Response to Fasting of Hepatic Arginase, Alkaline Phosphatase, and Rhodanese in Protein-Depleted Rats.* (21163)

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Lightbody and Kleinman(1) reported that there was a positive correlation between the total amount of liver arginase in the rat and the protein content of the diet. Ashworth(2) first noticed the drop in liver arginase activity on protein starvation which he interpreted as enzyme deterioration by non-use. The relation of the activity level of hepatic arginase to the protein-intake of the animal has been repeatedly confirmed(3-7). Opinions are divided, however, regarding the significance of this relationship. Miller(6) considers the changes in the arginase content of rat liver as non-specific participation in the gain and loss of hepatic protein; whereas according to Mandelstam and Yudkin(7) the relationship between enzyme production and amount of dietary protein is in conformity with the mass action theory of enzyme adaptation.

Previous studies from this laboratory(8) supported the inference that the rate of restoration of liver arginase following 70% partial hepatectomy was geared to the protein catabolism of the animals inasmuch as the rate was the faster, the higher and the more rapidly the urinary nitrogen excretion rose above the preoperative level. Accordingly restoration of arginase was fastest in protein-depleted rats in which the increase in protein catabolism is relatively greater than in protein-fed animals. The rate was enhanced by fasting and reduced by supplying non-protein calories immediately after the operation. Since the protein catabolism of the protein-starved animal can be increased by fasting to approximately the same extent as by a partial hepatectomy, it was of interest to study the response of liver arginase to fasting.

Experimental. The experiments were carried out in conjunction with assays of alkaline phosphatase previously reported by us (9). The animals were male rats from the colony of the Wistar Institute which weighed approximately 250 g at the time of transfer to the protein-free semi-synthetic diet. After 2 weeks on this diet food was withheld and groups of animals were sacrificed after 2 or 4 days of fasting for enzyme assay and chemical analysis. The methods employed have been detailed in previous publications from this laboratory(5,9).

Results. The experimental results summarized in Table I are expressed in terms of enzyme units or of mg of protein per total liver per 100 g initial body weight; *i.e.* the weight on the day of transfer to the protein-free diet. The figures represent the means and standard errors† for the control groups and the differences between the means of the experimental and control groups with the standard errors of the differences.‡ To facilitate comparison of the magnitude of the changes due to fasting the differences and their standard errors are expressed as percentages of the respective control means.

It is seen that the arginase content of the protein-depleted livers increased significantly during the period of fasting concomitantly with, though somewhat slower than the rate of urinary nitrogen excretion. The individual arginase values obtained at the 2-day interval were still within twice the standard deviation of the control group, whereas all values at the 4-day interval were beyond this range and

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† $\pm\sqrt{\frac{\Sigma(\Delta x)^2}{n(n-1)}}$

‡ $\pm\sqrt{\frac{\Sigma(\Delta x_1)^2+\Sigma(\Delta x_2)^2}{n_1+n_2-2}\left(\frac{1}{n_1}+\frac{1}{n_2}\right)}$

TABLE I. Effect of Fasting on Enzyme and Protein Content of Protein-Depleted Rat Livers.

Period	Arginase, units*	Alkaline phosphatase, units	Rhodanese, units	Protein, mg	Urinary nitrogen exer., mg/day
Control	1083 (76)† ±22.1‡	6.16 (81) ±.155	246.5 (4) ±9.1	385.7 (80) ±2.64	56.3 (4) ±2.20
2 days fast	±19.6% (4) ±9.01 P<.05	−7.3% (4) ±11.54 P>.05	+25.8%(4) ±9.75 P<.05	+10.3% (4) ±3.18 P<.01	+53.8%§(4) ±5.10 P<.01
4 " "	+57.5% (8) ±6.59 P<.01	−58.2% (8) ±8.01 P<.01	+27.5%(4) ±6.82 P<.01	+2.8% (8) ±2.62 P>.05	+73.2% (4) ±13.21 P<.01

* Units = μM of reaction product formed per min. at 25°C and pH 9.5 for arginase, at 37.5°C and pH 9.4 for phosphatase, and at 20°C and pH 7.47 for rhodanese.

† No. of experiments in parentheses.

‡ Stand. error of the mean.

