Chapter V

Comparative studies on the activities of certain enzymes of amino acid metabolism in normal, neoplastic, newborn, and regenerating liver

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As pointed out in the preceding chapter, comparative data on the amino acid utilization of normal liver and hepatoma cultures are suggestive of underlying differences in the metabolism of a number of amino acids including arginine and glutamine. The present studies were directed towards further investigations on the metabolism of these two amino acids in normal and neoplastic liver. As newborn and regenerating liver were also found to show an increase in glutamine utilization over normal tissue, the studies were extended to these tissues as well.

For this purpose, studies were made of the activities of arginase, glutaminase I, glutamine synthetase, glutamyltransferase and glutamic-pyruvic transaminase in the different tissues referred to. Details of these investigations are recorded in this chapter.

Experimental

Preparation of enzyme extracts

The methods for the isolation of the tissues and the

preparation of the homogenates have been described in Chapter III. KCl extracts were used for assaying arginase and glutaminase I, while bicarbonate extracts were used for assaying glutamine synthetase, transferase and glutamic-pyruvic transaminase.

Enzyme assay

Arginase: Arginase activity was determined by measuring the disappearance of arginine. The reaction mixture consisted of : tris buffer, pH 9.0, 50 micromoles; L-arginine hydrochloride, 10 micromoles; enzyme extract; and water to a total volume of 2.0 ml. The mixture was incubated at 37° for 15 minutes and the reaction stopped by the addition of 1.0 ml. of 95% ethanol after which the tubes were kept in a warm water bath for 5 minutes. The precipitated protein was removed by centrifugation and the left over arginine determined in the supernatant by the circular paper chromatographic method using butanol-acetic acid-water (4:1:5) as the solvent system. The reaction in the blank was stopped immediately after the addition of enzyme.

A unit of enzyme activity is defined as the amount of enzyme which causes the disappearance of one micromole of arginine in 15 minutes at 37°, and specific activity, as units per milligram protein.

Glutaminase I : The enzyme was assayed in presence of phosphate at pH 8.0. The disappearance of glutamine was followed chromatographically.

The reaction mixture consisted of : tris buffer, pH 8.0, 30 micromoles; L-glutamine, 5.0 micromoles; phosphate as phosphate buffer, pH 8.0, 50 micromoles; enzyme extract; and water to a total volume of 3.0 ml. The incubation was carried out at 37°. The reaction was stopped after 0 and 30 minutes by adding one ml. of 95% ethanol to the assay tubes and keeping them in a warm water bath for 5 minutes. The precipitated protein was removed by centrifugation and the left over glutamine in the supernatant determined by circular paper chromatography using butanol-water-acetic acid (40:7:5) as the solvent system.

A unit of enzyme activity is defined as the amount of enzyme which deamidates one micromole of glutamine in 30 minutes at 37°, and specific activity, as units per milligram protein.

Glutamine synthetase : Glutamine synthetase activity was determined by the method described by Gothoskar *et al.* (199).

The reaction mixture consisted of : tris buffer, pH 7.4, 25 micromoles; sodium glutamate, 25 micromoles; magnesium

sulphate, 20 micromoles; neutralized cysteine, 20 micromoles; adenosine triphosphate (Na salt), 2 micromoles; hydroxylamine hydrochloride (neutralized), 50 micromoles; enzyme extract; and water to a final volume of 2.2 ml. The mixture was incubated at 50° for 5 minutes before tipping in the enzyme. The reaction was then allowed to continue for another 15 minutes after which it was terminted by the addition of 0.8 ml. of ferric chloride reagent (the reagent contained equal volumes of 10% $FeCl_3.6H_2O$ in 0.2N HCl, 24% TCA and 50% v/v HCl). The precipitated protein was removed by centrifugation and the color of the ferric-hydroxamate complex formed read off in a Klett-Summerson colorimeter at 540 mμ. The blank contained all the components except the substrate. Synthetic glutamyl-hydroxamic acid (GHA) was used as the standard.

