Changes in Hepatic Metabolism Associated with Carcinogenesis or Regeneration in Rat Liver*

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SUMMARY

The technic of isolated liver perfusion was used to study protein synthesis and amino acid catabolism in normal rat livers, rat livers undergoing azo dye carcinogenesis, and in regenerating rat livers. The results demonstrate that rat livers undergoing carcinogenesis or regeneration have decreased catabolism of amino acids and increased synthesis of protein as compared with normal livers. Although the livers undergoing carcinogenesis and the regenerating livers had a qualitative similarity in amino acid metabolism, they showed quantitative differences when the metabolism of specific amino acids was investigated.

For several years we have been investigating the development of liver tumors in rats maintained on a diet containing 3'-methyl-4-dimethylaminoazobenzene.1 We have reported results indicating that, during the precancerous period, the isolated, perfused livers of such rats show increased synthesis of liver and plasma protein and decreased catabolism of amino acids. Associated with this increased synthetic activity, the livers contain an increased level of free amino acids. We have postulated that these changes are related to a biochemical block or enzyme deletion occurring during carcinogenesis (3-6).

Two primary objectives of the present investigations were to extend our studies to include the production of urea nitrogen from the nitrogen of a number of specific amino acids by the perfused liver and to compare the metabolism of specific amino acids in normal livers with metabolism in livers undergoing carcinogenesis or regeneration. We expect such an approach to enable us to locate changes in amino acid nitrogen metabolism at the level of the biochemical reactions involved and to distinguish those metabolic changes intimately associated with carcinogenesis from changes related to concomitant alterations in the organ such as cellular proliferation or changes in the proportions of liver cell types as they occur during regeneration.

MATERIALS AND METHODS

The technic of isolated rat liver perfusion has been described previously in detail (3, 20, 21).

The rats used were adult, 200- to 300-gm., Sprague-Dawley or Wistar males, obtained commercially and fasted 24 hours prior to use. Blood for the perfusate was drawn from the abdominal vena cava of normal rats under ether anesthesia.

D-Glucose (100 mg.) and the particular amino acid to be studied were dissolved in Ringer's solution. L-Lysine-6-C14 was added in all experiments. The pH of the supplement was adjusted to 7, and this solution was added to the blood perfusate. The circulating blood glucose level was 105-135 mg. per cent throughout the perfusion.

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1Obtained from Hemlock Hollow Farms, Wayne, New Jersey, or Hilltop Caviary, Scottsdale, Pennsylvania.
Experimental liver carcinogenesis was initiated by feeding rats for 8 weeks a basal diet containing 0.06 per cent 3'-Me-DAB (3). The normal and partially hepatectomized rats were fed the same diet without added carcinogen.

Regenerating livers were removed for perfusion from rats 24 hours following subtotal hepatectomy. In order not to complicate our results by using regenerating livers which were considerably smaller than the other livers in the study, we removed only one lobe (about 3.5 gm.) from relatively large livers (about 12 gm.). Thus, regenerating liver weight was comparable to the weight of the other livers used.

Blood urea nitrogen was determined by the Conway microdiffusion method (11). Blood glucose was measured by the Nelson method (23). Blood amino acid nitrogen was determined by Cocking and Yemm’s (10) modification of the method of Moore and Stein (22). Total liver and plasma proteins were assayed by Oyama’s modification (24) of the method of Lowry and Rosenbraugh (18).

L-Lysine-6-C14 was obtained from Dr. Leon Miller. Five mg. (5 μc.) were added to the perfusate in each experiment. Radioactivity in liver and plasma proteins was assayed by converting them to carbon dioxide with the oxidizing mixture of Van Slyke (34) and by measuring the activity in an ionization chamber coupled with a Carey Model 31 vibrating reed electrometer and suitable linear recorder (Texas Instrument Co.). Expired carbon dioxide from each perfusion experiment was trapped in 40 per cent potassium hydroxide. The CO2 released from the latter solution by sulfuric acid was assayed for radioactivity in the same manner.

