ARGINASE, ADENOSINEPYROPHOSPHATASE, AND RHODANASE LEVELS IN THE LIVER OF RATS*

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Previous studies from this laboratory (2, 3) have dealt with the chemical composition of resting and regenerating rat liver under various dietetic conditions. The major emphasis was placed upon changes in the amount of protein, since the quantity of this tissue component appeared to offer the most appropriate estimate of the functionally effective liver mass. It was felt that supplementation of these data by enzymatic assays could provide more direct information on the functional capability of this organ. Recent reports from several laboratories (4–6) indicate that the change in the activity of an individual enzyme may differ considerably in its magnitude from the change in total liver protein.

The enzyme assays to be reported were carried out on liver tissue from rats which were kept either on a protein-rich, semisynthetic diet or on an analogous protein-free régime for various intervals of time. The animals were then subjected to a partial hepatectomy and sacrificed during the first 8 postoperative days. The present paper deals with the effect of protein starvation on the enzymatic activity of the resting liver tissue that was obtained at operation.

The three enzymes under investigation were selected for metabolic reasons as well as for the sake of accuracy and simplicity of assay. The liver arginase plays a major role in the protein catabolism of the animal. The amount and concentration of this enzyme are known to depend on nutritional conditions (4, 5, 7). The enzymatic hydrolysis of adenosine triphosphate is a ubiquitous property of cells which is possibly of importance for the intracellular transfer of energy. Since it is known that liver extracts and homogenates split off both labile phosphorus groups of the nucleotide (8, 9), we shall refer to the enzyme or enzymes involved as adenosinepyrophosphatase rather than as adenosinetriphosphatase. Rhodanese (10) represents a characteristic liver enzyme of about as high an

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activity as arginase. No other rat tissue, with the exception of kidney cortex, displays more than 6 per cent of the rhodanese activity of liver tissue (11). The transfer of sulfur from thiosulfate to hydrocyanic acid, which is catalyzed by the enzyme, is not known to be linked to major metabolic processes. Hence rhodanese furnished a useful control system in the study of dietary effects on the levels of liver enzymes.

EXPERIMENTAL

Young male rats of the Wistar strain, weighing approximately 250 gm., were used for the experimental series. They were fed ad libitum. The composition of the diets, the technique of the partial hepatectomy, and the handling of the excised liver tissue have been previously discussed by Gurd, Vars, and Ravdin (3).

The major portion of the excised liver tissue was placed for 10 minutes in an ice-cooled container. It was then rapidly minced with scissors and weighed in a glass homogenizer of the Potter-Elvehjem type (12). 4 volumes of ice-cold glass-distilled water were added per gm. of mince and the mixture homogenized for 3 minutes, the tube being immersed in ice water. Upon aliquots of this 20 per cent homogenate, determinations of the total nitrogen, non-protein nitrogen, and total lipides were done as previously described (3).

Arginase was determined according to the directions of Van Slyke and Archibald (13), except that the entire procedure was carried out in Warburg vessels, the urea being determined with the method of Krebs and Henseleit (14). Immediately following the preparation of the 20 per cent homogenate an aliquot was diluted 100-fold with a mixture of equal parts of 0.1 M MnSO₄ solution and 0.9 per cent NaCl solution. 1 ml. samples of this 0.2 per cent homogenate were incubated at 56° in rubber-stoppered conical Warburg vessels for a period of 20 minutes. Thereafter the vessels were incubated at 25° with 0.5 ml. of a 5 per cent arginine solution (pH 9.5) for 5 minutes. The arginase action was stopped by the addition of 1 ml of 0.25 M H₂SO₄. 0.3 ml. of a 5 per cent solution of urease (Arlco) in 0.3 M acetate buffer at pH 5 was placed in the side bulb and 0.5 ml. of 1.5 M sodium acetate solution added to the contents of the main compartment. The urea was then determined manometrically in the usual fashion at 37°. The assays were run in duplicate, which usually agreed within 3 per cent. Whenever deviations greater than 5 per cent were en-

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1 One vessel, to which the arginine had been added after the sulfuric acid, served as a control. The "blank" pressure, on addition of urease, amounted to but 3 to 4 mm. and did not vary significantly among the individual homogenates. Hence a single thermobarometer control suffices for a set of determinations upon individual homogenates.
countered, the determinations were repeated on one of the following days. This was feasible as the 0.2 per cent homogenates kept their arginase activity for at least 72 hours when stored in the cold.

