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Metabolic Adaptations in Higher Animals VI. Liver Arginase Activity During Adaptation to High Protein Diet.* (26563)

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Changes in liver arginase activity that represent adaptive responses to dietary changes have been reported from several laboratories. Liver arginase activity per unit of tissue decreases when rats are fed low-protein or protein-free diets(1-4) and increases when they are fed a high-protein diet(4) or are starved (5).

Lightbody and Kleinman(6) first observed that liver arginase activity was responsive to protein intake. They reported that activity per unit of dry liver or per unit of body weight increased with each increase in the dietary level of protein in rats fed on diets containing 6, 25, 60 and 75% protein. Mandelstam and Yudkin(7) also showed that liver arginase activity, per unit of liver or per unit of body weight, in rats was proportional to the amount of protein in their diet and that the response to protein was linear for rats fed on diets containing 20, 40 and 60% of protein. However, Maramatsu and Ashida(8) reported that, although this was true of rats fed on diets containing more than 20% of protein, it did not hold for those fed on diets containing less than 20%.

Little has been reported on the relationship between total arginase activity and amount of urea excreted. A high positive correlation between liver arginase activity and urea excretion was found in an experiment on rats fed for 2 weeks on diets containing 0, 5, 10, 15, 25, 40, and 60% of casein(8), but the results of Rosenthal and Vars(5) who used protein-depleted rats subjected to 2 and 4 days of starvation do not reveal such a correlation. There is also a lack of information about the time-course of the change in arginase activity in response to a change in diet. Mandelstam and Yudkin(7) measured activity at weekly intervals and observed that the response to a change in dietary level of protein reached a maximum within 2 to 3 weeks.

Recently Freedland and Harper(9) reported that a substantial response in liver glucose-6-phosphatase activity occurred within 24 hours after fructose or protein was substituted for "dextrin" in the diet of rats. Therefore, the adaptation of liver arginase during the first few days after feeding rats a high-protein diet has been investigated and the

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relationship between total arginase activity and amount of urea in the urine during the adaptation period has been determined. The latter was of particular interest because the food intake of rats falls for one or 2 days when the protein content of their diet is greatly increased. The effect of a high protein intake on activity of kidney arginase was also measured.

Experimental procedure. Animals and diets. Male Holtzman rats weighing from 60 to 65 g were used in all experiments. The 3 different diets used throughout this study were as follows: (1) a control diet containing 64.6% of dextrin (moist cornstarch heated in an autoclave at 115° for 2 hours, then dried and ground) and 25% of casein; (2) a diet containing 44.35% of dextrin and 45% of casein; (3) a diet containing 19.1% of dextrin and 70% of casein. All diets contained 4.5% of corn oil, 5% of mineral mixture, 0.15% of choline chloride, and 0.5% of fat-soluble vitamin mixture in corn oil(10). A mixture of water-soluble vitamins in sucrose(10) was added as follows: to the 25% casein diet, 0.25%; to the 45% casein diet, 0.5%; to the 70% casein diet, 0.75%.

Rats used to study the change in arginase activity with time were fed *ad libitum*. The food intake of those used to study the relationship between arginase activity and urea excretion was restricted and the groups were pair-fed. Each rat was placed in an individual metabolism cage and urine was collected in dilute sulphuric acid for 2 days following the first, 5th, and 9th days after the casein content of the diet had been increased from 25% to 70%. The funnels were rinsed with distilled water at the end of each period and the urine was stored at 4°C.

When the effect of changing the casein content of the diet from 70% to 25% was studied, the animals were fed the 70% casein diet for 8 days, then were divided into 2 groups; the first group was continued on the 70% casein diet and served as a control, the second was offered the 25% casein diet.

All rats were fed the control diet for 4 days from the time they were received until they were offered the experimental diets, to compensate for any lack of uniformity in previous dietary history. Rats were weighed, either daily, or every other day, depending upon type of experiment.

