ARGINASE, ADENOSINEPYROPHOSPHATASE, AND
RHODANESE IN REGENERATING RAT LIVER*

By OTTO ROSENTHAL, CHARLES S. ROGERS,† HARRY M. VARS,
and COLIN C. FERGUSON†

With the Assistance of JOAN W. ZERBE AND BEATRICE G. NOVACK

(From the Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania)

(Received for publication, November 7, 1950)

Gurd, Vars, and Ravdin (2) have shown that the restoration rate of liver protein following surgical removal of 70 per cent of the organ was greater in protein-depleted than in well nourished rats. These authors suggested that the stimulus to recovery of liver protein was more potent in the animal with the more severe reduction of liver substance. The nature of the stimulus to regeneration is not known. It seemed to us that information on the mechanism of growth responses could be obtained by studying the relationship between restoration of enzyme activity and of total liver protein in different states of nutrition. This paper deals with the restoration of arginase, adenosinepyrophosphatase, and rhodanese after partial heptectomy. Data on the decrease of these enzymes on protein depletion have been presented previously (3).

Procedure

Male rats of the Wistar strain, weighing approximately 250 gm., were kept either on a semisynthetic 18 per cent casein-containing diet or on a corresponding protein-free régime for the periods of time indicated in Tables I and II. 70 per cent of the liver was then removed surgically and the animals were sacrificed at intervals varying from half a day to 8 days after operation. Postoperatively, two types of nutritional states were studied: (1) feeding of the preoperative diet ad libitum, and (2) fasting for sacrifices up to the 4 day interval, and 4 days fasting followed by 4 days feeding ad libitum for the 8 day interval. The composition of the diets, operative procedure, and methods of chemical analysis have been described by Gurd, Vars, and Ravdin (2). The methods of enzyme assay will be found in our previous publication (3).

* This study was carried out under contract between the Department of the Army and the University of Pennsylvania. A preliminary report has appeared (1).
† Harrison Fellow in Surgical Research.
As in the previous publication (3), results of the analyses have been expressed in terms either of concentration per gm. of liver protein or of content of total liver. The term content refers to the quantity of a constituent or the number of enzyme units per total liver mass per 100 gm. of initial body weight. The initial body weights of protein-fed and protein-starved animals were taken as the weight at operation and the weight on the day of transfer to the protein-free diet, respectively. The total liver weight on the day of operation was computed by multiplying the amount excised (left lateral and median lobes) by the factor 1.43 (3). Enzyme units are expressed as micromoles of assayed reaction product formed per minute under the standard conditions previously defined.

Since this study is concerned with relative rates of regeneration rather than with absolute enzyme activities or amounts of constituents, the majority of results are recorded as the ratio of the sacrifice value to the value obtained at partial hepatectomy, the latter value being taken as unity. In this way each value has its individual internal control. In Tables I and II the ratios are denoted by the quotients s/h. In the case of concentrations, the quotient equals unity if the restoration of the quantity under examination is proportional to that of the total liver protein. With reference to content values, unity quotients signify complete restoration of a given entity, while a quotient of 0.30 indicates complete failure to regenerate. It should be remembered that the value of 0.30, the relative size of the remnant left in situ at operation, represents an average with a standard deviation of ±0.015 (3). A considerable variation of the s/h values must therefore be expected and their reliability as an index of growth is comparatively low as long as regeneration is small. The quotients computed on the basis of concentrations, on the other hand, are derived from quantities determined directly and will be mainly used for estimating relative restoration rates of entities other than protein.

Table I provides information on the restoration of liver enzymes and liver protein in protein-depleted rats after partial hepatectomy. The data cover the first 2 postoperative days, during which period the animals were supplied with protein-free food and water ad libitum. The results are expressed in terms of relative contents of liver constituents at the day of sacrifice.

Inspection of the data shows that on the average the protein content of the liver remnant increased by 43 per cent (from 0.3 to 0.43) in 1 day and by 90 per cent (from 0.3 to 0.57) in 2 days. Restoration of rhodanese and apyrase paralleled that of the protein, although enzyme restoration
was somewhat slower. Neither postoperative caloric intake nor duration of preoperative protein depletion greatly affected the restoration rate of protein and the two enzymes. In contrast, arginase restoration was