**RESULTS**

In Table 1 are data on the production of urea nitrogen from a number of specific amino acids added as supplements to the perfusate. With the exception of experiments in which glutamine or ammonia was used, there was a marked impairment of urea nitrogen production in the perfused livers of rats fed 3'-Me-DAB. This confirms our previously published observations on livers undergoing carcinogenesis. We interpreted our earlier results as indicative of a biochemical block on the metabolic pathway from amino acid nitrogen to urea nitrogen.

Several other points relative to the data in Table 1 should be noted. There is a marked decrease in the production of urea nitrogen from citrulline, aspartic acid, or arginine in the perfused livers of azo dye-fed rats. This is compatible with our earlier suggestion of arginase inhibition or depletion during carcinogenesis. Conversion of the nitrogen of the added amino acid to urea nitrogen is decreased in the azo dye livers with the exceptions noted above, even though, as we have pointed out, individual amino acids differ in their contribution to urea synthesis (2). Others have recognized this variation in the contribution of different amino acids to urea synthesis in the normal animal (17).

A further point illustrated in Table 1 is the decreased production of urea nitrogen from some amino acids in the perfused, regenerating liver.

**TABLE 1**

<table>
<thead>
<tr>
<th>Amino acid added</th>
<th>Normal</th>
<th>3'-Me-DAB</th>
<th>4-Hour regenerating</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arginine†</td>
<td>34 (8)</td>
<td>16 (7)</td>
<td>30 (4)</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>31 (10)</td>
<td>30 (12)</td>
<td>32 (4)</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>30 (4)</td>
<td>9 (4)</td>
<td>7 (4)</td>
</tr>
<tr>
<td>Glycine</td>
<td>20 (4)</td>
<td>18 (4)</td>
<td>16 (4)</td>
</tr>
<tr>
<td>L-Asparagine</td>
<td>25 (4)</td>
<td>8 (6)</td>
<td>16 (4)</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>24 (4)</td>
<td>12 (4)</td>
<td>12 (4)</td>
</tr>
<tr>
<td>L-Citrulline</td>
<td>21 (4)</td>
<td>14 (4)</td>
<td>24 (4)</td>
</tr>
<tr>
<td>Ammonia</td>
<td>20 (4)</td>
<td>20 (4)</td>
<td>10 (4)</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>16 (5)</td>
<td>10 (4)</td>
<td>9 (4)</td>
</tr>
<tr>
<td>L-L-aspartic acid</td>
<td>16 (4)</td>
<td>7 (5)</td>
<td>12 (4)</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>18 (4)</td>
<td>7 (4)</td>
<td>7 (2)</td>
</tr>
<tr>
<td>L-Valine</td>
<td>12 (4)</td>
<td>7 (3)</td>
<td>7 (2)</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>15 (4)</td>
<td>5 (4)</td>
<td>7 (2)</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>11 (6)</td>
<td>5 (5)</td>
<td>10 (5)</td>
</tr>
</tbody>
</table>

* 1½ mmoles amino acid added.
† 1 mmoles amino acid added.
* These livers, however, in contrast to the livers undergoing carcinogenesis, are capable of producing from arginine and citrulline an amount of urea nitrogen equivalent to that produced by the normal livers. They are also capable of producing normal amounts of urea nitrogen from glutamine and ammonia.

We conclude from these data that the impairment of urea production present in these regenerating livers is the result of a derangement of the synthetic process at some metabolic reaction occurring outside the Krebs-Henseleit urea cycle. This is in contrast to the explanation suggested for livers undergoing carcinogenesis. In the latter case decreased arginase activity could account for the depression of urea-nitrogen production.

In Chart 1 are shown curves illustrating the cumulative per cent of the added L-lysine-6-C14 converted to and expired as C14O2 by normal, precancerous, and regenerating livers during 4-hour perfusion. The over-all oxidative catabolism of the
added lysine appears to be quantitatively identical in the latter two liver types and only 50 per cent of that in the normal livers.

The data in Charts 1–3 represent averages derived from all experiments. The results were not affected by variation in the amino acid supplement.

The data in Chart 2 show the per cent of added carbon-14 activity incorporated into the plasma proteins of the blood perfusate. The precancerous livers showed enhanced incorporation as reported previously. The regenerating livers showed incorporation into plasma protein similar to that of normal livers.