Arginase activity. Animals were killed at intervals after being given the experimental diets as indicated in the results. The livers, and in some cases the kidneys, were removed, weighed, and placed in ice. A portion of the chilled liver or kidney was homogenized in 9 volumes of water and filtered through cheesecloth. The liver homogenate was diluted 100-fold with water and the kidney homogenate 10-fold. One portion of the diluted homogenate was used for the arginase assay, and another for determination of protein. Arginase activity was measured by the method of Greenberg(11) without activation, because, in another series of experiments, arginase of homogenates from livers of rats fed the control or the high protein diet was found to be activated in proportion to the initial activity. For the enzyme determination, 0.5 ml of 5% arginine solution, pH 9.5, was added to 0.5 ml of the 0.1% homogenate of liver or to 1 ml of the 1% homogenate of kidney, both of which had been incubated at 25°C for 3 minutes. After incubation at the same temperature for 5 minutes, the reaction was stopped by addition of 1 ml of glacial acetic acid. Then the activity was estimated by measuring the amount of urea produced(12). A portion of the diluted homogenate was dissolved in 1 N sodium hydroxide and protein content was estimated by the method of Lowry *et al.*(13).

The amount of urea in urine was determined by the method used for the estimation of arginase activity(12).

Results. Arginase activity is expressed as micromoles of urea formed in one minute at 25°C and pH 9.5 per mg of liver protein nitrogen (specific activity) or per g of body weight.

Change in liver arginase activity after feeding high-protein diets. Changes in liver arginase activity 1, 2, 4, 7, and 10 days after increasing the casein content of the diet are shown in Fig. 1 and 2. Liver arginase activity of rats increased within one day when the casein content of the diet was increased from 25% to 45 or 70% whether the results were expressed as activity per mg of liver protein

TABLE I. Effect of Increasing Protein Content of Diet on Food Consumption.

Diet	Food consumption, g/rat						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
25% casein (control)	10.0 ± .4* (10)†	12.6 ± .4 (8)	13.5 ± .2 (6)	14.0 ± .6 (6)	13.8 ± 1.8 (4)	15.5 ± .4 (4)	15.2 ± .4 (4)
45% casein	7.1 ± .2 (20)	9.7 ± .2 (16)	11.3 ± .4 (12)	12.3 ± .5 (12)	11.5 ± .5 (7)	13.8 ± .4 (7)	13.2 ± .5 (7)
70% casein	5.6 ± .3 (10)	7.2 ± .3 (8)	8.1 ± .2 (6)	9.4 ± 1.2 (6)	9.7 ± .2 (4)	11.1 ± .4 (4)	10.2 ± .3 (4)

* Stand. error of mean. † Numbers in parentheses show No. of animals used.

nitrogen (specific activity) (Fig. 1) or per g of body weight (Fig. 2). The activity per g of body weight of the group receiving 45% of casein continued to increase for 4 days and that of the group receiving 70% of casein continued to increase for 7 days. For each group and at each time interval the increase in arginase activity per unit of body weight was much greater than the increase per unit of liver nitrogen. This occurred because the livers of rats fed the high protein diets were larger than those of rats fed the control diet. Similar effects of a high protein intake on glucose-6-phosphatase activity were observed by Freedland and Harper(14).

Effect of high protein diet on body weight gain and food consumption. Growth curves for rats receiving the high protein diets are shown in Fig. 3. Growth was retarded for one day when the casein content of the diet was increased to 45%, and for 2 or 3 days when it was increased to 70%, thereafter growth rates of the 3 groups were similar. The slightly lower gain of the control group for one day may have been due to the rats becoming disturbed when they were redistributed in order to equalize the average body weights of the groups.

The food consumption figures in Table I show that there was an inverse relationship between protein content of the diet and food intake. Thus caloric intake was depressed for a few days after the protein content of the diet was raised.