A unit of enzyme activity is defined as the amount of enzyme which catalyses the formation of one micromole of GHA in 15 minutes at 50°, and specific activity, as units per milligram protein.

Glutamyltransferase : The enzyme activity was determined by the method described by Waelsch (200).

The reaction mixture consisted of : acetate buffer, pH 5.0, 30 micromoles; L-glutamine, 40 micromoles;

hydroxylamine hydrochloride, 10 micromoles; manganese chloride, 5 micromoles; adenosine triphosphate, 1 micromole; enzyme extract; and water to a final volume of 2.2 ml. After incubation for 30 minutes at 37°, the reaction was stopped by the addition of 0.8 ml. of ferric chloride reagent and the ferric-hydroxamate complex formed measured as described under glutamine synthetase. Blanks contained all the components except the substrate.

A unit of enzyme activity is defined as the amount of enzyme which, under the conditions of the assay, forms one micromole of GHA, and specific activity, as units per milligram protein.

Glutamic-pyruvic transaminase : The enzyme activity was determined from the amount of alanine formed which was assayed by circular paper chromatography using butanol : water : acetic acid (40:7:5) as the solvent system.

The reaction mixture consisted of : phosphate buffer, pH 7.0, 50 micromoles; L-glutamic acid, 20 micromoles; sodium pyruvate, 20 micromoles; enzyme extract; and water to a final volume of 2.0 ml. After incubation for 30 minutes at 30°, the reaction was stopped by the addition of 1.0 ml. of 95% ethanol and the tubes were

placed in a warm water bath for 5 minutes. The precipitated protein was removed by centrifugation and an aliquot of the supernatant removed for amino acid assay. Boiled enzyme replaced fresh enzyme in the blanks.

A unit of enzyme activity is defined as the amount of enzyme which forms one micromole of alanine in 30 minutes at 30°, and specific activity, as units per milligram protein.

Results and discussion

Tables 6 and 7 give the activities of enzymes on the basis of wet weight and protein content respectively. The specific activities are also compared in Fig. 4. It can be seen that essentially the same picture is obtained from both.

The data confirm the existence of considerable differences between hepatoma and normal liver in the activities of the enzymes studied. The additional observation is made that the enzyme levels in newborn liver are closer to those in hepatoma than normal liver, with regenerating liver occupying an intermediate position. However, there are considerable differences in enzyme levels between newborn liver and hepatoma, the specific activity in one being from 2 to 7 times that in the other. In other words, a quantitative difference is observed inspite of the existence of a qualitative similarity.

Table 6

Comparative data on the activities of certain enzymes of amino acid metabolism in normal, neoplastic, newborn and regenerating liver

Tissue	Enzyme activity*				
	Arginase	Glutamine synthetase	Glutamyl-transferase	Glutaminase I	Glutamic-pyruvic transaminase
Normal	2360	174	115	28	420
Neoplastic	150	1.8	7.0	125	22
Newborn	335	13.0	23	50	90
Regenerating	2100	91.0	53	38	290

* Expressed as units per gram of wet tissue.

Table 7

Comparative data on the activities of certain enzymes of amino acid metabolism in normal, neoplastic, newborn and regenerating liver

Tissue	Enzyme activity*				
	Arginase	Glutamine synthetase	Glutamyl-transferase	Glutaminase I	Glutamic-pyruvic transaminase
Normal	10.9	0.81	0.50	0.13	1.9
Neoplastic	1.0	0.01	0.05	0.90	0.2
Newborn	1.8	0.07	0.12	0.27	0.5
Regenerating	10.5	0.45	0.26	0.18	1.5

* Expressed as units per milligram of protein.