**Table 1**

**Enzyme and Protein Restoration after Partial Hepatectomy of Protein-Depleted Rats**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Depletion period</th>
<th>Body weight</th>
<th>Total postoperative intake</th>
<th>Protein content of liver at operation</th>
<th>Related content of liver at sacrifice, ( \frac{t}{s} )</th>
<th>Mitotic index*, †</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial*</td>
<td>At operation</td>
<td>At sacrifice</td>
<td>Protein</td>
<td>Arginase</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>251</td>
<td>76</td>
<td>73</td>
<td>16</td>
<td>386</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>238</td>
<td>76</td>
<td>73</td>
<td>16</td>
<td>301</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>244</td>
<td>86</td>
<td>83</td>
<td>12</td>
<td>351</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>260</td>
<td>84</td>
<td>84</td>
<td>3</td>
<td>386</td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>273</td>
<td>70</td>
<td>69</td>
<td>14</td>
<td>298</td>
</tr>
<tr>
<td>6</td>
<td>31</td>
<td>263</td>
<td>73</td>
<td>69</td>
<td>10</td>
<td>336</td>
</tr>
<tr>
<td>7</td>
<td>49</td>
<td>260</td>
<td>58</td>
<td>56</td>
<td>13</td>
<td>306</td>
</tr>
<tr>
<td>8</td>
<td>49</td>
<td>258</td>
<td>61</td>
<td>62</td>
<td>0</td>
<td>346</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 days after operation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|               |                  |             |                            |                                       |         |          |             |         |
| 9             | 14               | 244         | 83                         | 79                                   | 62      | 365      | 0.60        | 0.77    | 0.57    | 0.52   | 4      |
| 10            | 14               | 270         | 80                         | 77                                   | 40      | 341      | 0.60        | 0.87    | 0.42    | 0.52   | 4      |
| 11            | 14               | 238         | 73                         | 70                                   | 30      | 386      | 0.51        | 0.75    | 0.44    | 0      |
| 12            | 14               | 248         | 83                         | 80                                   | 26      | 365      | 0.54        | 0.75    | 0.52    | 0      |
| 13            | 13               | 201         | 84                         | 78                                   | 6       | 355      | 0.66        | 1.55    | 0.47    | 0.65   | 1      |
| 14            | 49               | 246         | 65                         | 60                                   | 26      | 305      | 0.52        | 1.24    | 0.47    | 0      |
| Mean          |                  |             |                            |                                       |         |          |             |         |         |

*Initial body weight (i.b.w.) = the weight on the day of transfer to the protein-free diet.
† (Content of total liver at sacrifice)/(content of total liver at operation). Contents of total liver and of liver remnant at operation taken as 1.00 and 0.30, respectively.
‡ Number of mitoses per 1000 nuclei of liver cells.

markedly influenced by the metabolic state of the animal. If food intake was negligibly small (Experiments 4, 8, and 13), the arginase content of the liver remnant at sacrifice exceeded by 40 to 80 per cent the estimate for the total liver at the time of operation. If, on the other hand, the caloric intake approached the minimum requirement of 20 to 25 calories per day
for protein-depleted rats, the rate of arginase restoration was not greater than that of protein restoration (see Experiments 1 and 2). The data show, furthermore, that at equal caloric intake arginase restoration was greater in the more severely depleted animal (cf. Experiments 3 and 7 or Experiments 12 and 14); i.e., in the liver with lower initial arginase activity (3).

The inverse relationship between caloric intake of the protein-starved animal, and the arginase level of the regenerating liver appeared to indicate that the restoration rate of the enzyme was related to the postoperative rise of the endogenous nitrogen catabolism. To test this interpretation, arginase restoration was compared with urinary nitrogen excretion under the conditions of enforced caloric intake and of fasting. Results in terms of relative liver contents and relative rates of nitrogen excretion are recorded in Table II. In the animals of Groups 1 and 2 the postoperative caloric intake was raised to 80 to 90 per cent of the preoperative level through oral and parenteral administration of a glucose-saline solution. The protein catabolism, as judged from the unchanged urinary nitrogen excretion, did not rise and the restoration of arginase remained about proportional to that of the liver protein. In the fasted groups, Nos. 3 and 4, on the other hand, in which urinary nitrogen excretion almost doubled following the partial hepatectomy, the arginase content of the regenerating liver remnant was nearly 30 per cent higher than that of the total liver at the time of operation. The data show, furthermore, that high intake of non-protein calories also reduced protein restoration. This is to be expected from the protein-sparing action of glucose, since in protein starvation the formation of new liver protein occurs at the expense of extrahepatic protein breakdown. The diminution of protein restoration in Group 1, however, was greater than could be explained by the observed caloric intake and nitrogen output. Edema formation, an unavoidable side effect of glucose-saline administration, was not responsible for this result. For in the fasted rats of Group 4, in which parenteral sucrose application combined with oral supply of plain saline produced marked fluid retention and edema, protein restoration was not impaired. It is possible that in the animals of Group 1 the greatly increased urinary volumes due to excessive fluid intake (up to 80.0 ml. of glucose-saline per day) resulted in a too rapid loss of nitrogenous metabolites in the urine.