Loss of arginase activity after changing from a high protein diet to a low protein diet. The results presented in Fig. 4 represent the change in liver arginase activity per unit of body weight when the diet was changed from 70% casein to 25% casein. Activity of liver arginase decreased, rate of loss being almost the inverse of rate of increase observed when the diet was changed from 25% casein to 70% casein.

Effect of changing from a high protein diet to a low protein diet on body weight gain and food consumption. The body weight gain for one day when the diet was changed from 70% casein to 25% casein was well above that of rats fed 70% casein diet, and thereafter growth rates in both groups were almost the same (Fig. 5). Food consumption of the former on the day when the diet was changed was also above that of the latter, 13.3 ± 1.0 g. compared to 11.3 ± 0.2 g. The slight growth retardation observed in the 70% casein group for one day may have been due to the animals being disturbed when the groups were redistributed as mentioned previously (Fig. 3).

Relationship between liver arginase activity and urinary urea production. Rats that were fed the control diet (25% casein) for 4 days were divided into 2 groups; one was fed the 70% casein diet and the other was continued on the control diet. The amount of each diet fed was restricted to approximately the amount consumed by the 70% casein group under *ad libitum* feeding conditions (Table I) so that each group would have the same caloric intake. The quantities fed daily from the first to the tenth days were as follows: 5, 7, 8, 9, 9.5, 10, 11, 12, 13 and 13 g. Rats were killed 2, 6, and 10 days after changing diet and urine was collected for 2 days before killing rats. Weight gains of the two groups were almost the same (Table II) but average liver weight of the group fed the 70% casein diet was significantly greater than that of the group fed the 25% casein diet.

