Effect of Ethionine on Histidine Metabolism.* (28388)

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Recent observations(1-3) of children with histidinemia, in whose tissues the enzyme histidase is absent due to a genetic defect, have led to a consideration of procedures which might reproduce the effects of the disorder in animals. As it has not been possible specifically to inactivate histidase *in vivo*, attention was drawn to urocanase, the enzyme which catalyzes the second step in the straight deamination pathway of histidine catabolism. Silverman, Gardiner and Bakerman(4) found that rats fed 0.05% of DL-ethionine excreted considerable amounts of urocanic acid in the urine and had low levels of liver urocanase. Recently it has been shown(5,6) that rats adapted to diets which contained ethionine; *i.e.*, after an initial loss of weight, animals fed sub-lethal amounts of the antagonist grew at rates comparable to those of control rats.

To inquire into the specificity of the ethionine-effect and to determine whether changes in the levels of enzymes of the alternative transamination pathway for the degradation of histidine accompany a block in the straight deamination pathway, levels of liver urocanase, histidase and histidine-pyruvate transaminase were measured in ethionine-fed rats at regular time intervals during the pre-adaptive stage and the period of subsequent growth.

Experimental. Male rats of the Sprague-Dawley strain (initial weight 82-98 g) were divided into 3 groups of 22 rats each. The basal diet for the animals in the *ad libitum*-fed and pair-fed groups contained casein, 9%; salts(7), 5%; corn oil plus fat-soluble vitamins(8), 5%; water-soluble vitamin supplement(8), 0.25%; choline chloride, 0.15%, and sucrose as the carbohydrate to make 100%. In the diet for the ethionine-fed group, 0.25% of DL-ethionine was added in place of an equivalent amount of sucrose. One rat from each group was killed daily starting on the first day after initiation of the experimental diets (except for the 18th and 19th days). The initial body weights of the 3 rats killed on a same day differed by less than one gram.

Histidase, urocanase and histidine-pyruvate transaminase were measured as described previously(9) in supernatant fractions obtained by high-speed (105,000 $\times$ *g*) centrifugation of 20% homogenates of the liver from each animal. When necessary, 2 levels of supernatant were assayed to insure that the reaction velocity was proportional to enzyme concentration. The protein content of the supernatant fractions from the homogenates was measured by the method of Lowry *et al.* (10). The DNA contents were determined in an aliquot of the whole homogenate by the hot TCA extraction procedure of Schneider(11); the extracts were analyzed for DNA by the diphenylamine(12) reaction.

One unit of enzyme activity was defined as the amount of enzyme which catalyzed the destruction of appropriate substrate or formation of product at the rate of one mμmole per minute under the conditions used. The data were calculated on the basis of units per 0.1 μmole of DNA, per mg of supernatant protein, per gram of fresh liver and per 100 g of rat. Plots of activity *vs* days of ingestion of the antagonist gave the same pattern of changes in all cases; for convenience, the enzyme activities are recorded as units per 0.1 μmole of DNA.

Results. The ethionine-fed rats lost weight for the first 5-7 days, then grew at the same rates as animals fed the control diet *ad libitum*. Most of the pair-fed rats grew at rates similar to those of the corresponding ethionine-fed animals. The growth curves of the rats killed on the last (24th) day of the experiment are shown in Fig. 1, top. The variation in body weight with time of ingestion of the antagonist shown for the ethionine-fed rat illustrates the growth status of other ethionine-fed rats killed daily throughout the experiment.

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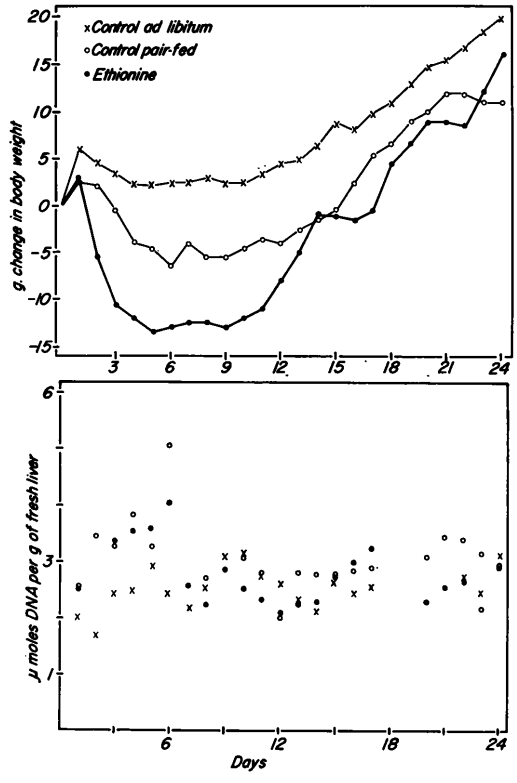

