Comparative Biochemistry of Hepatomas

V. Studies on Amino Acid Incorporation in Liver Tumors of Different Growth Rates*

SHREEPAD R. WAGLE, HAROLD P. MORRIS, AND GEORGE WEBER

(Department of Pharmacology, Indiana University School of Medicine, Indianapolis, Ind.; and Laboratory of Biochemistry, National Cancer Institute, N.I.H., P.H.S., Bethesda, Md.)

SUMMARY

The incorporation and oxidation of alanine, aspartate, glycine, serine, isoleucine, and valine were compared in normal and regenerating liver and in hepatomas of different growth rates. For the incorporation of amino acid into protein a rough correlation with the growth rates of liver tumors was observed.

For all amino acids tested regenerating liver showed an increased incorporation (125–168 per cent) as compared with the values found in the liver of sham-operated rats. The incorporation into the slowly growing H-35 was in normal range; in the more rapidly growing 7288-C there was an increased incorporation (127–155 per cent); in the two most rapidly growing tumors, 3924-A and 3683, there was a very marked increase (174–194 per cent).

The oxidation of amino acids was essentially unaltered in the regenerating liver and in hepatomas H-35 and 7288-C. In the rapidly growing tumors the oxidation of isoleucine and valine was increased (145–197 per cent). The purpose of these studies is to contribute to the elucidation of the metabolic significance of biochemical parameters which underlie the different biological behavior of liver tumors of different growth rates. Preceding reports in this series demonstrated that the activities of certain enzymes involved in carbohydrate metabolism show a correlation with growth rates (11–14). It has also been shown by means of isotopic methods that certain carbohydrate metabolic activities also exhibit a correlation with the biological behavior of liver tumors (1, 9, 15). On the other hand, these investigations have drawn attention to the fact that some enzyme systems as well as certain metabolic parameters had no apparent relationship with the growth rate of the examined liver tumors. In an effort to discover further biochemical tools to characterize the metabolic properties of liver tumors, studies were carried out to explore protein metabolism in a series of liver tumors of different growth rates. The present work describes the incorporation of various amino acids into protein and the oxidation of amino acids in normal resting and regenerating liver, in slowly growing (H-35, 7288-C) and in rapidly growing (3924-A, 3683) hepatomas.

MATERIALS AND METHODS

Animals.—Male, Buffalo strain, and ACI/N rats, weighing 180–250 gm., were used in these experiments. The tumor-bearing and control animals were shipped by air express from Dr. H. P. Morris of the National Cancer Institute, Bethesda, to Indiana University, Indianapolis. The biochemical studies were carried out 3–10 days after arrival of the animals. The transplantable tumors used were the slowly growing Reuber (H-35) carried in ACI/N rats; Morris hepatoma 7288-C with more rapid growth rate, carried in Buffalo rats; and the most rapidly growing tumors in the series, the Morris hepatomas 3924-A and 3683.
transplanted in ACI/N rats. The biology and growth properties of these tumors were described previously (6, 7).

For studies on regenerating liver, male Wistar rats weighing 180–250 gm. were partially hepatectomized (66 per cent) under light ether anesthesia, according to the method of Higgins and Anderson (3). Sham operations were performed at the same time on rats of the same weight. The regenerating and sham-operated animals were sacrificed 1 day after the operations.

Exactly 0.5 gm. of liver or tumor slices were weighed on a torsion balance and incubated in a Ringer-bicarbonate medium equilibrated with 95 per cent O\textsubscript{2}–5 per cent CO\textsubscript{2} (2). Uniformly C\textsuperscript{14}-labeled amino acids, alanine, aspartate, glycine, serine, isoleucine, or valine, were added to the medium to give an initial concentration of 1 mg/ml. The tissue slices were incubated in 6 ml. of medium containing from 2.0 to 2.5 × 10\textsuperscript{4} counts/min of labeled substrate. After 90 minutes' incubation, the medium was assayed for CO\textsubscript{2} activity, and tissues were analyzed for C\textsuperscript{14} activity into protein. The radioactive CO\textsubscript{2} was isolated as BaCO\textsubscript{3} for radioassay. Tissue proteins were isolated by trichloroacetic acid precipitation followed by extraction with hot trichloroacetic acid, per- formate digestion, and reprecipitation with trichloroacetic acid according to the method of Manchester and Krahl (4). The labeled amino acids were obtained from Nichem, Inc., Bethesda, Md.