The relationship between total liver argi-

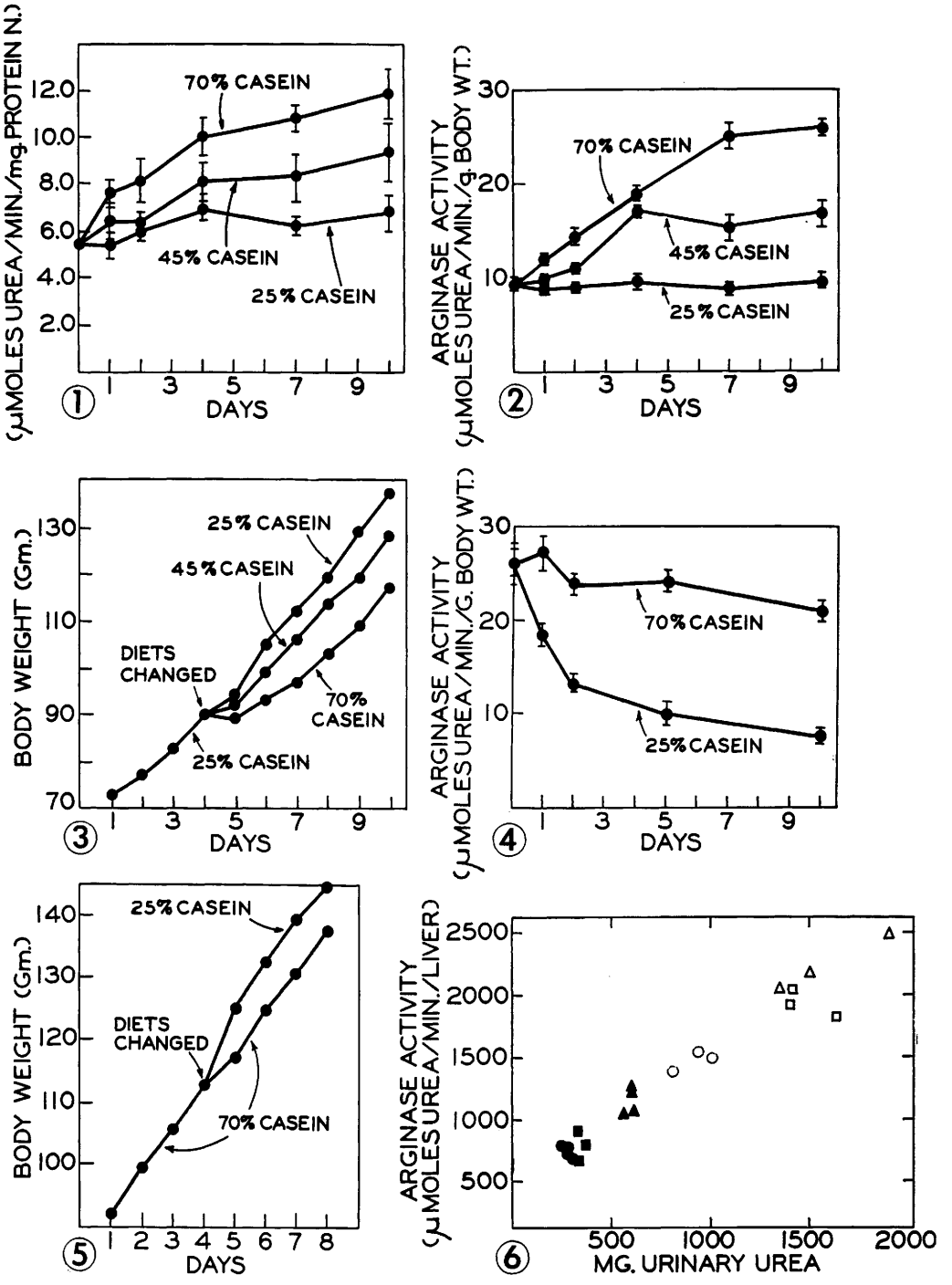

FIG. 1. Change in liver arginase activity after feeding high protein diets. Values expressed as activity per mg of liver protein nitrogen (specific activity). Each value is the average for 4 to 7 animals. Vertical lines represent standard errors of means.

FIG. 2. Change in liver arginase activity after feeding high protein diets. Values expressed as activity per g of body weight. Each value is the average for 4 to 7 animals. Vertical lines represent standard errors of means.

TABLE II. Comparison of Liver Weights of Rats Fed Equal Quantities of Medium or High Protein Diets.

Days after changing diet	Body wt, g		Liver wt, g	
	25% casein	70% casein	25% casein	70% casein
2	84 ± 2*†	83 ± 2	3.18 ± .22	4.89 ± .32
6	105 ± 5	102 ± 4	4.08 ± .17	5.93 ± .07
10	132 ± 1	131 ± 3	5.05 ± .10	6.57 ± .20

* Stand. error of mean. † Each value represents avg for 4 animals.

TABLE III. Effect of High Protein Diet on Kidney Arginase Activity and Kidney Weight of Rats Having Equal Food Intakes.*

Days after changing diet	Kidney wt, g		Kidney arginase activity			
			Urea produced per mg of kidney nitrogen, μmol		Urea produced per mg of body wt, μmol	
	25% casein	70% casein	25% casein	70% casein	25% casein	70% casein
2	.86 ±.03†	1.09 ±.03	.50 ±.06	.49 ±.02	.13 ±.01	.17 ±.01
6	1.08 ±.03	1.22 ±.03	.42 ±.04	.62 ±.04	.11 ±.01	.21 ±.01
10	1.21 ±.04	1.60 ±.06	.41 ±.02	.69 ±.09	.09 ±.01	.22 ±.01

* Each value represents avg for 4 animals. † Stand. error of mean.

nase activity and amount of urea excreted in the urine is shown in Fig. 6. Throughout the experimental period total liver arginase activity increased linearly with increasing urinary urea excretion.

Change in kidney arginase activity after feeding high protein diet. The activity of kidney arginase was also measured in rats used in the experiment on the relationship between activity of liver arginase and amount of urea in urine. The results are shown in Table III. The activity of kidney arginase per mg of kidney protein and also per g of body weight increased between the second and the sixth day in rats fed the 70% casein diet. The kidneys of rats fed the 70% casein diet increased in size in about the same proportion as their livers.

