Comparative Uptake of Free Amino Acids by Mouse-Ascites Carcinoma Cells and Normal Tissues*

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The growth of a tumor in a wasting animal demands that the normal tissues yield their structural units to the neoplastic tissue. Because such structural elements are constantly released and recombined, the neoplastic cell can gain them from the normal cell merely by seizing upon them with greater avidity, without direct attack upon the normal tissue.

In the investigation reported here, we have considered the behavior of the amino acids, the most prominent of the protoplasmic structural units. The cells of the higher animals (as well as many microbial species) maintain internal environments which are characteristically enriched in the amino acids (7). They do this by taking the various amino acids into the cells against strong concentration gradients. This concentrative activity is in some ways as impressive as the similar and related (8) behavior by which the potassium ion is introduced into the cell against a concentration gradient. It is in the enriched cellular medium that protein synthesis occurs. Several observations have led us to propose that the concentrative process for amino acids may play a significant part in the control of growth.

In the pregnant animal, for example, the placenta enriches the fetal circulation in amino acids at the expense of the maternal blood. Not only do the fetal cells live in a richer extracellular medium; they outdo the cells of the adult in further enriching their internal environment (6). This is in spite of their extremely rapid utilization of amino acids for growth. Similarly, the amino acid levels of the liver are sharply elevated during the period of experimental regeneration of this organ (5). The flow of amino acids from the wasting muscles to the growing liver appears to be a result of stepped-up concentrative activity of the liver for amino acids.

Several other cases of association between rapid growth and elevated tissue amino acid concentrations have been reported. No one has yet reported the response which is more to be expected—namely, that cellular amino acid levels are dragged down during rapid growth.

According to the above viewpoint, if a tissue were to acquire an increased concentrative activity for amino acids, overgrowth could result. We are presenting the thesis that unusual concentrative powers permit a tumor to capture amino acids from the normal tissues of the host.

As a subject for comparative study of concentrative activity we have used the Ehrlich mouse-ascites carcinoma cell. A mouse inoculated with this tumor may in a week develop cells weighing one-tenth as much as the whole animal did to begin with. These carcinoma cells in vitro show concentrative activity so intense as perhaps to explain their competitive success. For example, when we added large amounts of glycine to the cell suspension removed from a mouse, the cellular glycine level rose to a 90-millimolar level with the extracellular at 30 mM—a gradient of 60 HIM/liter, enough to cause a considerable osmotic gradient, as indicated by the transfer of water to the cells (3). These gradients surpass any observed in vitro with mammalian tissue. The objection may be raised that the uptake of amino acids should be compared in the whole mouse where the competition actually occurs. This has been done with the results recorded below.

EXPERIMENTAL

Male mice of strain A (Jackson Memorial Laboratory, Bar Harbor, Maine) were inoculated with 0.2 ml. of ascitic fluid from a mouse having a well developed carcinoma-cell ascites. Two to 4 days later the animal was fasted 3 hours, and then fed the glycine or L-alanine in two doses, the first one 25 mm/kg of body weight, and the second one an hour later, 15 mm/kg. The animals were sacrificed for analysis 1 hour after the last dose. The purpose of this dosage schedule was to maintain a nearly maximal rate of absorption of the amino acids.

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acid over nearly 2 hours. Analysis of the gut contents at the end of the experiments showed the presence of 16–26 per cent of the glycine fed. When 40 mM of alanine was fed according to the same schedule, only 2.3 per cent of the alanine remained unabsorbed. Therefore, the dosage of alanine was increased to 60 mM/kg, in two doses of 40 and 20 mM. In this case 17 per cent of the alanine was recovered in the alimentary canal.

The animals were killed by decapitation. In most cases blood was collected in a heparinized tube. The ascitic fluid was collected rapidly after opening the abdomen. The liver and the musculature of the hind legs were removed quickly, weighed on a direct-reading torsion balance, and ground in a mortar with sand in the presence of 10 parts of saturated aqueous picric acid. The tumor cells were separated by centrifugation in a tared tube at 38°C, then weighed and extracted with saturated picric acid. The blood plasma and the cell-free ascitic fluid were treated with 5 volumes of aqueous picric acid. The extracts were analyzed for glycine or alanine by the methods of Alexander, Landwehr, and Seligman (1, 4) and of Alexander and Seligman (2).

