The Effect of Ethionine on Metabolism of Adenosine Triphosphate* (33888)

LIELA KOTITE[1] AND H. TARVER

Department of Biochemistry, University of California, San Francisco Medical Center, San Francisco, California 94122

Previous work which has been reviewed by Farber (1) and Stekol (2) has shown that the injection of ethionine into female rats leads to a reduction in protein synthesis in the liver. At the same time there is a reduction in ATP levels in the organ and it has been assumed that the reduction in synthesis is attributable to the lack of ATP. However, this is not necessarily the case because a higher rate of phosphorus turnover in ATP might compensate for a lower level of the energy transducer. Consequently, we have investigated the levels of adenine nucleotides and their turnover in liver after the injection of labeled phosphorus into both normal and ethionine-treated female rats.

Experimental Methods. Two groups of rats were used: Group I to establish the effect of ethionine on ATP levels in rat liver at different time intervals after the injection of ethionine, Group II to investigate the metabolism of adenine nucleotides in the livers of rats after the injection of ethionine. The experiments with Group I rats showed that ATP levels in the liver diminished to a minimum value at 4 hr after the injection of ethionine. Therefore, phosphorus turnover in the nucleotides was measured, over a period of 20 min, 4 hr after the injection of ethionine.

Animals. Female Sprague-Dawley rats weighing 150–200 g were employed after subjecting them to a 16-hr fast. They were injected intraperitoneally with 200 mg of ethionine in 8 ml of saline, divided into two doses, given with an interval of 1 hr between doses. Control animals were given saline. For the phosphorus turnover studies with the animals of group II, ^{32}P was injected intravenously as a 0.5-ml dose of 4×10^{-4} *M* sodium phosphate at pH 7 with an activity of 4.17×10^7 cpm.

Procedure. The animals were killed using a standard procedure designed to minimize losses in ATP (3). They were anesthetized with ether in a plastic bag containing pure oxygen. The liver was exposed and a lobe was squashed between two metal plates precooled in a dry ice–acetone mixture. A weighed sample was then homogenized in a precooled Teflon homogenizer with a cold solution of 0.8 *M* perchloric acid using 7 ml /g of liver. The acid-soluble supernatant obtained by centrifugation was neutralized with about one-tenth its volume of 7 *M* KOH. Potassium chlorate was removed by centrifugation and the supernatant was used for the determination of ATP (3) and other nucleotides, inorganic phosphorus (4) and radioactivity. For the measurement of the nucleotides 60–100 μl of the supernatant were required for the chromatographic method described below (5, 6). When only ATP levels were being measured a modified version of the firefly assay was employed (7). Radioactivity measurements were made with a liquid scintillation counter.

Chromatography. Glass plates (20 × 20 cm) were coated with a 0.5-mm thick layer of cellulose powder (MN-300) as follows: A 10% (w/v) polyethyleneimine hydrochloride solution (pH 5.5–6.0) was purified by dialysis against a large volume of distilled water for 20 hr. The solution was diluted to 1% and 20 g of cellulose powder were homogenized in 133 ml of the solution. Using a Desaga-Brinkmann applicator plates were coated with the suspension. The plates were allowed to dry overnight and then washed by ascending chromatography, 4 cm with 10% NaCl,

* Supported in part by National Institutes of Health Grant No. 5RO1-CA-02366-12 and by funds allocated by the Research Committee, School of Medicine, University of California.

[1] Present address: Department of Chemistry, American University of Beirut, Beirut, Lebanon.

TABLE I. Adenine Nucleotides in the Liver of Normal and Ethionine Treated Rats.[a]

Treatment[b]	ATP	ADP	AMP	Total	Inorganic phosphate
	(μmoles/g)				
Control (11)	1.58 ± 0.06	0.68 ± 0.05	0.55 ± 0.03	2.84 ± 0.08	3.37 ± 0.004
Ethionine (8)	0.38 ± 0.04	0.17 ± 0.02	1.52 ± 0.12	1.90 ± 0.05	5.87 ± 0.007

[a] Values given are means ± SD.

[b] Treatment: ethionine (200 mg) or saline given 4 hr prior to killing animals; all animals fasted 16 hr; number of animals used given in parentheses.

dried and then followed by distilled water. After redrying they were developed with water.

Five-μl samples of the nucleotides in a 2.5 m*M* solution were applied to the plates in a line 2 cm from the lower edge. With the dried plates 1 cm deep in a 0.2 *M* LiCl solution the solvent was allowed to ascend to the 2-cm line. A second solvent containing 1 *M* LiCl saturated with boric acid adjusted to pH 7.0 with concentrated NH_4OH was allowed to rise an additional 14 cm. After redrying, the plates were desalted by soaking in dry methanol for 15 min with occasional agitation (6). Chromatography was then carried out in the second dimension using 0.1 *N* acetic acid up to 4 cm from the origin followed by 0.3 *M* LiCl to 10 cm from the origin. The separation achieved in the first dimension was not good but became satisfactory in the second.

The nucleotides in the spots were transferred to paper wicks, using 75–100 μl of 2.0 *M* LiCl for the ATP and ADP and 50–75 μl of 1.0 *M* LiCl for the AMP. The wicks were dried, cut into small pieces into a centrifuge tube. The ATP and ADP were eluted overnight with 1 ml of 1.2 *M* LiCl and 10 μl of 0.125 *M* phosphate buffer at pH 7.0. The AMP was eluted with 0.012 *M* phosphate at the same pH. The optical densities of the eluates were measured at 260 mμ. Blanks were prepared from wicks made from parts of the papers adjacent to the spots. Recoveries of the nucleotides were almost quantitative.