### RESULTS

The results of this investigation are presented in Tables 1 and 2. Tumor values are compared with liver data of the corresponding normal control rats killed at the same time. The data on the regenerating liver are compared with those of the sham-operated controls. The statistical evaluation of the significance of the differences between

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Alanine</th>
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<th>Glycine</th>
<th>Serine</th>
<th>Isoleucine</th>
<th>Valine</th>
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<tbody>
<tr>
<td>Normal liver (Buffalo):</td>
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<td></td>
<td></td>
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<tr>
<td>Control for 788-C</td>
<td>51 ± 4</td>
<td>45 ± 4</td>
<td>70 ± 3</td>
<td>83 ± 6</td>
<td>108 ± 4</td>
<td>95 ± 3</td>
</tr>
<tr>
<td>Sham-operated</td>
<td>44 ± 3</td>
<td>39 ± 4</td>
<td>74 ± 4</td>
<td>78 ± 3</td>
<td>102 ± 4</td>
<td>109 ± 4</td>
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<tr>
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<tr>
<td>Control for H-35</td>
<td>46 ± 4</td>
<td>40 ± 2</td>
<td>65 ± 3</td>
<td>76 ± 3</td>
<td>89 ± 3</td>
<td>84 ± 4</td>
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<tr>
<td>Control for 3663</td>
<td>43 ± 2</td>
<td>38 ± 3</td>
<td>59 ± 3</td>
<td>72 ± 4</td>
<td>77 ± 8</td>
<td>58 ± 5</td>
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<tr>
<td>Control for 3924-A</td>
<td>39 ± 3</td>
<td>60 ± 2</td>
<td>68 ± 4</td>
<td>45 ± 4</td>
<td>79 ± 3</td>
<td>83 ± 6</td>
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<tr>
<td>Regenerating liver</td>
<td>74 ± 3</td>
<td>60 ± 2</td>
<td>100 ± 5</td>
<td>101 ± 4</td>
<td>105 ± 3</td>
<td>105 ± 3</td>
</tr>
<tr>
<td>H-35 (Reuber)</td>
<td>49 ± 11</td>
<td>38 ± 3</td>
<td>68 ± 3</td>
<td>61 ± 6</td>
<td>82 ± 3</td>
<td>71 ± 2</td>
</tr>
<tr>
<td>7888-C</td>
<td>79 ± 4</td>
<td>68 ± 3</td>
<td>86 ± 4</td>
<td>105 ± 3</td>
<td>184 ± 16</td>
<td>182 ± 4</td>
</tr>
<tr>
<td>3924-A</td>
<td>102 ± 4</td>
<td>150 ± 1</td>
<td>108 ± 8</td>
<td>161 ± 9</td>
<td>239 ± 21</td>
<td>295 ± 29</td>
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<tr>
<td>3663</td>
<td>105 ± 4</td>
<td>88 ± 8</td>
<td>127 ± 10</td>
<td>125 ± 9</td>
<td>239 ± 11</td>
<td>260 ± 10</td>
</tr>
</tbody>
</table>

*Statistically significant difference from the respective control (P = <0.05).

The tumor-bearing and control animals were kept in separate cages upon arrival in the Indiana University laboratory. Purina Laboratory Chow and water were available ad libitum.

Preparation of livers and tumors for biochemical studies.—When the tumor-bearing rats were killed, normal rats of the same strain and sex were sacrificed at the same time to supply control livers. The livers of the tumor-bearing rats were not used, because it was shown that the host livers exhibited marked biochemical differences from the normal liver (14). The animals were stunned, decapitated, and exsanguinated. Livers and tumors were rapidly removed and blotted on filter paper. Tumors were carefully dissected free of necrotic, hemorrhagic, and nontumorous material. Tissues were chilled in a beaker on cracked ice for 5 minutes, then slices were prepared as described previously

