Metabolic Adaptations in Higher Animals. 10. Glutamic Dehydrogenase Activity of Rats Consuming High Protein Diets.¶ (29316)

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Adaptation of the rat to a high protein intake results in enzymatic changes and increased relative liver and kidney size. Not only do the activities of enzymes involved in the catabolism of specific amino acids increase, *e.g.*, threonine dehydrase and tryptophan pyrrolase(1); but also essentially all enzymes of central importance in the catabolism of amino nitrogen, such as the urea cycle enzymes, glutamic-pyruvic transaminase and glutamic-oxaloacetic transaminase, are increased(2-5). However, little or no increase in glutamic dehydrogenase (GDH) has been observed when protein intake is increased(2, 4,5). The key position of GDH in the scheme of amino nitrogen catabolism has prompted a closer examination of the activity of this enzyme in the tissues of rats adapting to a high protein intake.

Methods. Male rats of Holtzman or Charles River strain weighing 100-150 g were used unless otherwise noted. They were housed individually in suspended cages with screen bottoms and given food and water *ad libitum*. The control diet used in the high protein studies contained 25% casein, 65% sucrose, 5% corn oil, and 5% salts. The salt and vitamin mixtures(6) which were used initially were later replaced by Wesson salts and the vitamin mixture of Miller *et al*(7). In diets containing 50% or more of casein the quantity of vitamins added to the diet was doubled. Adjustments in the level of casein were made at the expense of sucrose. Pair-fed animals received a restricted amount of the 25% casein control diet so that their daily food intake was the same as that of the high protein group. The control diet was fed to all animals for at least 3 days before start of the experiment.

The diet was modified for the amino acid studies by replacing the sucrose with dextrin (autoclaved corn starch) and the casein with a mixture of crystalline L-amino acids. The basal mixture of essential amino acids contained 4.6% arginine·HCl; 4.9% histidine·HCl·H_2O; 21.7% lysine·HCl; 9.5% methionine; 10.4% isoleucine; 13.1% leucine; 13.7% phenylalanine; 9.5% threonine; 2.1% tryptophan; and 10.4% valine and constituted 8.7% of the diet.† The main components of the diets for the low carbohydrate study are given in Table IV.‡

At time of sacrifice the rats were stunned by a blow on the head and decapitated. The tissues were removed and chilled on ice. The intestinal samples consisted of approximately 8 inches of the upper small intestine which had been rinsed with isotonic saline to remove the contents. The mucosa was separated from the serosa with the aid of a spatula. Protein concentration was measured by the method of Lowry *et al*(9).

After examining a number of variables the following method for determination of GDH was developed. Tissues were homogenized for 2 minutes (3 minutes for muscle) in distilled water with a VirTis "45" tissue homogenizer.§ Centrifugation when used was at $1000 \times g$ for 5 minutes. Where dilution was required 0.1 M, pH 7.4 phosphate buffer was used.

GDH activity was determined spectrophotometrically. Cuvettes contained 1×10^{-4} M reduced nicotinamide-adenine dinucleotide

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† The amino acid experiments were part of a larger study of amino acid diets by Dr. W. P. Stucki(8).

‡ The tissues for this study were provided by Dr. M. A. Waldorf.

§ The dial setting was 50 which yielded approximately 16,000 rpm.

TABLE I. Tissue Distribution of Glutamic Dehydrogenase Activity.

Tissue	Units/g tissue* ± standard error
Liver	102 ± 4
Kidney	40 ± 1
Intestinal mucosa	9.4 ± .7
" serosa	4.9 ± .4
Spleen	4.4 ± .1
Heart	1.9 ± .2
Diaphragm	1.7 ± .2
Gastrocnemius muscle	.3 ± .05

* Tissues from 8 rats which had been raised on a stock diet and weighed approximately 250 g were analyzed.

(NADH), 2.5×10^{-1} M NH_4Cl, 5×10^{-3} M α-ketoglutarate, 1×10^{-4} M ethylenediamine tetraacetic acid, 0.1 M, pH 7.4 phosphate buffer and homogenate. Activity was measured at room temperature by the rate of change in optical density at 340 mμ. Blanks contained no α-ketoglutarate. A unit of activity was defined as 1 μmole of NADH oxidized per minute under the assay conditions.