Fig. 1 illustrates the time-course of liver protein restoration in protein-fed and protein-starved animals during the first 8 days following partial hepatectomy. It also compares the effect of a postoperative fast on liver protein regeneration in both states of nutrition. From the shape of the curves it is evident that the initial rate of regeneration was greatest in the protein-depleted state and that regeneration came to a virtual standstill.
<table>
<thead>
<tr>
<th>Group No.*</th>
<th>Postoperative diet</th>
<th>Oral†</th>
<th>Parenteral‡</th>
<th>Caloric intake</th>
<th>Relative N excretion</th>
<th>Relative liver content at sacrifice, (\frac{t}{F})</th>
<th>Mitotic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (4)</td>
<td>D. + 10% glucose in saline</td>
<td>20% glucose in saline</td>
<td>60</td>
<td>±5.2</td>
<td>1.18</td>
<td>±0.032</td>
<td>±0.031 ±0.028 ±0.020 ±0.041 ±0.051</td>
</tr>
<tr>
<td>2 (4)</td>
<td>D. + tap water</td>
<td>Same</td>
<td>60</td>
<td>±9.1</td>
<td>1.00</td>
<td>±0.050</td>
<td>±0.029 ±0.032 ±0.017 ±0.009 ±0.054</td>
</tr>
<tr>
<td>3 (4)</td>
<td>Tap water</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>1.98</td>
<td>±0.106</td>
<td>±0.121 ±0.020 ±0.032 ±0.035 ±0.218</td>
</tr>
<tr>
<td>4 (2)</td>
<td>Plain saline</td>
<td>20% sucrose in H₂O</td>
<td><strong>&quot;</strong></td>
<td>1.84</td>
<td>±0.265</td>
<td>±0.015 ±0.020 ±0.032 ±0.025 ±0.010</td>
<td>9</td>
</tr>
</tbody>
</table>

* Number of animals given in parentheses. 2 weeks preoperative protein depletion. Sacrificed 2 days after operation.
† D = protein-free diet. Saline = 0.85 per cent NaCl solution. Food and drink supplied ad libitum.
‡ Either 6 × 2 ml. or 5 × 2.5 ml. injected at 2 hour interval during the day time, starting 2 hours after operation.
§ The caloric intake for the last 2 preoperative days averaged 76 kilocalories.
|| (Postoperative urinary N excretion)/(N excretion during last 2 preoperative days). The latter averaged 98 mg.
between the 2nd and 4th postoperative days unless protein was supplied with the food. This is confirmation of previous studies from this laboratory (2). In addition the graph shows that a postoperative fast accelerated the initial rate of protein restoration in the depleted liver, while slightly depressing it in the animal which was protein-fed up to the time of operation.

It is of importance to note that under no nutritional condition was increased mitotic activity observed before the 24 hour interval, which was also the point of highest activity. Although the mitotic indices varied from 0 to 65 at this interval of time (see Fig. 2), the averages of 20 to 30 were of the same order in the individual nutritional groups. There was thus no definite correlation between rate of protein synthesis or absolute amount of protein laid down and mitotic activity, with the sole limitation that low mitotic indices, between 0 and 5, occurred in livers in which protein restoration was below the average for the group. Since there is good evidence (4, 5) that protein depletion does not cause a decrease in the number of liver cells, it would appear that the restoration of the cytoplasmic protein which has been lost during the period of depletion is not an essential preliminary of cell division.

Fig. 3 serves to illustrate to what extent the regeneration of enzyme
activity deviated from that of the total liver protein. Activity levels have been expressed in terms of enzyme concentration per gm. of protein and are presented as the ratios of concentration at sacrifice to concentration at operation.

It is evident from Fig. 3 that the regeneration of rhodanese lagged behind that of the total liver protein under all the nutritional conditions studied. Comparison of Fig. 3 with Fig. 1 reveals that the concentration of this enzyme started to fall when protein regeneration began to increase, passed through a minimum during the subsequent period of rapid protein regeneration, and gradually rose when protein regeneration slowed down, although levels at the time of operation were in general not reached during the period of observation. While in the protein-depleted rats which were fed ad libitum after operation (Group II) the individual means at the 1st and 2nd postoperative days were not significantly smaller than unity, the combined average of 0.85 ± 0.042 for the two intervals was significantly reduced. The graph also shows that the concentration changes of apyrase closely followed those of rhodanese.