RESULTS AND DISCUSSION

Chart 1 shows the powerful uptake of glycine by the carcinoma cells in vitro (cf. 3). The line drawn on this chart represents a 50-mM gradient, that is, the cell water richer than the extracellular fluid by 50 mM of glycine/kg. It should be emphasized that the gradient does not remain constant at low concentrations; actually, the curve slopes off toward the origin. The indications are that at zero extracellular glycine concentration there will be no free glycine in the cells.

The results obtained with the intact tumor-bearing mouse are shown in Table 1 (for glycine) and Table 2 (for alanine). Because the content of

<table>
<thead>
<tr>
<th>Condition of mouse</th>
<th>Blood</th>
<th>Liver</th>
<th>Muscle</th>
<th>Carcinoma cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycine-fed</td>
<td>0.4*</td>
<td>1.8(±0.3)*</td>
<td>2.5(±0.2)*</td>
<td>0.5*</td>
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<tr>
<td></td>
<td>11.9</td>
<td>35</td>
<td>6.6</td>
<td>11.9</td>
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<td>17.5</td>
<td>51</td>
<td>15.9</td>
<td>12.0</td>
</tr>
</tbody>
</table>

* These averages were obtained with tumor-bearing mice of the same strain. The values in parentheses are standard deviations (five observations).

<table>
<thead>
<tr>
<th>Condition of mouse</th>
<th>Blood</th>
<th>Ascitic</th>
<th>Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine-fed</td>
<td>0.8*</td>
<td>5.5(±1.8)*</td>
<td>3.6(±0.6)*</td>
</tr>
<tr>
<td></td>
<td>7.7</td>
<td>10.0</td>
<td>9.0</td>
</tr>
</tbody>
</table>

* Averages obtained with ascites tumor-bearing mice of the same strain. The values in parentheses are standard deviations (five observations).
greater than that of the muscle cells. In the experiments with alanine feeding, the three tissues fall in the same order as to alanine uptake in one case; in the other, the liver surpasses the neoplastic cell with regard to alanine accumulation. There can be no question that the net synthesis of protein had been proceeding much faster in the multiplying neoplastic cells than in the other tissues of these mice. Were isotopically labeled alanine administered, undoubtedly the more rapid incorporation of the amino acid into protein in the neoplastic cell than in other cells could be demonstrated, as was done by Zamecnik, Frantz, Loftfield, and Stephenson with rat hepatomas (8). The present experiments have a quite different purpose. They show that the neoplastic cells have a greater ability to accumulate free amino acids than other cells; this should give the protein-synthetic mechanisms in the cancer cells an advantage, which is perhaps expressed in their rapid growth and in the flow of amino acids from normal tissues to tumor in advanced neoplasia.

The liver occupies a special position in amino acid metabolism, not only as a protein-synthesizing organ, but also in its capacity of destroying amino acids by deamination and by other reactions. It is not maintained that the greater accumulation of added amino acids by the liver than by the muscle, or by almost any other tissue, implies that the liver ought to grow faster than muscle or other tissues. Certain concentrative activities are characteristic of each organ for each amino acid. Growth has been shown in several instances to be associated with increases of these characteristic concentrative activities. Unfortunately, the concentrative activities of epithelial cells which are homologous to the carcinoma cell are not yet subject to measurement. We do regard as significant the finding that the carcinoma cells exceed even the highly active liver cells with regard to the accumulation of free amino acids.

**SUMMARY**

When mice bearing the Ehrlich ascites tumor were fed glycine or L-alanine, the carcinoma cells were more active in the accumulation of the free amino acid than the cells of liver or muscle. This superiority in amino acid accumulation is considered a significant factor in the growth and multiplication of the neoplastic cell in a wasting animal.

**REFERENCES**

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