Seifer et al. (5) reported that the arginase activity of liver homogenates greatly depended upon the length of time between excision of the liver and homogenization. We have found no differences in the activity of samples from the same liver specimen when the storage time on ice prior to homogenization was increased from the usual 10 minutes up to 60 minutes.

The incubation of the 0.2 per cent homogenates at 56° for a period of 20 minutes resulted in an increase of but 15 to 25 per cent in the arginase activity. This is considerably less than the increments obtained by Van Slyke and Archibald (15) and Hunter and Downs (16) with dog liver extracts or by ourselves with extracts from acetone-dried rabbit liver. Apparently the arginase activity of rat liver homogenates remained well preserved in the cold manganese saline medium. Roberts (17) reported that for the complete activation of mouse liver homogenate incubation times of 5 hours at 50° were needed. We have found that the arginase activity of homogenates from livers of both protein-fed and protein-depleted rats was not further increased when the incubation period at 56° was extended up to 2 hours. Likewise, Roberts' technique of incubating 1 per cent homogenates at 50° for extended periods of time failed to result in higher activities than those obtained with our standard procedure. It thus appears justifiable to assume that by our method the full arginase activity in resting and regenerating liver tissue, under various nutritional conditions, is determined.

Adenosinepyrophosphatase (Apyrase) was assayed by means of a slight modification of the method of DuBois and Potter (18). 0.2 ml. of a 1 per cent liver homogenate, prepared immediately before use from the 20 per cent homogenate by dilution with redistilled water, was mixed in a micro ignition tube with 0.2 ml. of 0.05 M barbiturate buffer (pH 7.4), 0.2 ml. of 0.012 M adenosine triphosphate solution (ATP), and 0.2 ml. of water or salt solution. The mixture was repeatedly stirred with a small glass rod. After 15 minutes incubation at 37° the reaction was stopped by the addition of 0.2 ml. of ice-cold 25 per cent trichloroacetic acid and the tubes chilled in ice water and centrifuged. Upon 0.5 ml. samples of the supernatant the inorganic phosphorus was determined by means of the method of Fiske and Subbarow as modified by Lohmann and Jendrassik (19). Readings were made with the Klett-Summerson photoelectric colorimeter, the total reaction volume being 5.2 ml. The reproducibility of the duplicate determinations was better than 5 per cent.

* Unpublished experiments.
The homogenates lost about 20 per cent of their Apyrase activity during 6 hours storage in the cold. All assays reported here were started within 50 to 90 minutes from the time of homogenization.

Fig. 1 provides information on the phosphatase activity of normal liver homogenates toward diverse substrates. Details regarding the compounds used can be found in the legend to Fig. 1.

It is obvious from Fig. 1 that there was but negligible activity towards substrates of the alkaline phosphatase, such as β-glycerophosphate and adenosine-3-phosphate. Of compounds that may either be present as impurities of ATP or be formed in the enzymatic breakdown, inorganic pyro-

![Graph](http://www.jbc.org/)

**Fig. 1.** Hydrolysis of phosphorus esters by liver homogenates from protein-fed rats. Temperature at 37°C; pH 7.4. All substrates were employed at concentrations of 3 mM per liter in the form of their sodium salts adjusted to pH 7.4. Pyr., sodium pyrosphosphate (Baker); ATPa, sodium adenosine triphosphate (Rohm and Haas); ATPb, barium adenosine triphosphate (Sigma); A5P, adenosine-5-phosphoric acid (My-B-den, Bischoff); A3P, adenosine-3-phosphoric acid (Schwarz); BG1, sodium β-glycerophosphate (Eastman); Amb., filtered through Amberlite IR-100 (sodium salt, Rohm and Haas).

phosphate was hydrolyzed 3 to 5 times as rapidly as ATP, while adenosine-5-phosphate was split at about one-third to one-fifth the rate of ATP.