The regeneration pattern of arginase was just the inverse of that of the other two enzymes. The highest rates of regeneration, up to twice that of

---

1 Standard error of the mean.
the total liver protein, were obtained during the 1st postoperative days. After the 2nd day the rate of arginase regeneration slowed down much faster than that of the total liver protein. This resulted in a return of the arginase concentrations to almost normal levels during the subsequent 6 days. In the protein-depleted animals the fall of the arginase concentration was due in part to an absolute loss of arginase activity. At the 2nd postoperative day the relative arginase content of the regenerating livers was 1.29 ± 0.158 and 0.78 ± 0.029 in Groups I and II, respectively. At the 8th day, the corresponding mean values were 0.88 ± 0.076 and 0.61 ± 0.032. The respective differences of 0.41 ± 0.175 and 0.17 ± 0.043 were statistically significant.

To demonstrate the relationship of arginase regeneration to the protein catabolism of the animal, Fig. 4 shows the mean values for the relative
rates of urinary nitrogen excretion during the entire postoperative period in the four nutritional groups. It is obvious that there was a remarkable similarity of the nitrogen excretion curves and the corresponding arginase concentration curves as given in Fig. 3.

Although, on the average, arginase regeneration exceeded that of the total liver protein only if the nitrogen catabolism rose significantly above the preoperative level, increments of the arginase concentration from 10 to 60 per cent were found in half of the eight livers from Group IV of Fig. 3 which were assayed during the first 24 postoperative hours. In these instances the average increase in total liver protein amounted to but 7 per cent, as compared with 20 per cent in the other four livers in which no rise of the arginase concentration took place. It appears possible that there was always an initial increase in arginase activity of the liver remnant following partial hepatectomy, but that this increase became detectable only if it was particularly large, either absolutely or with respect to the subsequent increase in total liver protein. This view is supported by the fact that under all nutritional conditions the initial rate of arginase regeneration was significantly greater than that of rhodanese. For instance in Group III of Fig. 3 the relative enzyme concentrations for the first 2 postoperative days averaged 0.93 ± 0.031 and 0.75 ± 0.0067 for arginase and rhodanese respectively. The difference of 0.18 ± 0.032 was highly significant statistically.
Fig. 5 furnishes information on the changes of lipide phosphorus and total lipide concentrations after partial hepatectomy. As in the case of enzyme concentrations, the protein has been used as the basis of reference. Temporary increases of the lipide phosphorus concentration, ranging from 10 to 30 per cent, were noticed in all groups during the 1st postoperative days. While the initial rate of phospholipide regeneration was thus somewhat faster than that of the total liver protein, no relation to the regeneration pattern of enzymes was found.

The increase of the total lipide concentration generally exceeded that of the lipide phosphorus to such an extent that the contribution of phospho-
lipides to the increase can be ignored. The curves thus illustrate the rapid accumulation of neutral fat in the liver remnant after partial hepa-
tectomy. The maximum was reached at the end of the 1st postoperative
day. The relative increases in the fasted groups, Nos. I and IV, were
larger than in the corresponding Groups II and III fed ad libitum. The
relative increase also greatly depended on the fat concentration in the
liver tissue at the time of operation, being smaller in the protein-depleted
than in the protein-fed animals, which had less liver fat preoperatively.
Not too much significance should be attached to the average increases
recorded in the graph, since the individual variations of the total lipide
concentrations were extremely large in all groups, both before and after
operation. These individual variations did not appear to have any direct
bearing on the rates of protein and enzyme regeneration or on the amount
of liver protein laid down. The main significance of the data lies in the
fact that they demonstrate how greatly and in what unpredictable manner
the chemical composition of liver tissue may change after partial hepatec-
tomy. It is obvious that during the early phases of regeneration the
weight of the liver remnant is not a reliable index of tissue growth or an
adequate basis of reference for judging enzyme regeneration.

