Effect of Ethionine Treatment on the Activity of Enzymes concerned with Urea Synthesis in Rat Liver

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1. The activities of the enzymes of the urea cycle were measured in rat liver after feeding with a diet containing ethionine for 2 weeks. This treatment resulted in a decrease in the activity of ornithine transcarbamoylase, which fell to half the control value, while arginase increased by about 40%. The remaining enzymes of the urea cycle were unchanged at this time of treatment. These results are discussed in relation to the known effects of ethionine on nucleic acid synthesis. 2. The activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were measured in rat liver after feeding with a diet containing ethionine for 2 or 5 weeks. At the shorter time-period glucose 6-phosphate-dehydrogenase activity was more than doubled but 6-phosphogluconate-dehydrogenase activity remained unchanged. 3. The results are compared with similar measurements made after feeding with diets containing 4-dimethylamino-3'-methylazobenzene for 2 weeks. Striking similarities were found in the pattern of change in the enzyme activities of each of the pathways in the two treatments.

In a previous study measurements were made of the effect of carcinogenic azo-dyes on the activity of enzymes of the urea cycle in rat liver (McLean, Reid & Gurney, 1964). An early change then observed was a depression in activity of ornithine transcarbamoylase and an increase in the activity of arginase after 2 weeks of feeding with a diet containing 4-dimethylamino-3'-methylazobenzene. Measurements of the dehydrogenases of the pentose phosphate cycle at this same time-interval revealed that, while glucose 6-phosphate dehydrogenase had almost doubled in activity, 6-phosphogluconate-dehydrogenase activity remained unchanged. Thus striking changes had occurred in the enzymic pattern of two important metabolic cycles and these appeared to be related to the carcinogenic nature of the azo-dyes since non-carcinogenic derivatives of 4-dimethylazobenzene did not produce such alterations.

The extensive studies of Farber and his colleagues have established that ethionine is a potent carcinogenic agent producing a high proportion of liver tumours in rats (see Farber, 1963). Increases in urea, ammonia and α -amino nitrogen excretion have been observed in experiments with acute ethionine administration to rats (Wu, 1955). It was therefore decided to determine the early pattern of change of enzymes in the urea cycle and the pentose phosphate pathway of carbohydrate metabolism in ethionine-treated animals to determine if there was a common sequence of events in the response of liver to carcino-

genic agents of such different structure. The present results do, in fact, show markedly similar changes in the activities of the enzymes of the urea cycle and of glucose 6-phosphate dehydrogenase in liver after feeding rats for short periods with diets containing ethionine or 4-dimethylamino-3'-methylazobenzene.

METHODS

Materials. DL-Ethionine was obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. All other chemicals were as described by McLean *et al.* (1964).

Animals. Female albino rats (body wt. approx. 140 g.) were fed on a diet compounded of powdered rat cube diet 41B (Bruce & Parkes, 1949) and ethionine in aqueous solution so as to produce a paste in which the DL-ethionine concentration was 0.25%. This diet was fed *ad lib.* to the treated animals, and control animals were pair-fed with these experimental animals by using a similar diet but which contained no ethionine. Treatment was continued for 2 or 5 weeks.

Enzyme assay. Measurements were made of the five enzymes of the urea cycle, namely carbamate kinase (carbamoyl phosphate synthetase; ATP-carbamate phosphotransferase; EC 2.7.2.2), ornithine transcarbamoylase (carbamoyl phosphate-L-ornithine carbamoyltransferase; EC 2.1.3.3), argininosuccinate synthetase [L-citrulline-L-aspartate ligase (AMP); EC 6.3.4.5], argininosuccinate lyase (argininosuccinase; L-argininosuccinate arginine-lyase; EC 4.3.2.1) and arginase (L-arginine amidinohydrolase; EC 3.5.3.1), and the two dehydrogenases of the pentose phosphate cycle, namely glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate-NADP oxidoreductase; EC 1.1.1.49)

and 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate-NADP oxidoreductase; EC 1.1.1.44). The methods used were as described by McLean *et al.* (1964).

The activities of the urea-cycle enzymes were all expressed in terms of a unit defined as the amount of enzyme catalysing the formation of 1 μ mole of citrulline or urea/hr. at 37° under the conditions of assay. Glucose 6-phosphate-dehydrogenase and 6-phosphogluconate-dehydrogenase activities were expressed in units defined as the amount of enzyme catalysing the formation of 1 μ mole of NADPH/hr. at 20° based on the initial velocity during the first 5 min.

Statistical treatment. The results are given as means $\pm$ S.E.M. Fisher's *P* values are given and are considered significant if *P* was no greater than 0.05. Where *P* is greater than 0.1 the values are quoted as not significant (N.S.).