The two commercial brands of ATP used were hydrolyzed at different rates. The enzymatic breakdown of Sample ATPa was not affected by the addition of calcium or magnesium. When solutions of Sample ATPa were first filtered through the cation exchange resin Amberlite, the rate of hydrolysis in the absence of added cations increased by 50 per cent. The average rates thus obtained exceeded slightly those found by DuBois and Potter (18) at calcium levels of 3 mM per liter, which provided optimal activation of their liver homogenates. With our system, on the contrary, 3 mM of calcium retarded the rate of reaction. We have nevertheless
carried out one set of experiments (Sample ATP, Table I) at this calcium level, rather than without added cations, in order to avoid the possibility that differences in the enzyme activity of individual liver homogenates could be due to varying cation levels rather than to changes in the amount of enzyme protein. Small variation of the calcium concentration, about 3 mM per liter, did not significantly alter the rate of hydrolysis.

Sample ATP, was a commercial barium salt which had been converted into the sodium salt through Amberlite treatment according to the direction of Kielley and Meyerhof (20). With this preparation both magnesium and calcium exhibited stimulatory effects, the former cation being more effective than the latter. The concentration optima were essentially the same for both cations. Experiments in Tables I and II have been carried out with Sample ATP, in the presence of magnesium chloride, 0.8 mM per liter, which provided optimal activation.

The reasons for the different activity of liver Apyrase towards the two ATP preparations are not clear. Possibly, because Sample ATP, contained an excess of calcium or magnesium which was only partially removed through Amberlite filtration, the measurements fell on the declining limb of the cation activity curve. At any rate, the results show that not much significance can be attached to the numerical values for Apyrase activity. The relative values obtained with the same ATP preparation on individual organs are significant and reproducible, however.

Rhodanese was determined with the method of Cosby and Sumner (22), adapted to the assay of small homogenate samples. In test-tubes were placed 3 ml. of 0.11 M phosphate buffer (pH 7.4), 0.5 ml. of 0.5 M Na₂S₂O₃, homogenate samples corresponding to 10 and 20 mg. of liver tissue from protein-fed and protein-starved rats respectively, and water to make a total volume of 4.5 ml. The tubes were immersed in a water bath at 20°. The reaction was started by the addition of 0.5 ml. of neutralized 0.5 M KCN from a tuberculin syringe and stopped, after 2 minutes, by dumping into the reaction mixture 5 ml. of 10 per cent trichloroacetic acid containing 3.6 ml. of 40 per cent formaldehyde solution per liter. The formaldehyde prevents sulfur formation from thiosulfate. To 3 ml. of clear filtrate, 5 ml. of water and 2 ml. of 5 per cent Fe(NO₃)₉H₂O, containing 5 ml. of concentrated HNO₃ per 100 ml., were added.

For the preparation of blank and standard the filtrate of a tissue sample was used to which cyanide was added after the trichloroacetic acid. One 3 ml. portion, serving as blank, was treated as described above. A second 3 ml. portion received 2 ml. of 0.001 M KSCN and 3 ml. of water

LePage and Potter (21) recently pointed out that commercial ATP preparations frequently contain trace impurities which inhibit oxygen consumption of tissue homogenates.
before the addition of the iron reagent. 15 minutes after the addition of the iron reagent the color was read in a Klett-Summerson colorimeter. With the procedure outlined above the color intensity follows the Beer-Lambert law.

While 10 to 20 per cent liver homogenates preserve their full rhodanese activity for at least a week when stored in the cold, activity is rapidly lost on greater dilution unless an excess of thiosulfate is present. It is essential, therefore, to add thiosulfate to the reaction tube before addition of the homogenate.

Units—All enzyme units are here expressed in terms of micromoles of assayed reaction product formed per minute under the standard conditions given above. Units per gm. of protein (enzyme concentration) as well as units per total organ per 100 gm. of initial body weight (enzyme content) will be used as measures of the enzymatic activity of liver. The quantity of other tissue constituents is expressed in analogous terms. Values for liver nitrogen were converted into terms of protein by multiplying the difference between total nitrogen and trichloroacetic acid-soluble nitrogen by the factor 6.25. The initial body weights of protein-fed and protein-starved rats were taken as the weights on the day of operation and on the last day on normal stock diet, respectively.