The data in Chart 3 on incorporation of lysine-\textsuperscript{14}C into liver proteins are in contrast to the results depicted in Chart 2. In this case protein synthesis in both precancerous livers and regenerating livers, estimated by lysine-\textsuperscript{14}C incorporation, was considerably higher than that in the normal livers.

The levels of free amino acid nitrogen in the three types of liver are shown in Chart 4. No attempt to determine intracellular volume or con-
centration was made; however, the "availability" of amino acid substrate for protein synthesis in the precancerous and regenerating livers is apparent. It is evident that free amino acid levels remained high in the regenerating liver, even though the total amount of liver decreased.

DISCUSSION

We have previously published data relative to decreased amino acid catabolism and increased protein synthesis in precancerous livers and in the livers of rats fed acetylaminofluorene (3-6). The data showing that this decrease is related to decreased arginase activity have relevance with respect to the deletion hypothesis of cancer development as proposed by Potter (25-27) and applied to azo-dye carcinogenesis by the Millers (19).

Our conclusion that arginase activity in regenerating liver is not reduced is at variance with the findings of Rosenthal that arginase activity was reduced 40-50 per cent 24 hours after subtotal hepatectomy (31). His rats, however, were protein-depleted before operation, and he determined arginase by enzyme assay. He also found urea formation uninfluenced by partial hepatectomy. Thomson found a negligible change in arginase activity following partial hepatectomy (32). Greenbaum has reported a decrease in glutamic dehydrogenase and glutamic-aspartic transaminase following partial hepatectomy (15). Our findings of decreased urea synthesis in such livers could be the result of decreased activity of such enzymes.

The increased level of free amino acids in regenerating livers represents a teleologically satisfying situation in view of the high rate of tissue protein synthesis in this organ. However, these differences between normal livers and livers 24 hours following subtotal hepatectomy are much greater than the differences found by Ferrari and Harkness (13). These authors found only small differences in the total or intracellular free amino acids 24 hours after partial hepatectomy. They also report decreases of about 20 per cent in the urea concentration in regenerating livers.

With regard to the synthesis of plasma protein during liver regeneration our data are in substantial agreement with the findings of other authors. Chanutin described a decrease in the concentration of all plasma proteins following partial hepatectomy (9). DeLamirande and Cantero found a decrease in plasma albumin and an increase in plasma globulins during liver regeneration and quantitatively similar changes in the precancerous livers of rats fed DAB (18). Roberts has demonstrated an over-all decrease in serum protein concentration after partial hepatectomy (29). These studies and those reported in this paper gain interest in view of the report by Glinos that the stimulus to cellular proliferation in the regenerating liver is related to a decrease in the concentration of circulating serum albumin as demonstrated by increased mitoses in the liver of normal rats following plasmapheresis (14). A lowered serum albumin level during the early period of regeneration, followed by a steady rise in this material, invites speculation that feedback related to serum albumin concentration may be responsible for the limitation of growth which occurs in liver regeneration as the tissue approaches normal size and function.

An increased incorporation of C\textsuperscript{14}-labeled glycine or leucine into the proteins of regenerating liver tissue has been reported by others (8, 16, 28, 39). However, Campbell has concluded from calculations based on the ratio of the radioactivity of free amino acid to that of the protein-bound amino acid that the rate of protein synthesis in normal liver is greater than that in DAB-induced liver tumors (7).

In conclusion we may point out that our data demonstrate similarities in amino acid anabolism and catabolism in the two types of rapidly growing liver studied. The two processes, however, have quantitative differences. The controlled growth during tissue regeneration, as contrasted with the relatively unrestrained proliferation of neoplastic tissue, certainly raises many questions. The mechanism exerting this control cannot be defined. However, biochemical feedback related to the synthesis and level of serum albumin may constitute the control over hepatic cell division (14). The role of depression of catabolism by enzyme deletion and an associated increase in synthetic activities is certainly worth further study in this connection. Tissue-specific factors which inhibit or stimulate cell division merit consideration. The work of Bucher, Scott, and Aub (1) and the contradictory data obtained by Rogers (30) are relevant to this aspect of the problem.

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REFERENCES


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