Homogenization with a VirTis homogenizer resulted in higher, more consistent activities than homogenization with a Potter-Elvehjem (teflon pestle) homogenizer. The low activity of the Potter-Elvehjem homogenates was probably due to insufficient disruption of the mitochondria which is required for the release of "latent" activity(10). Centrifugation at 16,000 × *g* decreased the activity and was therefore avoided. Freezing and thawing tended to inactivate as well as release "latent" activity.

Michaelis constants determined on liver homogenates (NH_4^+, 2.9×10^{-2}; NADH, 7.4×10^{-5}; α-ketoglutarate, 2.1×10^{-4}) were in agreement with literature values for purified beef liver enzyme(11,12). Although the pH optimum of 7.3 was lower than that reported for the purified enzyme, it agreed with that previously published for rat liver homogenate(13,14). With the exception of intestinal homogenates the activity was stable for at least 3 days when stored at 8°C.

Results. The GDH activities of several tissues were determined in a group of rats raised on a stock diet (Table I). Liver had the greatest activity, being about twice as active per g of tissue as kidney. The tissues of the small intestine and the spleen had 5-10% of the activity of liver. Muscle tissues had still lower activities with the skeletal muscle, gastrocnemius, having considerably lower activity than heart or diaphragm.

The effect of a high protein intake was studied by allowing rats to consume 25 or 80% casein diets for 7 to 14 days (Table II). There was a small but statistically significant ($P < .05$) increase in the livers of the rats consuming the high protein diet. A larger increase in GDH activity occurred in the kidneys of the rats adapted to the high protein intake. The other tissues showed no appreciable changes.

A more detailed examination was made of the liver GDH activity during the adaptation period (Table III). Feeding the 80% casein

TABLE II. Effect of an 80% Casein Diet on Glutamic Dehydrogenase Activity.

Tissue	Activity (%)* ± standard error
Liver	115 ± 5
Kidney	138 ± 3
Heart	113 ±15
Spleen	97 ± 7
Gastrocnemius muscle	85 ± 3

* The activity per g of wet weight was determined in tissues of 5 to 15 rats. Activity is expressed as % of that of the 25% casein group.

TABLE III. Liver Glutamic Dehydrogenase Activity During Adaptation to an 80% Casein Diet.

Days on diet	Activity (%)* ± standard error: 80% Casein	25% Casein, pair-fed
	Per g wet wt	
2	103 ± 5	108 ± 3
3	105 ± 3	119 ± 4
5	106 ± 3	121 ± 3
7	115 ± 5	122 ± 7
	Per g protein	
2	88	98
3	94	109
5	95	110
7	103	111
	Per g body wt	
2	113 ± 4	91 ± 3
3	117 ± 6	100 ± 4
5	118 ± 3	96 ± 2
7	132 ± 6	105 ± 5

* Values are averages for 8 to 15 rats and are expressed as % of the 25% casein group fed *ad libitum*.

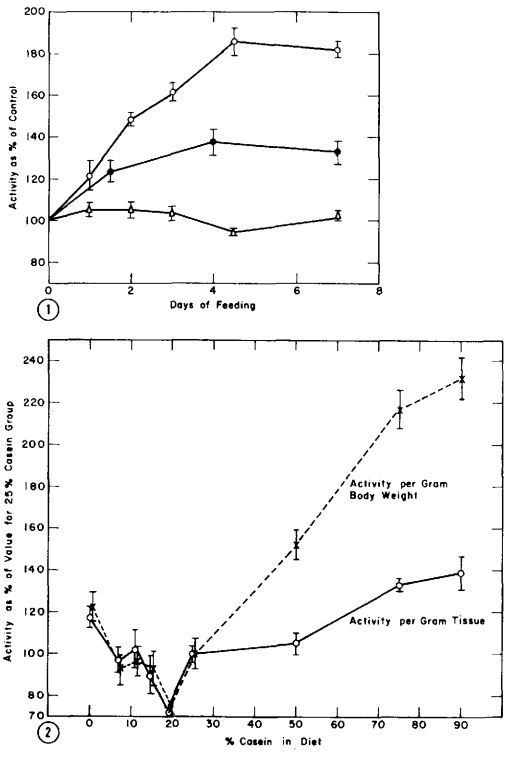

FIG. 1. Effect on activity of kidney glutamic dehydrogenase per unit of body wt of feeding high protein diets for varying lengths of time. Values are expressed as per cent of control and are averages for at least 8 animals. Rats were fed 80% casein (○——○), 50% casein (●——●, or were pair fed (△——△) a 25% casein diet. Vertical lines represent standard errors.