Discussion. The time-course of the adaptive response of liver arginase in rats fed a high protein diet has been followed and a relationship between response of the enzyme and response of the whole animal to a high protein intake has been demonstrated.

Within one day after the protein content of the diet was raised from 25% to 45 or 70% food intake of rats fed the high protein diets was depressed and their growth was retarded. Within 24 hours liver arginase activity per unit of liver protein increased. Since a reduction in caloric intake results in an increase in liver arginase activity(15) this rise may have been due in part to the lowered food intake. However, the activity of the enzyme continued to increase for between 7 and 10 days and during this time liver size also increased, an effect not seen in rats suffering caloric restriction, so that by the end of one week arginase activity per unit of body weight had increased 2.5-fold. Thus the adaptation involved both increased liver size and increased enzyme activity. As a metabolic adaptation should result in a change in the activity of a system on a body weight basis, the adaptation to the

FIG. 3. Effect of high protein diet on body weight gain. Each value up to the fourth day is average for 24 animals; each thereafter is average for 8 animals

FIG. 4. Loss of arginase activity after changing from high protein to low protein diet expressed per g of body weight. Each value is average for 4 to 6 animals. Vertical lines represent standard errors of means.

FIG. 5. Effect of changing from high protein to low protein diet on body weight gain.

FIG. 6. Relationship between total liver arginase activity and urinary urea production. ●, 25% casein group. ○, 70% casein group 2 days after changing diet; ■, 25% casein group, □, 70% casein group 6 days after changing diet; ▲, 25% casein group, △, 70% casein group 10 days after changing diet.

45% casein diet would appear to be complete within 4 days and that to the 70% casein diet within 7 days (Fig. 2).

By the second day, food intake and rate of growth of rats fed the high protein diets increased. Between the 7th and 10th days after the protein content of the diet had been raised, the rats receiving the high protein diets gained weight nearly as rapidly as the control rats receiving the 25% casein diet. Of particular interest in relation to this response is the observation that, for rats fed either 25% or 70% of casein and having the same caloric intake, the relationship between liver arginase activity and urinary urea excretion was nearly linear throughout the entire adaptation period.

Although arginase would not appear to be the most limiting of the urea cycle enzymes when their activities are measured *in vitro* (16), these observations indicate that activity of liver arginase is proportional to extent of protein catabolism in the body over the range of dietary protein levels studied. A further point indicating that the high enzyme activity represents an adaptive response of the animal to a high rate of protein catabolism is that within one day after the protein content of the diet was lowered liver arginase activity fell sharply. Although the evidence is indirect, these observations suggest that food intake is depressed when protein content of the diet is raised because the animal is unable to metabolize an excess of protein until it undergoes various adaptations, one of which is a substantial increase in arginase activity.

Summary. 1. Liver arginase activity of rats increased within one day when the casein content of their diet was increased from 25% to 45% or 70% whether the results were expressed as activity per liver or per g of liver protein nitrogen. Arginase activity of the group receiving 45% casein continued to increase for 4 days and that of the group receiving 70% casein continued to increase for 7 days. Average liver weight of the high protein groups also increased significantly above that of the 25% casein group. 2. Growth rate and food consumption were depressed for one day when the casein content of the diet was increased to 45%, and for 2 or 3 days when it was increased to 70%; thereafter, growth rates of the 3 groups differed only slightly. 3. When the casein content of the diet was dropped from 70% to 25% and rate of loss of arginase activity was plotted graphically the curve obtained was almost the reverse of that obtained when the casein content of the diet was raised from 25% to 70%. 4. A correlation between total activity of liver arginase and amount of urea in urine was observed throughout 10 days after changing the casein content of the diet from 25% to 70%. 5. Kidney arginase activity and kidney weight also increased when casein content of the diet was raised from 25% to 70%.

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