In thirty-eight partially hepatectomized normal rats the ratio of excised liver to total liver averaged 70 per cent, with a standard deviation of ±1.52. Accordingly, the factor 1.43 was used for computing the total liver weight from the weight of the excised portion.

Results

Experimental results concerning the effect of protein starvation on composition and enzymatic activity of liver tissue are summarized in Tables I and II. As in previous studies from this laboratory the majority of assays was performed upon animals depleted for a period of 2 weeks (Group II). The smaller experimental group, No. III, served to explore to what extent protein depletion, when continued almost to the limit of the survival time of the animals, would alter the enzymatic capability of liver tissue. This group included four rats depleted 28 to 35 days, three rats after 44 days of depletion, and four after 49 days.

In Tables I and II mean values of the different quantities under examination are listed, together with the coefficient of variation and the standard error of the mean. To facilitate the evaluation of the results the percentage differences between the group means and their standard errors are also presented so that direction and degree of a change and its significance may be readily seen. From the coefficients of variation it is evident that protein starvation led to an increased heterogeneity of the group.
<table>
<thead>
<tr>
<th>Group No.</th>
<th>Type and duration of diet</th>
<th>Designation*</th>
<th>Wet weight (gm.)</th>
<th>Dry weight (%)</th>
<th>Per 100 gm. wet weight</th>
<th>Per 1 gm. protein</th>
<th>Arginase</th>
<th>Rhodanese</th>
<th>Apyrase†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Protein (gm.)</td>
<td>Lipide P (mg.)</td>
<td>Total lipides (gm.)</td>
<td>Arginase (units × 10⁻⁴)</td>
<td>Rhodanese (units)</td>
<td>Apyrase (units)</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>18% casein, 7 days</td>
<td>Mean</td>
<td>3.31 (16)</td>
<td>18.1 (16)</td>
<td>96 (16)</td>
<td>3.69 (16)</td>
<td>522 (16)</td>
<td>913 (16)</td>
<td>102 (8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C.V.</td>
<td>±2</td>
<td>±5</td>
<td>±0</td>
<td>±16</td>
<td>±7</td>
<td>±8</td>
<td>±9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S.e.</td>
<td>±0.014</td>
<td>±0.21</td>
<td>±2.2</td>
<td>±0.149</td>
<td>±9.3</td>
<td>±17.2</td>
<td>±2.4</td>
</tr>
<tr>
<td>II</td>
<td>Protein-free, 13-17 days</td>
<td>Mean</td>
<td>3.26 (65)</td>
<td>13.3 (65)</td>
<td>73 (65)</td>
<td>5.87 (63)</td>
<td>302 (65)</td>
<td>524 (65)</td>
<td>91 (14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C.V.</td>
<td>±4</td>
<td>±10</td>
<td>±14</td>
<td>±30</td>
<td>±20</td>
<td>±14</td>
<td>±12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S.e.</td>
<td>±0.008</td>
<td>±0.16</td>
<td>±1.2</td>
<td>±0.22</td>
<td>±7.6</td>
<td>±9.2</td>
<td>±2.8</td>
</tr>
<tr>
<td>III</td>
<td>Protein-free, 28-49 days</td>
<td>Mean</td>
<td>3.34 (11)</td>
<td>13.4 (11)</td>
<td>87 (11)</td>
<td>5.82 (10)</td>
<td>213 (11)</td>
<td>520 (11)</td>
<td>106 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C.V.</td>
<td>±7</td>
<td>±13</td>
<td>±20</td>
<td>±28</td>
<td>±31</td>
<td>±9</td>
<td>±18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S.e.</td>
<td>±0.071</td>
<td>±0.53</td>
<td>±6.0</td>
<td>±0.505</td>
<td>±20.2</td>
<td>±13.7</td>
<td>±3.8</td>
</tr>
<tr>
<td>((I - I)/100)</td>
<td>% change</td>
<td>-2</td>
<td>-27</td>
<td>-24</td>
<td>+59</td>
<td>-42</td>
<td>-43</td>
<td>-11</td>
<td>-6</td>
</tr>
<tr>
<td>((III - II)/II)100</td>
<td>S.e.</td>
<td>±0.7</td>
<td>±1.5</td>
<td>±2.5</td>
<td>±7.2</td>
<td>±2.3</td>
<td>±2.1</td>
<td>±3.7</td>
<td>±3.9</td>
</tr>
<tr>
<td></td>
<td>% change</td>
<td>+2</td>
<td>+1</td>
<td>+19</td>
<td>+1</td>
<td>-30</td>
<td>-1</td>
<td>+16</td>
<td>-13</td>
</tr>
<tr>
<td></td>
<td>S.e.</td>
<td>±2.4</td>
<td>±4.1</td>
<td>±6.4</td>
<td>±10</td>
<td>±7.1</td>
<td>±3.3</td>
<td>±4.3</td>
<td>±5.9</td>
</tr>
</tbody>
</table>