FIG. 2. Effect of casein level of diet on kidney glutamic dehydrogenase activity. Activity as per cent of the value for the 25% casein group is plotted vs. per cent of casein in the diet. Vertical lines represent standard errors. Five rats were used per group.

diet resulted in an increased liver protein concentration and relative liver size (g liver per g body weight) during the first 5 days. The GDH activity kept pace with the increased liver size but lagged behind the increased protein concentration. By the seventh day the activity was elevated both per g of body weight ($P<.01$) and per g of tissue ($P<.05$). Although the activity of the livers of the pair-fed group was elevated per g of tissue, activity per g of body weight was not altered.

The rise of kidney GDH during the adaptation period was followed using rats consuming 25, 50, or 80% casein diets (Fig. 1). Food intake of the 25% casein group was restricted to the amount consumed by the 80% casein group. The activity of the high protein groups rose both per g of tissue and per g of body weight. As the relative size of the kidney also increased during this period the increases in activity were larger when expressed per g of body weight. The increase was greater at the higher protein level and reached a maximum at 4-7 days. The activity was not changed in the pair-fed group.

The relationship between protein level and kidney activity was further investigated (Fig. 2). Rats receiving an 18% casein diet had the lowest activity. Raising the dietary protein content above 18% resulted in increases in activity that were roughly proportional to the increases in protein intake. This figure also illustrates the effect on activity expressed per unit of body weight of the increase in relative size of the kidney.

Several dietary manipulations were carried out to determine which dietary factor was causing the enzyme responses. Feeding a diet in which carbohydrate was replaced by fat produced only a small rise in kidney GDH (Table IV) compared to that obtained with a high protein diet (Fig. 2). The effect of changing the amino acid composition of the diet was studied with diets in which crystalline amino acids and ammonium acetate were the only source of amino nitrogen (Table V). Kidney GDH was the same whether glutamic acid, ammonium acetate, or a mixture of dispensable amino acids provided the nitrogen for the synthesis of the amino acids not provided. When only indispensable amino acids were included in the

TABLE IV. Kidney Glutamic Dehydrogenase Activity of Rats Consuming Diets High or Low in Carbohydrate.

Diet	Activity (%)* ± standard error	
	Per g tissue	Per g body wt
Control	100 ± 4	100 ± 3
Low carbohydrate	104 ± 4	122 ± 6

* Six rats per group were maintained for 9 days on the control diet (25% casein, 65% dextrin) or the low carbohydrate diet (38% casein, 50% fatty acids from the hydrolysis of crisco). Values are expressed as % of the control group.

TABLE V. Kidney Glutamic Dehydrogenase Activity of Rats Consuming Diets with Differing Sources of Dispensable Amino Acid Nitrogen.

Dietary addition	Body wt (g)	Activity (%)* ± standard error: Per g wet wt	Per g body wt
7.6% Glutamic acid	117	100 ± 2	100 ± 3
4.0% Ammonium acetate	112	94 ± 3	99 ± 3
5.7% Non-essential amino acids†	110	98 ± 3	98 ± 3
5.7% Essential amino acids‡	84	136 ±10	166 ± 9

* Five weanling rats per group received for 19 days diets containing a mixture of indispensable amino acids plus various sources of nitrogen for synthesis of dispensable amino acids. Activity is expressed as % of the group receiving glutamic acid.

† The mixture consisted of 48.1% L-glutamic acid, 28.9% glycine, and 3.84% each of L-alanine, L-aspartic acid, L-cystine, L-proline, L-serine, and L-tyrosine.

‡ Same as basal mix given in the methods section.

diet the kidney GDH activity was higher but growth was depressed. In another trial in which proline or alanine was the sole source of nitrogen for the synthesis of the other dispensable amino acids, liver, kidney and intestinal glutamic dehydrogenase activities were not different from values obtained when glutamic acid was provided in the diet.

Discussion. As might be anticipated from the key role of GDH in amino nitrogen metabolism, there was a correspondence between level of GDH activity and relative importance of the tissue in amino acid catabolism. The activity was greatest in the liver, in agreement with previous reports(13,15) and least in muscle. Liver, which is the major site of amino acid catabolism, had 10 times the total activity of kidney, the next highest tissue. The values for muscle were relatively lower than those reported previously(15). This is probably a reflection of the greater specificity of the spectrophotometric method used here.

