Liver Arginase, Histidase and Phenylalanine Transaminase Following Administration of their Respective Substrates.* (23960)

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Several enzyme activities that catalyze the first step in catabolism of certain amino acids increase after administration of their substrates, for example, tryptophan peroxidase-oxidase(1), tyrosine (α-ketoglutarate) transaminase(2), phenylalanine (α-ketoglutarate) transaminase (E.C.C. Lin, personal communication) and threonine dehydrase(3). Arginase activity increases when the protein content of the diet is increased(4,5,6) although feeding of arginine itself in high amounts does not alter this enzyme level(7). L-Amino acid oxidase levels can also be increased by administration of amino acids(8). Koizumi(9) administered large doses of L-histidine to rabbits prior to isolation of histidase. No reason was given for this procedure, which led Knox (1) to the discovery of adaptive nature of tryptophan peroxidase-oxidase. We recently observed(10) that phenylalanine (pyruvate) transaminase activity of livers of leukemic rats was directly related to the blood level of phenylalanine. Experiments were designed to ascertain whether arginase, histidase and phenylalanine (pyruvate) transaminase adapt to their respective substrates, since this would be of considerable theoretical interest.

Materials and methods. Albino rats (Holtzman) weighing 100 g (250-300 g in the arginase experiment) and white or Himalaya rabbits weighing approximately 2 kg were used. Test substance was administered *via* intraperitoneal injection in the following dosages: L-arginine in solution adjusted to pH 7, 0.75 mmole/100 g body weight; L-histidine in solution adjusted to pH 7, 1 g/kg body weight of rat and 0.5 g/kg body weight of rabbit; L-phenylalanine 0.5 mmole/100 g body weight. This dose of histidine was the same as that used by Koizumi(9), and the dose of phenylalanine was sufficient to increase plasma phenylalanine by 100%, an amount comparable to elevation observed in leukemic rats(10). The arginine dosage was chosen to fall within this same general range, *i.e.* 0.3 to 1.0 mmole/100 g body weight. Five hours after injection, all animals were decapitated. Livers of normal and treated animals were excised, weighed, and 5 g portions were homogenized for 2 minutes in Waring blendor, fitted with stainless steel microhead, with 7 volumes of 0.14 N KCl containing 0.0025 N NaOH. Homogenates were centrifuged in the high speed attachment of International Refrigerated Centrifuge, Model PR-2, for 45 minutes at 25,000 x *g*. The supernatant fractions were then analyzed for enzyme activity. Arginase activity was determined at pH 9.1 in the presence of 2.5×10^{-4} M Mn^{++} ion. The urea produced was determined according to Archibald(11). Histidase activity was measured by direct spectrophotometric method of Tabor and Mehler(12). Phenylalanine (pyruvate) transaminase assays were carried out using borate-arsenate method of Lin(13). All assays were performed at 38°C. Dry weights of homogenates, corrected for KCl in the homogenizing fluid, were obtained on 5 ml samples dried for 2 hours at 110°C in tared aluminum moisture dishes. Data are expressed in terms of μmoles product formed/hr/g dry wt.

Results. It is readily apparent from the data of Tables I and II that none of the enzymes tested responded with any significant change to administration of their respective substrates. Changes in arginase activity as a function of protein intake of animals(4,5,6) may be a reflection of stimulation of the urea cycle with increased nitrogen load. However, it is known that of all enzymes of the urea cycle, arginase is present in amounts which are not rate-limiting(14). It is possible that injection of histidine in Koizumi's work(9)

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TABLE I. Liver Histidase following Histidine Injection.

		Histidase, μmoles urocanate/g/hr
Rat	Control, ♂	54.0 (3)*
	Treated, ♂	52.1 (4)
	Control, ♀	50.4 (3)
	Treated, ♀	45.0 (3)
Rabbit	Control, ♂	12.4 (1)
	Treated, ♂	9.6 (2)
	Control, ♀	14.8 (2)
	Treated, ♀	10.0 (1)

* Values in parentheses indicate No. of animals used.

led to stabilization of the enzyme. The lack of change in the phenylalanine (pyruvate) transaminase activity following phenylalanine administration indicates that the observed changes of this enzyme in leukemic rats(10) were not a result of changes in the circulating phenylalanine levels but were the direct consequences of the leukemic state.

It would thus appear that although the mechanism of substrate-induced adaptation of certain amino acid catabolizing enzymes may be the means by which certain of the circulating amino acid levels are regulated, this mechanism cannot be the one which is generally used by the animals for homeostatic regulation of *all* circulating amino acid levels, or we would have observed adaptive changes in the arginase, histidase and phenylalanine transaminase levels after the administration of their respective substrates.

TABLE II. Liver Arginase and Phenylalanine (Pyruvate) Transaminase following Arginine and Phenylalanine Injection.

Rat	Arginase, μmoles urea/g/hr	Phenylalanine (pyruvate) transaminase, μmoles phenyl-pyruvate/g/hr
Control, ♂	61,300 (3)*	218 (3)
Treated, ♂	73,600 (3)	207 (4)

* Values in parentheses indicate No. of animals used.

Summary. Rat liver arginase, histidase and phenylalanine (pyruvate) transaminase as well as rabbit liver histidase did not increase following injection of their respective amino acid substrates.

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