* C.V. = the coefficient of variation = (s/z)100, where z is the arithmetic mean and s = ±√((Σx²/n - (Σx/n)²). S.e. = the standard error of the mean = s/√n = ŝ; per cent change = (Δ̂x/̂x)100, where Δ̂x is the difference between the means ̂x₁ and ̂x₂; s.e. of per cent change = (sΔ̂x/ẑx)100, where sΔ̂x = ±√((Δx̂)² + (̂x̂)²). The per cent changes as well as the coefficients of variation have been abbreviated to the nearest whole number.
† See the legend to Fig. 1.
‡ The figures in parentheses indicate the number of rats.
The greatest variation was found in the total lipides and arginase levels. This variability of the responses to protein starvation occurred despite the fact that the population was uniform as to strain, sex, and dietary history and carefully selected as to body weight (see Table II). It follows, that comparatively large groups must be employed for the evaluation of dietary effects on composition and enzymatic activity of liver tissue.

Table I deals with the qualitative alterations which result from protein starvation. The data show the pronounced fall of the protein and lipide phosphorus concentration and the concomitant rise of the concentration of the total lipides. Although the rise in total lipides was thus due to an increase in neutral fat, there was but a negligible diminution of the ratio of wet weight over dry weight, corresponding to a reduction of the water concentration from 69.7 to 69.3 per cent. This indicates that reduction of tissue hydration by the accumulation of fat was counteracted by a rise of the glycogen concentration. The occurrence of such a rise has been demonstrated in previous studies from this (3) and other (5) laboratories. Protein and total lipide concentrations remained essentially constant over the entire period of protein depletion studied, while the lipide phosphorus showed a considerable, though statistically not highly significant, increase.

Table I shows, furthermore, that protein depletion led to an altered composition of the liver protein as evidenced by its reduced enzymatic activity and a changed enzyme pattern. After 2 weeks of protein depletion, the arginase and rhodanese concentrations had dropped 42 per cent, while the Apyrase concentration had decreased but little. Contrary to the protein concentration, the enzymatic activity of the liver protein continued to change when the period of protein starvation was extended to 4 to 7 weeks. There was a pronounced fall of the arginase activity, while the rhodanese concentration remained constant. Not much significance can be attached to the observed changes in the Apyrase concentration, since the number of assays is too small. In those livers in which decreased Apyrase concentration was found, the decrease was small in comparison to that of the arginase concentration.

Table II furnishes supplementary information on the net losses in individual constituents and total mass of the liver and in body weight of the animals. The percentage loss in liver protein here recorded for 2 weeks of protein depletion was numerically identical with that previously obtained with a smaller group of depleted animals (3). The excellent reproducibility attests to the usefulness of the protein content as an index of the potentially effective liver mass. This index does not suffice, however, to reveal the full extent of reduction in potential metabolic capability of the organ, since, with 43 per cent of the protein, 68 per cent of both arginase and rhodanese and 50 per cent of the Apyrase activity were
**TABLE II**

Body Weight, Liver Weight, and Total Protein, Lipide Phosphorus, Lipide, and Enzyme Content of Liver