§ Mean of the increments obtained in individual animals ± stand. error. Increments computed from the avg excretion rates for days 1 and 2 and days 3 and 4 respectively.

their mean significantly higher than that at the earlier interval.

The results of simultaneous assays of hepatic alkaline phosphatase show that the fall in the phosphatase content was the mirror image of the rise in arginase content. Since previous studies from this laboratory indicated that the activity levels of these 2 hepatic enzymes were affected by the metabolic state of the animal, some of the livers were assayed for rhodanese which did not display such a dependence. It is evident that the rhodanese content§ of the depleted livers increased on fasting although the increment was smaller and the maximum reached earlier than with arginase. It is of interest to notice that the protein content of the depleted livers did not diminish on fasting and even appeared to be slightly augmented at the second day interval. The tendency of the liver protein to increase during fasting in the protein-starved animal is in analogy to the high initial rate of hepatic protein synthesis following partial hepatectomy in this type of animal (8). It presumably results from the circumstance that in the state of protein starvation any extrahepatic protein breakdown raises the amino acid concentration in the liver above the level with which the "labile" liver cytoplasm was in equilibrium.

To find out whether the qualitative and quantitative differences in the response of the 3 enzymes to fasting were related to their intracellular distribution, a group of protein-depleted livers was separated by means of fractional centrifugation in isotonic sucrose solution into nuclear fraction, NW, mitochondrial fraction, MW_2, microsomal fraction, P, and soluble proteins, S_2, according to the procedure of Schneider and Hogeboom(10). In Table II relative specific enzyme activity and enzyme content of these fractions are recorded. Locations of maximum enzyme concentration were the mitochondrial fraction for rhodanese, the nuclear and microsomal fractions for arginase, and the microsomal fraction for alkaline phosphatase. The distribution of arginase and rhodanese in protein-depleted livers resembled closely that reported by Ludewig and Chanutin(11) for rat liver in general and also agreed with unpublished data from this laboratory regarding liver tissue from protein-fed rats. The alkaline phosphatase distribution, on the other hand,

§ All rhodanese determinations utilized in Table I were done at pH 7.47 whereas assays on the control group of 76 livers, from which the arginase values of Table I were taken, had been done at pH 7.37. Mean and standard error of the latter group was 217.5 ± 2.87. Unpublished experiments showed that around pH 7.4 a shift of the pH of 0.1 unit results in an activity change of 10%. It appeared advantageous, therefore, to assay hepatic rhodanese at pH 8.8 since there is a broad activity maximum between pH 8.6 and 9.0. The activity at the pH maximum is about 2.5 times that at pH 7.4. The rhodanese assays presented in Table II were obtained at pH 8.8 in 0.1 M 2-amino-2 methyl-1,3 propandiol sulfuric acid buffer.

TABLE II. Intracellular Distribution of Enzymes in Protein-Depleted Rat Liver.*

Enzyme	Nuclei (Nw)	Mitochondria (Mw_2)	Microsomes (P)	Supernatant liquid (S_2)
Rhodanese	.76† (11.9%)§	2.98 (79.4%)	.08‡ (4.3%)	
Arginase	2.21 (23.1%)	.68 (19.2%)	1.53 (42.3%)	.12 (4.1%)
Phosphatase	.66 (8.2%)	.4 (11.4%)	2.00 (51.7%)	.90 (31.8%)
Phosphatase (protein-fed)	.43 (6.7%)	.34 (10.6%)	.92 (23.5%)	2.30 (71.7%)

* Mean values of 2 to 3 experiments.

† Relative specific activity = (units/mg N in fraction)/(units/mg N in homogenate).

‡ Because of the low activity the supernatant from the mitochondrial fraction was not fractionated further.

§ Percentage distribution = [(Units in fraction)/(units in homogenate)] × 100.

differed from that reported by the previous workers in not displaying a concentration maximum in the nuclear fraction. As will be seen from Table II, the distribution differed still more from that found by us in protein-fed livers in which two-thirds of the total phosphatase activity was recovered in the soluble protein fraction. It has been previously pointed out by us(12) that the soluble liver phosphatase is activatable by magnesium ions and not inhibited by 0.01 Molar cyanide, whereas the component associated with particulate matter is highly sensitive to cyanide poisoning, but little activated by magnesium ions. It is the latter component that increases during protein depletion and decreases on fasting.