### TABLE 1

INCORPORATION OF AMINO ACIDS INTO PROTEIN IN NORMAL AND NEOPLASTIC LIVERS*

Means and standard errors represent four rats in each group. Incorporation is measured in counts/min/mg protein. Numbers in parentheses express data as percentages of the corresponding control values which are taken as 100 per cent.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Alanine</th>
<th>Aspartate</th>
<th>Glycine</th>
<th>Serine</th>
<th>Isoleucine</th>
<th>Valine</th>
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<tr>
<td>Normal liver (Buffalo): Control for 7888-C</td>
<td>51 ± 4</td>
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<td>70 ± 3</td>
<td>83 ± 6</td>
<td>108 ± 4</td>
<td>95 ± 3</td>
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<td>109 ± 4</td>
</tr>
<tr>
<td>Normal liver (ACI/N): Control for H-35</td>
<td>46 ± 4</td>
<td>40 ± 2</td>
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<td>68 ± 4</td>
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<td>83 ± 6</td>
</tr>
<tr>
<td>Regenerating liver</td>
<td>74 ± 3</td>
<td>60 ± 2</td>
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<td>260 ± 10</td>
</tr>
</tbody>
</table>
Experimental and control metabolic activities are indicated in the tables.

Protein synthesis in normal and regenerating liver.—The incorporation of amino acids into protein in normal and neoplastic livers is summarized in Table 1. The amino acid incorporation into protein was similar in the livers of normal fed rats of Buffalo and ACI/N strains. There appeared to be a tendency toward a somewhat lower incorporation of isoleucine and valine in the ACI/N rat liver. However, the presented data are not considered sufficient to represent a real strain difference for the incorporation of these amino acids until a larger number of rats is examined. Regeneration for the incorporation of these amino acids was found as compared with the respective ACI/N control livers. These values ranged between 250 and 494 per cent in the 3924-A and 174-453 per cent in the 3683 tumors. The increases were the most pronounced for alanine. Slices from normal ACI/N rats and H-35 Reuber tumor incorporated 0.8–1.2 per cent of the added C14 activity. Figures for regenerating liver and 7288-C were 1.2–1.4 per cent, whereas those for 3924-A and 3683 were 2–2.4 per cent.

**Oxidation of amino acids in normal and neoplastic livers**

Means and standard errors represent four rats in each group. Incorporation is measured in counts/min/umole of BaCO3. Numbers in parentheses express data as percentages of the corresponding control values which are taken as 100 per cent.

### TABLE 2

Oxidation of amino acids in normal and neoplastic livers

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Alanine</th>
<th>Aspartate</th>
<th>Glycine</th>
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<th>Isoleucine</th>
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<tr>
<td>Control for 7288-C</td>
<td>9,200 ± 446</td>
<td>10,775 ± 578</td>
<td>4,900 ± 183</td>
<td>4,500 ± 129</td>
<td>4,150 ± 171</td>
<td>4,150 ± 276</td>
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<tr>
<td>Sham-operated</td>
<td>10,888 ± 692</td>
<td>14,418 ± 1197</td>
<td>4,343 ± 927</td>
<td>4,388 ± 807</td>
<td>3,749 ± 422</td>
<td>3,788 ± 238</td>
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<tr>
<td>Normal liver (ACI/N):</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control for H-35</td>
<td>9,925 ± 602</td>
<td>11,123 ± 690</td>
<td>4,850 ± 171</td>
<td>4,750 ± 171</td>
<td>3,900 ± 41</td>
<td>3,450 ± 150</td>
</tr>
<tr>
<td>Control for 3683</td>
<td>11,775 ± 696</td>
<td>12,438 ± 809</td>
<td>5,123 ± 125</td>
<td>4,899 ± 450</td>
<td>3,519 ± 881</td>
<td>3,168 ± 232</td>
</tr>
<tr>
<td>Control for 3924-A</td>
<td>12,948 ± 889</td>
<td>10,705 ± 719</td>
<td>5,175 ± 25</td>
<td>4,925 ± 929</td>
<td>3,841 ± 904</td>
<td>3,005 ± 158</td>
</tr>
<tr>
<td>Regenerating liver</td>
<td>12,775 ± 975</td>
<td>15,538 ± 992</td>
<td>4,563 ± 345</td>
<td>4,400 ± 916</td>
<td>3,715 ± 90</td>
<td>3,438 ± 251</td>
</tr>
<tr>
<td>H-35 (Reuber)</td>
<td>8,988 ± 488</td>
<td>10,838 ± 442</td>
<td>3,875 ± 260</td>
<td>3,900 ± 129</td>
<td>3,800 ± 129</td>
<td>3,200 ± 183</td>
</tr>
<tr>
<td>7288-C</td>
<td>8,650 ± 312</td>
<td>9,350 ± 367</td>
<td>4,723 ± 111</td>
<td>4,500 ± 265</td>
<td>4,475 ± 105</td>
<td>4,150 ± 119</td>
</tr>
<tr>
<td>3924-A</td>
<td>8,480 ± 836</td>
<td>7,623 ± 191</td>
<td>5,893 ± 334</td>
<td>4,867 ± 290</td>
<td>6,542 ± 175</td>
<td>5,179 ± 316</td>
</tr>
<tr>
<td>3683</td>
<td>8,590 ± 735</td>
<td>9,124 ± 1,074</td>
<td>5,440 ± 429</td>
<td>5,835 ± 256</td>
<td>5,113 ± 198</td>
<td>5,938 ± 267</td>
</tr>
</tbody>
</table>