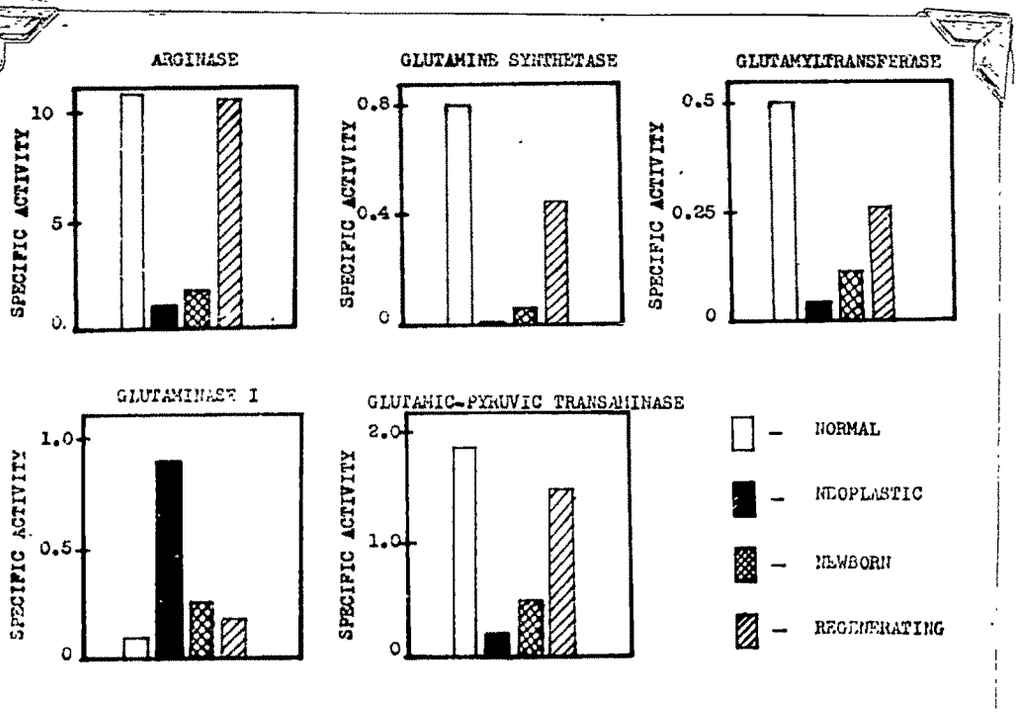

Fig. 4. Specific activities of certain enzymes of amino acid metabolism in normal, neoplastic, newborn, and regenerating liver.

A comparison of the enzyme levels with the data on amino acid utilizations reveals, however, some striking observations. To elaborate, the low level of arginase in newborn liver is hardly fitting with the high rate of arginine utilization by this tissue, if we consider the difference in enzyme level as the sole determinant of nutritional characteristic. However, the possibility remains that either an adaptive synthesis of this enzyme takes place in the cultures during proliferation or that the arginine utilized is channeled into the pathway of protein synthesis. The observation regarding the low level of arginase in hepatoma, however, is consistent not only with the observed decrease in the utilization of arginine by this tissue but also with the reported decrease in urea synthesis from arginine in the liver of azo-dye fed rats (141c).

Similarly, the differences in glutamine utilization between newborn and regenerating liver on the one hand and normal liver on the other are considerably greater than those between hepatoma and normal liver but the direction of differences is not the same with regard to enzyme levels. It would thus appear that while nutritional characteristics are determined to a large extent by the

enzyme make-up of the tissue, other factors are also operative. However, though no direct relation between the utilization of an amino acid and the activity of a related enzyme is observed the changes observed in the activities of glutaminase I and glutamine synthetase in newborn, regenerating and neoplastic liver tissues are suggestive of more utilization of glutamine in general by these tissues and its decreased synthesis.

The similarity of newborn and neoplastic liver with regard to these enzyme levels inspite of differences in nutritional characteristics is in contrast to the reverse observation made in regard to glucose utilization and enzymes concerned with glucose metabolism.