Discussion. To be designated as a specific response to metabolic stimuli a change in the enzyme content of an organ should be out of proportion to or of a different direction than changes in the content of functionally unrelated enzymes and of the total protein. The fall in the content of the cyanide-sensitive alkaline phosphatase satisfies these criteria. Its possible mechanism and significance have been discussed previously(9,12).

The small increment of the arginase content at the 2 day interval of time has the characteristics of a non-specific response since increments of a similar order were found in the total liver protein and in rhodanese, an enzyme of no known functional relationship and of different location from arginase. The more significant increment of the arginase content between the second and fourth day of fasting, on the other hand, is indicative of a specific response since it was not accompanied by simultaneous increases in rhodanese or total protein content.

It is of interest to recall that a similar sequence of nonspecific and specific responses of arginase as contrasted to consistently nonspecific responses of rhodanese characterized the loss of enzyme activity during extended periods of protein depletion(5). Under both conditions the phase of specific change in arginase content appeared to be the consequence rather than the cause of the altered protein catabolism of the animal. This is in keeping with reports of Folley and Greenbaum(13) and Kochakian and Robertson(14) regarding increases in hepatic arginase following hormonal stimulation of the protein catabolism. The arginase responses, though more than a mere non-specific participation in the protein turnover, are always initiated by simultaneous gains or losses of hepatic protein. They may be designated as excessive formation or excessive deterioration of an enzyme due to a functional overload or nonuse rather than as a strictly adaptive enzymatic response. Their main interest lies in demonstrating that the functional capability of an organ can be altered specifically by nutritional means.

Summary. When protein-depleted rats were subjected to 4 days of fasting, the arginase content of the liver increased by 58% while the alkaline phosphatase content decreased by a similar percentage. A simultaneous smaller increment of the rhodanese content was limited to the first 2 days of fasting. The experiments indicate that the initial increase in arginase activity was due to a nonspecific increase in hepatic protein while the

later rise was a specific response probably related to the increased protein catabolism.

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Effect of Cortisone and other Steroids upon *in vitro* Synthesis of Chondroitin Sulfate. (21164)

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It is known that cortisone inhibits the formation of granulation tissue(1) and that this tissue has a capacity to synthesize chondroitin sulfate(2,3). Layton(4) has further demonstrated that cortisone alcohol inhibits the *in vitro* synthesis of chondroitin sulfate by embryonic and wound tissues. From related physiological information regarding the effects of cortisone in the whole organism(5,6), its action by way of inhibiting chondroitin sulfate formation might well be capable of explaining a variety of observations. While the data presented by Layton(4) provided a reasonable inference that the material being measured was chondroitin sulfate, subsequent studies(7-11) have markedly increased the probability that the measurements are those representing chondroitin sulfate, and confirmation of the inhibition by cortisone alcohol has been provided(9,10).

One of the difficulties inherent in the study of the mode of action of the steroid hormones is the lack of an *in vitro* activity which bears any relation to the action of the hormone in the whole animal. It appeared possible that this inhibition of chondroitin sulfate synthesis by the addition of cortisone alcohol *in vitro* might be sufficiently related to its action in the whole animal, where such inhibition is also observed(12), to provide the *in vitro* tool which is so necessary for this type of problem. However, even the system employed by Layton(4) was somewhat too complex as an initial *in vitro* approach, since it was dependent upon embryonic (granulation) tissue, under conditions essentially comparable to those of tissue culture and required incubation periods of 45 hours. It would be desirable to obtain a somewhat similar system which would employ essentially a tissue slice. This was done by Boström and Mansson(8,9) but a more rapid system was still necessary. This has been achieved as described under methods. Having such a system, which in a period of 5 hours synthesized considerable quantities of chondroitin sulfate, it was found possible to inhibit the chondroitin sulfate synthesis by the *in vitro* addition of cortisone alcohol confirming the results reported by Layton(4) and Boström and coworkers(9,10). However, further studies of this system throw some doubt on the specificity of the effect of the steroid cortisone and on the question of whether this action is really related to the action of cortis-