The high protein intake caused an increase in activity per g of liver that was small and on the same order as the rise in protein concentration. However, the increase in total activity was substantial as liver size also increased. The limited GDH response contrasted with the marked increases reported for other liver enzymes, *e.g.*, urea cycle enzymes(2), glutamic-pyruvic transaminase(2, 3,4), and threonine dehydrase(1). The low GDH response was more comparable with the response of glutamic-oxaloacetic transaminase, another enzyme that is very active in liver (2-5).

The increased GDH activity per g of kidney demonstrated that adaptation to a high protein intake results in changes in enzyme activity as well as in size of this organ. Arginase has been reported to be increased when protein intake is raised(16); but the transaminases remain the same per g of kidney(3). The rise in kidney GDH followed the pattern reported for several liver enzymes(2,3). It was proportional to the elevation in dietary protein level and reached a maximum in 4-7 days. Lowering the carbohydrate intake or varying the glutamic acid intake did not affect the kidney GDH activity. It appears that the enzyme was responding to changes in protein intake *per se*.

Summary. The activity of glutamic dehydrogenase (GDH) has been determined in the tissues of rats consuming normal or high protein diets. In rats consuming a stock diet the distribution of GDH activity was as follows in order of decreasing activity: liver, kidney, intestinal mucosa, intestinal serosa, spleen, heart, diaphragm, and gastrocnemius muscle. Rats adapted to an 80% casein diet for 7-14 days had a higher activity per g of tissue in liver and kidney but not in spleen, heart or gastrocnemius muscle. Compared with rats consuming a 25% casein diet the activity of rats placed on an 80% casein diet rose 15% per g of liver, the same as the liver protein concentration, and 32% per g of body weight. Pair-fed rats had a higher activity per g of liver but no change per g of body weight. The increase in kidney GDH reached a maximum of 35% per g of tissue (85% per g of body weight) 4 days after changing the dietary casein level from 25 to 80%. The activity was proportional to the casein content of the diet for groups receiving more than 18% of casein. The activity of the kidney was not significantly affected by feeding a low carbohydrate-high fat diet or by substituting various dispensable

amino acids for the glutamic acid in an amino acid diet.

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Uptake of Ethionine-Ethyl-H^3 by the Rat Conceptus.* (29317)

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Recent studies have shown that ethionine is transported across the placenta of rats and mice and that this amino acid analogue is incorporated into the proteins of the fetus (1-3). Our studies(3) have indicated that the maximum concentration of free ethionine in the fetus and amniotic fluid occurs some time after that of the placenta and the plasma. The purpose of this study is to determine the relative rates of movement of labeled ethionine into the free amino acid pools and into the proteins of the placenta, fetus, and amniotic fluid of the pregnant rat.

Materials and methods. Intraperitoneal injections of 200 mg DL-ethionine (Nutritional Biochemicals) were given to eight 12-day pregnant rats (Sprague-Dawley). The rats were anesthetized 6 hours later and were injected intravenously with 250 μc (1.5 mg) L-ethionine-ethyl-H^3 (New England Nuclear Corp.). The urethra was clamped off at time of injection. At 5, 15, 30 and 120 minute intervals after injection of the tracer, blood was drawn from the vena cava inferior, the bladder was removed, and amniotic fluid, fetuses and placentae were collected.

Four pregnant rats were injected with 200 mg DL-ethionine and with 250 μc of L-ethionine-ethyl-H^3 intraperitoneally 12 and 72 hours prior to autopsy on day 12 of pregnancy. The rats were placed in metabolic cages and the urine was collected at 12 hour intervals. Blood and tissues were obtained as before.

Absolute ethanol was added to the plasma, amniotic fluid, and to homogenates of 8 fetuses and 2 placentae from each animal to bring the final concentration to 80% ethanol. The precipitates were separated by centrifugation and washed twice with 80% ethanol. The supernatant from each tissue or fluid was combined with the washes and constituted the soluble activity. Fat was extracted from the precipitates with ethanol-ether and ethanol-chloroform. The precipitates were hydrolyzed in 6N HCl at 110°C for 18 to 24 hours, evaporated to dryness, and dissolved in water to yield the protein-bound amino acids. The protein samples were decolorized with norit. All samples were evap-

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