<table>
<thead>
<tr>
<th>Group No.*</th>
<th>Designation†</th>
<th>Body weight at operation</th>
<th>Change of initial body weight</th>
<th>Liver weight, per cent initial body weight</th>
<th>Content of total liver per 100 gm. initial body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>gm. per cent</td>
<td></td>
<td>mg. mg. mg. units X 10^{-3} units</td>
<td>Sample ATP&lt;sub&gt;A&lt;/sub&gt; Sample ATP&lt;sub&gt;B&lt;/sub&gt;</td>
</tr>
<tr>
<td>I</td>
<td>Mean</td>
<td>279 (16)†±6.0§(15)</td>
<td>3.75 (16)</td>
<td>678 (16) 3.62 (16) 138 (16) 355 (16) 621 (16)</td>
<td>67 (8) 155 (8)</td>
</tr>
<tr>
<td></td>
<td>C.v.</td>
<td>±3 ±33</td>
<td>±8 ±13 ±87 ±9 ±10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S.e.</td>
<td>±2.3 ±0.5</td>
<td>±0.074 ±13.4 ±0.121 ±9.1 ±8.1 ±15.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Mean</td>
<td>211 (65) −19 (65)</td>
<td>2.87 (65)</td>
<td>377 (65) 2.06 (65) 165 (65) 114 (65) 200 (65)</td>
<td>33.5 (14) 76 (11)</td>
</tr>
<tr>
<td></td>
<td>C.v.</td>
<td>±10 ±16</td>
<td>±13 ±7 ±13 ±36 ±19 ±12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S.e.</td>
<td>±2.5 ±0.5</td>
<td>±0.046 ±3.2 ±0.034 ±7.4 ±2.7 ±3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Mean</td>
<td>166 (11) −34 (11)</td>
<td>±2.59 (11)</td>
<td>342 (65) 2.19 (11) 150 (10) 73 (11) 177 (11)</td>
<td>31.4 (2) 60 (2)</td>
</tr>
<tr>
<td></td>
<td>C.v.</td>
<td>±11 ±10</td>
<td>±16 ±10 ±34 ±33 ±10 ±27</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S.e.</td>
<td>±5.6 ±1.1</td>
<td>±1.122 ±10.8 ±0.121 ±16.3 ±7.3 ±5.3</td>
<td>±2.6 ±9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% change</td>
<td>−24</td>
<td>−23 −43 −43 +20 −68 −68 −50</td>
<td>−51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I/100</td>
<td>S.e.</td>
<td>±3.3</td>
<td>±2.3 ±2.0 ±3.5 ±10.6 ±2.4 ±2.6</td>
<td>±3.9 ±3.7</td>
</tr>
<tr>
<td></td>
<td>% change</td>
<td>−21</td>
<td>−10 −9 +6 −9 −36 −12 −6</td>
<td>−21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II/100</td>
<td>S.e.</td>
<td>±2.9</td>
<td>±4.5 ±3.0 ±6.1 ±10.8 ±6.9 ±3</td>
<td>±9.8 ±11.8</td>
</tr>
</tbody>
</table>

* Group I, 7 days on 18 per cent casein diet; Group II, 13 to 17 days on protein-depleted diet; Group III, 28 to 49 days on protein-depleted diet.
† See foot-notes to Table I.
‡ The figures in parentheses indicate the number of rats.
§ Initial body weight here, as in the other groups, is the weight on the last day before transfer of the animals to the semisynthetic experimental diet, whereas for all other data concerning Group I the weight on the day of operation is taken as the initial body weight.
lost. It is of interest to note that the percentage losses due to extension of the period of protein starvation beyond 2 weeks amounted in most instances to about 10 per cent (see (III—II)/II, Table II). Exceptions showing significantly greater losses were the arginase content of the liver and the body mass of the animals. This correlation may possibly be of interpretative significance.

DISCUSSION

It has been noticed by previous investigators (23, 24) that changes in the nutritional state of the animal are paralleled by alterations of the enzymatic activity of liver tissue. Although these observations remain of value in so far as they refer to alterations of the enzyme pattern, they do not permit conclusions as to loss or gain of individual enzyme activities, since no correction had been made for concurrent changes in the composition of the unit mass of tissue which served as the basis of reference. Schultze (25) and Axelrod et al. (26), while studying liver enzymes in nutritional deficiencies, were the first to recognize this limitation of earlier investigations and to demonstrate that the total enzyme content of the organ furnished a better estimate of quantitative changes in enzyme activity.