DISCUSSION

Our experimental results indicate that restoration of protein and resto-
ration of cell number occurred in response to different stimuli. The rate
of protein restoration appeared to depend on how rapidly, and to what
extent, the level of nitrogenous metabolites in the circulation rose above
the preoperative equilibrium level with the hepatic protein. This in-
terpretation is consistent with the accepted concept of a dynamic equilib-
rium between protein and free amino acids. In the maintenance of such
equilibrium, however, the rate of supply of metabolites to the liver consti-
tutes an important factor, since amino acids are constantly withdrawn
from the equilibrium by irreversible reactions. It follows that, even in
the absence of elevated amino acid levels in the portal blood, the in-
creased blood supply to the liver remnant after partial hepatectomy will
stimulate protein synthesis provided that the increased supply of metabo-
lites exceeds the loss through oxidation and urea formation. Such a
mechanism offers a reasonable explanation of the absence of liver regenera-
tion in experimental animals in which the portal blood flow was partially
dverted from the liver remnant through a by-path between the portal
vein and the vena cava (6).

As to the stimulus for cell division, our experiments demonstrated
that the amount of protein laid down was not the decisive factor. From
the changes in the enzymatic activity of the regenerating organ it appears
possible that an altered composition of the newly formed protein could have been of greater importance. The stimuli to preferential restoration of individual enzymes thus deserve special consideration.

The most striking enzymatic change during the premitotic phase of liver regeneration concerned the activity of arginase. It was shown that the rate of restoration of this enzyme was geared to the protein catabolism of the animal. Oppenheimer and Flock (7) reported that the concentration of alkaline phosphatase in the liver remnant rose rapidly after partial hepatectomy of normal rats fed ad libitum. We (8) have confirmed this observation under the four nutritional conditions employed by us. Although the degree of the rise was influenced by the metabolic state of the animal, the precise interrelationship remains to be established. Evidence in support of hormonal control of the liver levels of both arginase and alkaline phosphatase has been presented by several investigators (9, 10). Opinions are divided in regard to the question whether the enzyme responses are primary effects of hormonal stimulation or whether they are secondary to the stimulation of the metabolism (11).

We have shown that under all nutritional conditions the concentration of rhodanese and apyrase started to fall toward the end of the premitotic phase of liver regeneration, to pass through a minimum during the subsequent period of highest mitotic activity. Novikoff and Potter (12) apparently observed a similar phenomenon with malic dehydrogenase, cytochrome c reductase, succinoxidase, and oxalacetate oxidase. The fall in nitrogen content of cytoplasmic liver fractions, as reported by Price and Laird (13), coincides with the period of reduced enzyme concentrations. This indicates that differential growth rates of morphological tissue components rather than specific enzymatic responses are involved.

These two-directional changes in the enzyme activity of regenerating liver tissue lead to a distortion of the original enzyme pattern which becomes maximum at the onset of increased mitotic activity and is alleviated in the course of cell division and additional protein synthesis. In view of the fact that the rise in arginase activity is conditioned by the metabolic state of the animal, one may tentatively suggest that excessive production of individual enzyme proteins in response to an increased functional load initiates the process of cell division, in the course of which balance between the cellular components is restored. A clearer understanding of the mutual interrelationship of these processes will require more information on enzyme location, as well as on the relation of amount and activity of enzymes to levels of substrates and endocrine factors.

*Its significance is not quite clear since enzyme concentrations are reported on the basis of dry weight. The pitfalls of this method have been pointed out above.*
SUMMARY

The initial rate of restoration of hepatic protein after partial hepatectomy was greater in protein-depleted than in protein-fed rats, accelerated by fasting in the former animals, and slightly reduced in the latter. The time of onset and rate of cell division, on the other hand, were identical in the nutritional groups, suggesting that composition rather than quantity of the regenerated protein was the factor determining mitotic activity. Changes in composition were evidenced by the fact that the arginase activity of the protein tended to increase during the premitotic phase of regeneration, while, toward the end of this phase, the concentration of rhodanese and adenosinepyrophosphatase started to decrease. It was shown that the regeneration of arginase was stimulated by increases in the protein catabolism of the animal, whereas the reduced restoration of the other two enzymes was independent of the nutritional state. The interrelationship of protein restoration, cell division, and enzymatic responses has been discussed.

BIBLIOGRAPHY

ARGINASE,
ADENOSINEPYROPHOSPHATASE, AND
RHODANASE IN REGENERATING RAT
LIVER

Otto Rosenthal, Charles S. Rogers, Harry M.
Vars, Colin C. Ferguson and With the
assistance of Joan W. Zerbe and Beatrice G.
Novack


Access the most updated version of this article at
http://www.jbc.org/content/189/2/831.citation

Alerts:

- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail
alerts

This article cites 0 references, 0 of which can be
accessed free at
http://www.jbc.org/content/189/2/831.citation.full.h
tml#ref-list-1