Materials. Nucleotides (ATP, ADP, AMP) in the form of their sodium salts were obtained from Schwarz Bioresearch Inc. Radioactive phosphorus with a specific activity of 250 mCi/mmole was from New England Nuclear Corp. C. A. Brinkmann Co. was used as a source of materials for thin-layer chromatography.

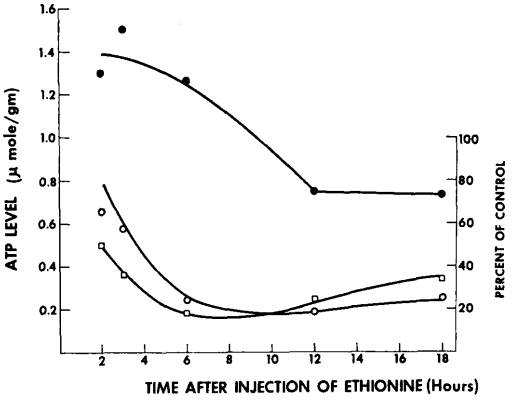

FIG. 1. ATP levels in the livers of control and ethionine-treated rats killed at intervals up to 18 hr after treatment: (●), control; (○), ethionine treated; (□), Percentage of control.

Results and Discussion. The data given in Fig. 1 show the changes in ATP levels which occur following the treatment of female rats with ethionine. There is first a rapid fall in ATP which reaches a minimum level 4–6 hr after the start of treatment. Thereafter, the ATP remains at a low level until the conclusion of the experiment after 18 hr. Although the persistent fast results in a lowering in ATP levels in the control animals the effect of ethionine is apparent over the whole period.

The fall in ATP after 4 hr is accompanied by a fall in ADP and a rise in AMP and inorganic phosphate (Table I). It is also clear that the concentration of total nucleotides containing adenine is much less in the ethionine-treated animals than in the controls. Twenty min after the injection of labeled phosphate the total phosphorus recov-

TABLE II. Uptake of Inorganic Phosphate-^{32}P by Livers of Normal and Ethionine-Treated Rats.[a]

No. of animals	Time after injection of ^{32}P (min)	Activity recovered (%/g)			
		Total	Inorganic	ATP	ADP
Control animals					
4	5	1.18 ± 0.13	0.94 ± 0.05	0.17 ± 0.005	0.027 ± 0.0007
4	7	1.38 ± 0.06	1.11 ± 0.07	0.21 ± 0.02	0.064 ± 0.001
3	10	1.75 ± 0.14	1.36 ± 0.15	0.32 ± 0.02	0.085 ± 0.02
4	15	1.44 ± 0.06	1.14 ± 0.07	0.25 ± 0.07	0.054 ± 0.006
4	20	1.89 ± 0.026	1.43 ± 0.15	0.38 ± 0.01	0.075 ± 0.001
Ethionine-treated animals					
4	5	1.25 ± 0.07	1.18 ± 0.06	0.05 ± 0.006	—
4	7	1.37 ± 0.19	1.26 ± 0.05	0.09 ± 0.003	0.016 ± 0.001
3	10	1.29 ± 0.06	1.23 ± 0.03	0.05 ± 0.01	0.009 ± 0.0004
4	15	1.48 ± 0.16	1.26 ± 0.12	0.20 ± 0.05	0.26 ± 0.001
4	20	1.62 ± 0.04	1.39 ± 0.22	0.16 ± 0.02	0.028 ± 0.0004

[a] Dose of ^{32}P; 4.17 × 10^7—ethionine (200 mg) given 4 hr before ^{32}P to animals fasted 16 hr. Control animals were given saline. Concentration (μmoles/g) of nucleotides in control and ethionine treated animals: ATP, 1.45 ± 0.06; ADP, 0.65 ± 0.04; AMP, 0.51 ± 0.06; inorganic phosphate, 3.93 ± 0.02; ethionine: 0.48 ± 0.04; 0.23 ± 0.06; 1.59 ± 0.04; 5.93 ± 0.3.

ered in the livers of the experimental and control animals are very similar being 1.6 and 1.9% of the dose, respectively (Table II). However, the percentage of the activity in the form of ATP in the controls is always more than twice that in the ethionine-treated animals in spite of the fact that the ATP levels in the controls are much higher than in the experimental animals. This is shown also in Fig. 2: the specific activities of the phosphorus in ATP in control animals are well above those in the animals given ethionine. Thus the control animals have more ATP in which the phosphorus turns over faster than that in the experimental animals. From these results it is concluded that the ethionine treatment leads to a reduction in the rate of ATP production which is probably due to a diminution of oxidative phosphorylation.

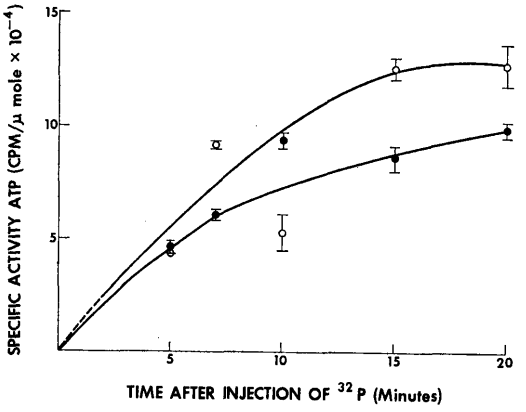

FIG. 2. Specific activities of ATP in normal and ethionine treated rat livers measured at intervals from 5 to 20 min following the injection of ^{32}P: (●), control; (○), ethionine treated.

Summary. When adult female rats are treated with ethionine, the metabolism of adenine nucleotides is changed as follows: (a) both ATP and ADP levels fall after 4 hr; (b) ATP remains at a low level for at least 18 hr; and (c) the turnover of P in the nucleotides is reduced.

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Received Feb. 3, 1969. P.S.E.B.M., 1969, Vol. 131.