In recent investigations from several laboratories estimations of the total enzyme content as well as of enzyme concentrations per unit weight of liver protein have been utilized. From the work of Miller (4) and of Schultz (27) on acute inanition it may be deduced that the losses in alkaline phosphatase, cathepsin, and catalase activity are approximately proportional to that of the liver protein, while the losses in xanthine dehydrogenase and cathepsin II (27) are much greater. Published data on complete protein starvation are scanty. Seifter et al. (5) found that D-amino acid oxidase and arginase were lost more rapidly than the liver protein. Benditt et al. (6) reported that in chronic protein deficiency the alkaline phosphatase content of the total liver remained constant, while cytochrome oxidase and succinoxidase decreased at a somewhat faster rate than the total liver protein.

The 30 to 40 per cent of liver protein that is rapidly lost during the initial stages of inanition or protein starvation is usually designated "labile" liver protein. Kosterlitz (28) has presented experimental evidence indicating that the labile protein arises from the breakdown of the cytoplasm of the parenchymatous cells. The author refers to the easily lost portion of the cytoplasm as "labile" cytoplasm. It is obvious that enzymes situated mainly in the "labile" cytoplasm will display a greater percentage loss than the total protein, those evenly distributed between labile and "stable"
cell components will decrease proportionally to the total protein, while the quantity of enzymes residing only in stable regions will remain constant.

From the data quoted from the literature as well as from our own results it appears that enzymes such as xanthine dehydrogenase, d-amino acid oxidase, rhodanese, and arginase, which are concentrated particularly in liver tissue and, presumably, mainly cytoplasmic constituents of parenchymatous cells, are lost more rapidly than the total liver protein. Phosphatases, on the other hand, which are detectable in cytoplasm and nucleus of parenchymatous and non-parenchymatous cells, decrease proportionally to or less than the total liver protein. It would seem, therefore, that the differential loss of individual enzymes roughly parallels their distribution between "labile" and "stable" cellular entities. Conversely, the percentage net losses may furnish an estimate as to what proportion of a given entity is lost in nutritional deficiencies. If, for instance, we assume that rhodanese and arginase are localized exclusively in the cytoplasm of the liver cells, then the data in Table II would indicate that 68 per cent of the cytoplasm was lost during 2 weeks of protein depletion. It is of interest to note that these enzyme assays fail to reveal any qualitative difference between the "labile" and the residual "stable" cytoplasm, since the ratio of rhodanese to arginase activity in the tissue from rats depleted of protein for 2 weeks was identical with that obtained in the casein-fed series. While this is in keeping with the results of chemical analyses of cytoplasmic constituents (28), conclusive evidence would require comparative assays upon a greater variety of enzymes and firmer knowledge as to their distribution.

There are indications that "labilty" of cell constituents may not be an inherent chemical characteristic, but may rather be conditioned by the metabolic state of the organism. In Miller's experiment (4) on acute inanition the alkaline phosphatase content of the liver decreased proportionally to the protein, while according to Benditt prolonged restriction of dietary protein resulted in similarly great losses of liver protein but failed to reduce the phosphatase content of the organ. The functional difference between both states of protein depletion apparently lies in the type and quantity of substrates which are metabolized by the liver cells. Our own experiments have shown that in advanced stages of complete protein starvation arginase activity diminished faster than rhodanese activity or total liver protein. It is possible that the degree of saturation with substrate is responsible for the differential preservation of enzyme activity under various conditions of protein depletion. Evidence in support of this view will be presented in a forthcoming paper.
LIVER ENZYMES IN PROTEIN DEPLETION

SUMMARY

After a 2 week period of protein depletion of rats the livers had lost 43 per cent of their protein and lipide phosphorus, whereas the amount of total lipides remained fairly constant. The loss of 68 per cent in total arginase and rhodanese activity of the organ greatly exceeded that in total protein, while the reduction in adenosine pyrophosphatase activity of 50 per cent more nearly approached the protein loss.

Extension of the period of depletion up to 4 to 7 weeks resulted in additional small losses of the order of 10 per cent in most liver components, with the exception of the total arginase activity, which decreased an additional 30 per cent.

The significance of the differential losses in enzyme activity has been discussed.

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ARGINASE, ADENOSINEPYROPHOSPHATASE, AND RHODANENSE LEVELS IN THE LIVER OF RATS

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