionine administration. It appears therefore that ethionine treatment does not appreciably affect the proportion of an injected dose of chylomicron fat which enters the liver. The percentage recovered from the liver was decreased when the ethionine-treated rats were given glucose and at the same time the amount which appeared in the adipose tissue was markedly increased. In similar experiments with normal rats the amount of radioactivity deposited in the adipose tissue was also increased after glucose administration (Table 8 and Bragdon & Gordon, 1958). These observations suggest that in ethionine-treated, as well as in normal, rats the tissue distribution of injected chylomicron fat is determined primarily by nutritional state.

SUMMARY

1. Some effects of the subcutaneous injection of between 150 and 350 mg. of DL-ethionine, over a period of between 1 and 3 days, have been studied in the rat.

2. The triglyceride content of the liver was increased to as much as five times the normal, whereas the phosphatide and cholesterol contents of the liver were essentially unchanged. The rise in liver-triglyceride concentration was greater in female than in male rats.

3. The plasma-protein concentration was decreased to 75% of the normal level. The plasma-triglyceride, -phosphatide and -cholesterol concentrations were decreased to between 10 and 50% of the normal values. The plasma unesterified fatty acid concentration was approximately doubled.

4. The rate of removal of injected ^{14}C -labelled chylomicron fatty acids from the circulation was decreased after ethionine treatment. The mean time of removal of 25% of an injected dose of 42 mg. of esterified fatty acids rose from 2.5 min. (normal) to 20 min. (ethionine-treated).

5. There was no marked effect of ethionine treatment alone on the percentage of labelled material deposited in the liver after the injection of ^{14}C -labelled chylomicron fatty acids. However, after feeding glucose to ethionine-treated animals, the uptake of label in the liver was decreased and that by the adipose tissue was increased.

6. No significant effects of ethionine administration on the rise in the concentration of plasma esterified fatty acids during fat absorption or on

the release of clearing-factor lipase into the blood after the intravenous injection of heparin were observed.

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