RESULTS

The changes in the activities of the urea-cycle enzymes after rats were fed on a diet containing ethionine for 2 weeks are shown in Table 1. The most marked change is the fall in ornithine-transcarbamoylase activity. This is decreased in the ethionine-treated group to approximately half the activity of the control group of rats. This enzyme is located in the mitochondrial fraction of the cell (Grisolia & Cohen, 1953) but it is clear that the activity of the other enzyme of the urea cycle also located in the mitochondria, carbamate kinase, does not change as a result of this treatment. The

other three enzymes of the urea cycle are located in the soluble fraction of the cell (Ratner, 1955) and of these only arginase is changed; this enzyme is increased by approx. 40% when expressed as units/g. of liver or as total units/100 g. body wt.

The activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were measured at two different time-intervals, when ethionine was fed for 2 weeks and after 5 weeks' treatment, and the results of these experiments are shown in Table 2. The shorter period of treatment resulted in a marked increase in glucose 6-phosphate-dehydrogenase activity, whereas 6-phosphogluconate-dehydrogenase activity remained unchanged. These results are in good agreement with those of Sie & Hablanian (1965), who found that glucose 6-phosphate-dehydrogenase activity was increased after feeding with a diet containing 0.5% of ethionine for only 4 days. In contrast with these short-term effects, when treatment with the diet containing ethionine was continued for 5 weeks glucose 6-phosphate-dehydrogenase and 6-phosphogluconate-dehydrogenase activities were unchanged (see Table 2).

DISCUSSION

One purpose of the present work was to examine the effects of ethionine on enzymes of the urea cycle

Table 1. *Effect of feeding with a diet containing ethionine for 2 weeks on the activities of rat-liver enzymes of the urea cycle*

Female rats were fed on a diet containing DL-ethionine (0.25%) for 2 weeks. Results are expressed as μ moles of citrulline or urea formed/hr. at 37° and are given as means $\pm$ S.E.M. Fisher's *P* values are given, but where *P* is greater than 0.1 the values are given as N.S. (not significant). Details are given in the Methods section.

	Control group	Ethionine-treated group	Fisher's <i>P</i>
No. of animals	8	8	
Initial body wt. (g.)	140 $\pm$ 5	132 $\pm$ 5	
Final body wt. (g.)	161 $\pm$ 5	131 $\pm$ 8	
Liver wt. (g.)	8.04 $\pm$ 0.70	6.39 $\pm$ 0.50	
Carbamate kinase			
Units/g. of liver	333 $\pm$ 22	336 $\pm$ 35	N.S.
Total units/100 g. body wt.	1640 $\pm$ 140	1630 $\pm$ 230	N.S.
Ornithine transcarbamoylase			
Units/g. of liver	14400 $\pm$ 1300	8240 $\pm$ 650	0.008
Total units/100 g. body wt.	70900 $\pm$ 5800	41200 $\pm$ 4800	< 0.001
Argininosuccinate synthetase			
Units/g. of liver	100 $\pm$ 8	95 $\pm$ 7	N.S.
Total units/100 g. body wt.	490 $\pm$ 42	460 $\pm$ 30	N.S.
Argininosuccinate lyase			
Units/g. of liver	273 $\pm$ 13	300 $\pm$ 24	N.S.
Total units/100 g. body wt.	1360 $\pm$ 136	1470 $\pm$ 152	N.S.
Arginase			
Units/g. of liver	93000 $\pm$ 6500	135000 $\pm$ 20000	0.06
Total units/100 g. body wt.	464000 $\pm$ 32000	634000 $\pm$ 174000	0.05

Table 2. *Effect of feeding with a diet containing ethionine for 2 or 5 weeks on the activities of rat-liver glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase*

Female rats were fed on a diet containing DL-ethionine (0.25%) for 2 or 5 weeks. Results are expressed as μ moles of NADPH produced/hr. at 20° and are given as means $\pm$ S.E.M. Fisher's *P* values are given, but where *P* is greater than 0.1 the values are given as N.S. (not significant). Details are given in the Methods section.

	Control group	Ethionine-treated group	Fisher's <i>P</i>
Treated with ethionine for 2 weeks			
No. of animals	6	6	
Initial body wt. (g.)	146 $\pm$ 4	146 $\pm$ 6	
Final body wt. (g.)	156 $\pm$ 10	126 $\pm$ 15	
Liver wt. (g.)	7.07 $\pm$ 0.51	5.81 $\pm$ 0.07	
Glucose 6-phosphate dehydrogenase			
Units/g. of liver	98 $\pm$ 9	230 $\pm$ 20	< 0.001
Total units/100 g. body wt.	440 $\pm$ 30	1100 $\pm$ 130	< 0.001
6-Phosphogluconate dehydrogenase			
Units/g. of liver	95 $\pm$ 10	98 $\pm$ 5	N.S.
Total units/100 g. body wt.	426 $\pm$ 38	466 $\pm$ 37	N.S.
Treated with ethionine for 5 weeks			
No. of animals	5	5	
Initial body wt. (g.)	150 $\pm$ 5	149 $\pm$ 6	
Final body wt. (g.)	183 $\pm$ 8	143 $\pm$ 17	
Liver wt. (g.)	8.57 $\pm$ 0.66	6.40 $\pm$ 0.54	
Glucose 6-phosphate dehydrogenase			
Units/g. of liver	127 $\pm$ 9	131 $\pm$ 8	N.S.
Total units/100 g. body wt.	596 $\pm$ 50	594 $\pm$ 30	N.S.
6-Phosphogluconate dehydrogenase			
Units/g. of liver	106 $\pm$ 8	108 $\pm$ 4	N.S.
Total units/100 g. body wt.	556 $\pm$ 60	487 $\pm$ 12	N.S.