FIG. 1. Adaptation to dietary ethionine. Top: Growth curves of 3 rats killed on 24th day of experiment described in text. The curve for the ethionine-fed rat shows the typical adaptation to the antagonist. Growth curve during days 1-13 for pair-fed control animal shown was exceptional. Most of the pair-fed animals gained or lost weight at same rate as did corresponding ethionine-fed rat. Bottom: Concentration of DNA in liver of rats fed the basal diet or 0.25% DL-ethionine diet for number of days indicated.

It has been observed that when rats are fed diets which contain sublethal levels of ethionine for more than 4-5 weeks, an increase in DNA content of their liver occurs (13,14) concomitantly with proliferation of ductular cells and a relative decrease in number of hepatocytes(15,16). In the present experiment, there was a relative increase in DNA concentration of the livers of the ethionine-fed and pair-fed control animals during the first 6 days of the experimental period (probably as a result of the reduced food intake and subsequent loss of liver glycogen). After 6 days, however, when the appetite of the ethionine-fed rats was restored to normal, there were no significant differences in the DNA content of the livers of the animals of the 3 groups (Fig. 1, bottom). Thus, the present measurements were made before the severe pathological damage, characteristic of longer-term ethionine administration, had occurred.

Levels of urocanase in the livers of the rats fed the experimental diets for periods of from one to 24 days are shown in Fig. 2, top, and may be compared with the levels of histidase and histidine-pyruvate transaminase (Fig. 2, center and bottom). Urocanase activity decreased during the first 8 days of ethionine feeding and remained at very low levels thereafter. There was no indication of restoration of urocanase activity during the period of subsequent growth adaptation of the animals. Administration of ethionine did not significantly alter the activities of liver histidase or histidine-pyruvate transaminase. While urocanase activities in livers of the control rats and histidase activities in the livers of all 3 groups of animals did not show significant changes, histidine-pyruvate transaminase levels in livers of all 3 groups decreased toward the end of the experimental period. It appears, therefore, that there may be an inverse relationship between the level of histidine-pyruvate transaminase in the liver and rate of growth.

Discussion. The specific and rapid decrease in urocanase activity following ingestion of ethionine is remarkable. In the present experiments, if the action of the antagonist were to inhibit protein synthesis in general, either by inhibiting the incorporation of the natural amino acid into proteins(17) or by producing a rapid fall in concentration of ATP(18), one might expect a similar decrease in the levels of other enzymes studied unless the half-life of urocanase is phenomenally shorter than those of histidase and histidine-pyruvate transaminase (and of the proteins required for growth). Alternatively, if ethionine were incorporated into the enzyme-protein in lieu of methionine, as has been shown to occur in other proteins(17), replacement of a methionine residue by ethionine at the active site of urocanase, or at some critical location which prevents proper orientation of the molecule, might result in formation of an inactive form of the enzyme. It would follow that methionine residues are