* Statistically significant difference from the respective control (P = <0.05).
intermediate growth rate in this series, there were no significantly different alterations in amino acid oxidation from the values found in the control rats. In the most rapidly growing tumors in this study the oxidation of alanine and aspartate was decreased to 61 and 70 per cent in 3924-A and 73 per cent in 3683. However, glycine and serine were oxidized in the normal range. On the other hand, the oxidation of isoleucine and valine was markedly increased in 3924-A (197 and 173 per cent) and 3683 (145 and 188 per cent).

DISCUSSION

The presented results demonstrated that there was an increase in the incorporation of amino acids into protein in the regenerating liver, not exceeding an increase of 168 per cent found for alanine. The values for aspartate, glycine, serine, isoleucine, and valine were lower than this level of elevated incorporation, but they were all significantly above the control values. The regenerating liver shows a very rapid growth rate, comparable to the most rapidly growing liver tumors. However, the amino acid incorporation in the rapidly growing hepatomas was much higher than that observed in the regenerating liver. This quantitative increase, especially marked for alanine (494 per cent and 453 per cent), may indicate a preferential use of some of these amino acids for the protein synthetic apparatus of the neoplastic livers. It is interesting that in the very slowly growing H-35 tumor the amino acid incorporation was in the normal range. This may suggest that the sensitivity of the analytic method used in this study is not high enough to indicate the presence of an increasing incorporation in this slowly growing tumor. On the other hand, hepatoma H-35 is a bile-secreting tumor, containing many macroscopic hemorrhages and necrotic areas, and it is difficult to obtain viable material rapidly enough after removal of the tumors. Microscopic examination of “viable” pieces, carefully chosen for biochemical studies, showed evidence of necrotic and hemorrhagic areas. This observation has to be taken into consideration, since the amino acid incorporation studies under the present conditions failed to reveal the expected slightly increased incorporation into protein which may exist in this tumor.

The increased utilization of alanine and aspartate, which are gluconeogenic amino acids, may be connected with the marked decrease or absence of gluconeogenic enzymes (11–14) and pyruvate to glucose production (9, 15) in these liver tumors.

One may expect a generally decreased amino acid oxidation in the tumors with rapid growth rate. Such a tendency can be seen for alanine and aspartate in tumors 3924-A and 3683. Interestingly, there is no change in the oxidation of glycine and serine, but there is a marked increase in the oxidation of isoleucine and valine in 3924-A and 3683.

Since isoleucine can give rise to acetoacetyl CoA and thus enter the fatty acid cycle this amino acid as well as valine may give rise to fatty acids which are known to be utilized by various tumors (5). In fact, Medes et al. estimated that a large percentage of the endogenous respiration may be due to the combustion of tissue fatty acids. Similar findings were also brought by Scholtefield et al. (8). Thus, the increased isoleucine and valine incorporation in the rapidly growing tumors may reflect an increased capacity for utilizing these amino acids through fatty acid formation.

In conclusion, in evaluating the patterns of amino acid incorporation into protein it appears that a rough correlation with the growth rate of the liver tumors is discernible. Further detailed studies are in progress to establish more clearly the mechanism and significance of these findings.

REFERENCES

9. Sweeney, M. J.; Ashmore, J.; Morris, H. P.; and Weber, G. Comparative Biochemistry of Hepatomas. IV. Isotope Investigation of Carbohydrate Metabolism in Liv-


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