and the pentose phosphate pathway and to compare these changes with those produced by other hepatocarcinogens such as the azo-dyes (McLean *et al.* 1964). Reid (1962) and Farber (1963) have stressed the difficulties in interpreting biochemical changes in liver induced by hepatocarcinogens because of changes in the relative proportions of parenchymal tissue and bile-duct tissue. It appears that the early stages of treatment with diets containing either ethionine or the carcinogenic azo-dye 4-dimethylamino-3'-methylazobenzene produce strikingly similar changes in the pattern of enzyme activities of these two pathways (see Fig. 1). These results are combined data from the present study with ethionine and from previous work with 4-dimethylamino-3'-methylazobenzene (McLean *et al.* 1964). Treatment with the non-carcinogenic azo-dyes 4-dimethylamino-4'-methylazobenzene or 4 - dimethylamino - 2 - methylazobenzene did not cause any alteration in the activities of the enzymes of the urea cycle (McLean *et al.* 1964).

The relative changes of carbamate-kinase and ornithine-transcarbamoylase activities in the ethionine-treated group of rats are such as to favour an increased availability of carbamoyl phosphate, a precursor of nucleic acids. A depressed activity of

ornithine transcarbamoylase and an increased activity of carbamoylaspartate transcarbamoylase have been observed in conditions of increased nucleic acid formation such as regenerating liver and certain hepatomas (Cohen & Sallach, 1961; Ono, Blair, Potter & Morris, 1963). In this context the work of Turner & Reid (1964) indicating a stimulation of RNA synthesis in liver after feeding rats with ethionine for 1-3 weeks is noteworthy and the present changes in ornithine-transcarbamoylase activity might be correlated with this change. The effect of ethionine on nucleic acid synthesis appears to be somewhat dependent on the method of treatment, i.e. whether acute or chronic administration is used. Farber, Shull, Villa-Trevino, Lombardi & Thomas (1964) observed an inhibition of RNA synthesis a few hours after the injection of ethionine.

The early increase in liver glucose 6-phosphate-dehydrogenase activity found after 4 days' treatment with ethionine (Sie & Hablanian, 1965) and after 2 weeks' treatment appears to be lost after 5 weeks' treatment (see Table 2). There are several examples of alterations in enzyme activities that persist during several weeks of feeding with carcinogen only to regress and finally reappear in

Fig. 1. Effect of feeding with diets containing ethionine (a) or 4-dimethylamino-3'-methylazobenzene (b) on the activities of enzymes of the urea cycle and the dehydrogenases of the pentose phosphate cycle in rat liver. Experimental animals were fed on diets containing DL-ethionine (0.25%) or 4-dimethylamino-3'-methylazobenzene (0.06%) for 2 weeks; control animals received the same diet without the carcinogens. The enzyme activities of the experimental groups, as units/g. of liver, are expressed as percentages of the corresponding control values (broken horizontal lines). The solid columns represent the enzymes of the urea cycle: A, carbamate kinase; B, ornithine transcarbamoylase; C, argininosuccinate synthetase; D, argininosuccinate lyase; E, arginase. The hatched columns represent the dehydrogenases of the pentose phosphate pathway: F, glucose 6-phosphate dehydrogenase; G, 6-phosphogluconate dehydrogenase. The results on the ethionine-treated rats (a) are from the present study; those on the azo-dye-treated rats (b) are from the work of McLean *et al.* (1964).

the hepatoma (see Reid, 1962). It would seem that glucose 6-phosphate dehydrogenase in ethionine-treated rats is another such example since in transplanted hepatomas induced with ethionine there is a threefold increase in glucose 6-phosphate-

dehydrogenase activity (McLean & Brown, 1966). There is an increase in the activity of glucose 6-phosphate dehydrogenase but not 6-phosphogluconate dehydrogenase as early as 2-4 weeks of feeding with a diet containing carcinogenic azo-dyes (Nodes & Reid, 1963; McLean *et al.* 1964), again a marked similarity with the early effects of ethionine treatment. These early changes in glucose 6-phosphate-dehydrogenase activity perhaps assume greater significance when considered in conjunction with the survey made by Weber, Henry, Wagel & Wagel (1964) of a number of biochemical parameters in a range of transplanted hepatomas of different growth rates, where it was found that, in general, glucose 6-phosphate-dehydrogenase activity was increased.

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