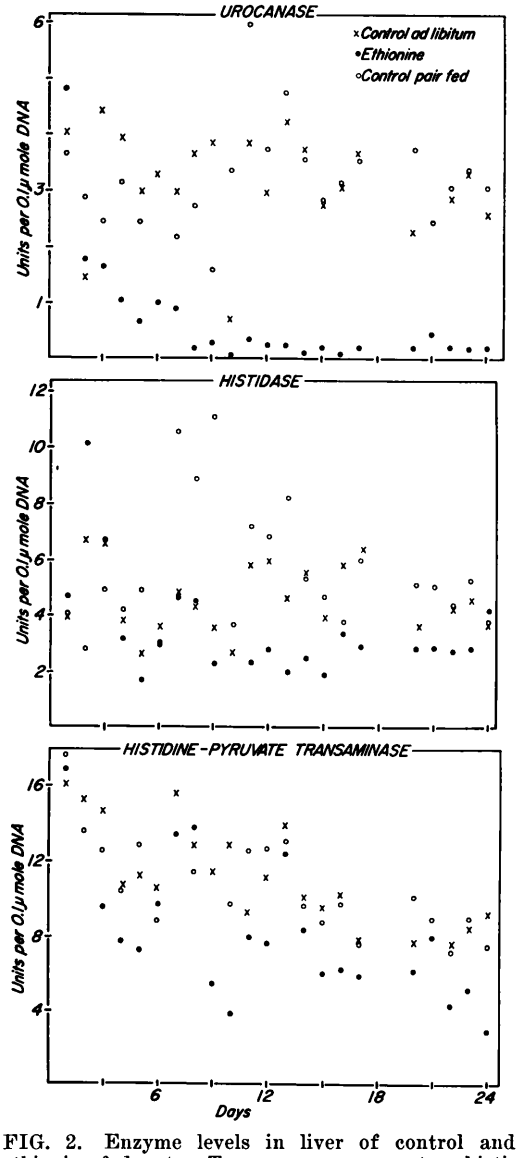

FIG. 2. Enzyme levels in liver of control and ethionine-fed rats. Top, urocanase; center, histidase; bottom, histidine-pyruvate transaminase. Rats were fed the control diet or a diet which contained 0.25% DL-ethionine for number of days indicated, were then killed and enzyme activities determined in homogenates of their livers. See text for definition of units used.

not present at the active centers of histidase and histidine-pyruvate transaminase. Other possibilities are alkylation by S-adenosyl-L-ethionine of some catalytically important functional grouping(s) on the urocanase molecule or alkylation of the substrate (urocanic acid).

It is of interest that liver urocanase is sensitive to a variety of factors other than ethionine; urocanase activity has been shown to be low in folic acid-deficient(19) and pantothenic acid-deficient animals(20) in the absence of any demonstrable cofactor role of the respective vitamins in the urocanase reaction; it is also low in adrenalectomized rats.† Excessive amounts of urocanic acid are excreted in the urine by persons in hepatic coma(21), and by children with kwashiorkor(22). Forced feeding of a kwashiorkor-inducing (maize) diet to rats for 3 days is followed by a precipitous fall in liver urocanase levels(23).

As the rats in the present experiment grew at a rapid rate in the absence of demonstrable urocanase activity in the liver, it appears that the histidase pathway of histidine catabolism, and products produced therein such as formimino-glutamic acid, are not essential for survival or growth of the rat.

Summary. Ethionine administration resulted in rapid and essentially complete inhibition of liver urocanase activity in the rat, while the activities of histidase and histidine-pyruvate transaminase were relatively unaffected. Rats receiving ethionine in their diet, after showing a severe weight loss during the first week, resumed a rate of growth parallel to that of the control animals; there was, however, no restoration of urocanase activity during the period of adaptation to dietary ethionine. Thus, the straight deamination pathway for the degradation of histidine is not essential for the growth of the rat.

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† R. C. Baldridge and R. Burket, unpublished.

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Antagonism of Hydrocarbon Anesthetic-Epinephrine Arrhythmias in Dogs by Nethalide, a Dichloroisoproterenol Analogue. (28389)

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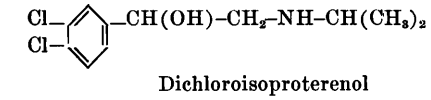
The synthesis of nethalide,* an analogue of the specific beta receptor antagonist dichloroisoproterenol(1), which differs from the latter in being free of intrinsic sympathomimetic activity(2), makes available a better pharmacological tool for studying adrenotropic receptors of the beta type(3). It was the purpose of the present study to utilize this compound in an effort to assess the importance of beta receptors in the mediation of hydrocarbon anesthetic-epinephrine arrhythmias.



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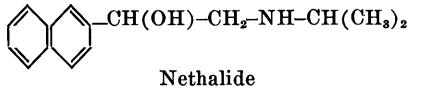

Nethalide

Methods. Unpremedicated dogs weighing 10-15 kg were given sufficient thiopental sodium (15-25 mg/kg) intravenously to allow tracheal intubation to be performed. An anesthetic mixture was then administered by means of a semi-closed system with a to and fro carbon dioxide absorber (recharged every 2 hours) and the animals allowed to breathe spontaneously throughout the experiment. With cyclopropane a flow rate of 600 ml/min (500 ml oxygen + 100 ml cyclopropane) was maintained, giving a concentration of approximately 16%. Concentrations of trichloroethylene and halothane (Fluothane†) which would produce myocardial sensitization were experimentally determined but actual vapor concentrations were not measured. For a 2 l/min oxygen flow rate, these were

* Nethalide is the generic name for (2-isopropylamino-1-[2-naphthyl]ethanol HCl) which was earlier referred to as I.C.I. 38,174 and now also has the trade name Alderein by Imperial Chemical Industries, Ltd., England. In addition, Ayerst Laboratories, who generously supplied the drug for this study, have employed the designation AY-6204.

† Ayerst Laboratories.