Growth of Carcinoma Implants in Fed and Fasted Rats

G. A. LePage, V. R. Potter, H. Busch, C. Heidelberger, and R. B. Hurlbert

(McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wis.)

A number of investigations have been made recently concerning the comparison of protein metabolism in normal tissues to that in neoplastic tissues. These were reviewed by Zamecnik (9) and by Mider (3). Mider et al. (4) have referred to tumor tissue as a "nitrogen trap," indicating that it probably tends to remove amino acids from the body pool without permitting any appreciable return to that pool. In his review, Mider raises several questions which are important in formulating a better concept of the tumor-host relationship with regard to tumor protein metabolism. These remained unanswered, and it was suggested that they would be amenable to research with isotopically labeled precursors. Among these questions were: "How much exchange exists between the neoplasm and its host with regard to amino acids? Is the nitrogen metabolism of the cancer essentially a one-way passage? Is the necrotic material in a tumor inert, or do split-products gain access to the blood stream?"

Shemin and Rittenberg (7) studied the incorporation of glycine-N16 into normal rat tissues and into sarcoma R39. Although the atoms percent excess of N14 at several times after the administration of glycine-N16 is given, the above questions are not answered, since data on the tumor weights and dilution due to rapid growth are not included. More recently, Norberg and Greenberg (5) have made a study of the incorporation of glycine-1-C14 into the tissues of normal and lymphosarcoma-bearing mice. This was presented as change of specific radioactivity of the proteins with time, over a period of 48 hours. The effect of fasting was studied, but total tissue content of the isotope cannot be calculated from their data.

With reference to the questions raised by Mider (3, 4), the tumor most likely to show a relatively small return of assimilated amino acid to the body pool would be a very malignant and rapidly growing tumor. Consequently, for the initial experiments we chose the Flexner-Jobling carcinoma, one of the most rapidly growing rat tumors available. Protein (amino acid) turnover was studied by means of isotope experiments, and fasting animals were used to limit protein turnover to a host-tumor relationship. Measurement of total tumor mass was also an important feature of the experiments.

EXPERIMENTAL

The animals used were female, albino rats obtained from the Holtzman-Rolfsmeyer Company (Sprague-Dawley strain) when they weighed 100-110 gm. They were kept in our laboratories 1 week on a Purina Fox Chow diet. Then Flexner-Jobling carcinoma implants were made subcutaneously at four sites on the abdominal area of each rat. Each batch of tumor mince was used for implants on twelve rats, and each such group was distributed equally through all experimental groups.

The first experiment was designed to determine the rate of tumor growth and tissue protein changes in fasted and fed tumor-bearing rats. Rats, each bearing four 10-day-old Flexner-Jobling carcinomas, were divided into three groups, twelve animals to a group. One group was sacrificed immediately to obtain initial tissue weights. A second group was fed Purina Fox Chow ad libitum. The third was fasted. After an additional 5 days, both of the latter groups were sacrificed and tissues weighed. At this time several of the rats of the fasted group were dying. Six of the 12 died on the morning of the fifth day, while the others were being sacrificed. Since no difference in tissue weights could be detected when these six were compared with the others, all twelve were grouped together in presenting the data. Tissues from each group were pooled into subgroups of three, and the total tissue pool in each case was broken up in a Waring Blender with 9...
volumes of water. Aliquots of the tumor and liver suspensions were treated with trichloroacetic acid to a final concentration of 5 per cent. The resulting precipitate was washed twice with 5 per cent trichloroacetic acid, twice with 95 per cent ethanol, and extracted 3 times with 3:1 alcohol-ether at 40° C. Finally, the precipitate was washed twice with ether and dried in vacuo. The resulting material was weighed and the weight used to compute per cent protein. The tissue weights and protein contents are presented in Table 1. The mean of results from twelve rats and the average difference from the mean have been presented in each case.

In 5 days the weight of tumor protein increased 345 per cent in the fed rats, 160 per cent in the fasted rats. The latter result is the more noteworthy when it is considered that these animals lost 31 per cent of their body weight and 39 per cent of their liver protein during the same period. It is thus apparent that the tumor was able to grow rapidly at the expense of the host tissues.

Further aliquots of the suspensions of liver and tumor were extracted and used for determinations of pentosenucleic acid (PNA) and desoxypentosenucleic acid (DNA), according to the method of Schneider (6). The results of these analyses are presented in Table 2. The liver DNA changed very little from that of the control group, but its PNA content decreased considerably with fasting. In the tumors, DNA increased approximately in proportion to the tissue weight, but the PNA content was held down by fasting. Hence the PNA/DNA ratios decreased with fasting in both tissues.

A second experiment was set up with an isotopically labeled protein precursor to provide more definitive results concerning the tumor-host tissue relationship in fed and fasted animals. The low

### TABLE 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Body</th>
<th>Tumor</th>
<th>Brain</th>
<th>Liver</th>
<th>Kidneys</th>
<th>Thymus</th>
<th>Spleen</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh wt. (gm.)</td>
<td>174 ±7.2</td>
<td>182 ±10</td>
<td>1.62 ±0.03</td>
<td>7.00 ±0.16</td>
<td>1.56 ±0.03</td>
<td>0.50 ±0.02</td>
<td>0.50 ±0.01</td>
<td>0.67 ±0.01</td>
</tr>
<tr>
<td>Protein content (per cent)</td>
<td>4.14 ±1.20</td>
<td>19.1 ±0.2</td>
<td>12.97 ±1.64</td>
<td>9.17 ±0.42</td>
<td>1.02 ±0.05</td>
<td>0.25 ±0.03</td>
<td>0.25 ±0.02</td>
<td>0.27 ±0.01</td>
</tr>
<tr>
<td>Fresh wt. (gm.)</td>
<td>173 ±5.0</td>
<td>182 ±10</td>
<td>1.62 ±0.03</td>
<td>7.00 ±0.16</td>
<td>1.56 ±0.03</td>
<td>0.50 ±0.02</td>
<td>0.50 ±0.01</td>
<td>0.67 ±0.01</td>
</tr>
<tr>
<td>Protein content (per cent)</td>
<td>17.2 ±0.7</td>
<td>21.9 ±1.8</td>
<td>7.5 ±0.5</td>
<td>5.0 ±0.12</td>
<td>1.0 ±0.06</td>
<td>0.05 ±0.01</td>
<td>0.05 ±0.01</td>
<td></td>
</tr>
</tbody>
</table>

* The mean with average mean deviation.
† Per rat (sum of four tumors).

### TABLE 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Tissue components</th>
<th>Nucleic acid analyses</th>
<th>Ratio of PNA/DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats sacrificed initially</td>
<td>Liver DNA</td>
<td>7.6 ±0.2</td>
<td>55</td>
</tr>
<tr>
<td>Rats fed 5 days</td>
<td>Tumor DNA</td>
<td>2.2 ±0.1</td>
<td>10.4</td>
</tr>
<tr>
<td>Rats fasted 5 days</td>
<td>Liver DNA</td>
<td>7.4 ±0.2</td>
<td>348</td>
</tr>
<tr>
<td>Rats fed 5 days</td>
<td>Tumor DNA</td>
<td>2.3 ±0.1</td>
<td>10.5</td>
</tr>
<tr>
<td>Rats fasted 5 days</td>
<td>Liver DNA</td>
<td>7.2 ±0.4</td>
<td>253</td>
</tr>
<tr>
<td>Rats fed 5 days</td>
<td>Tumor DNA</td>
<td>3.6 ±0.2</td>
<td>14.12</td>
</tr>
<tr>
<td>Rats fasted 5 days</td>
<td>DNA</td>
<td>4.8 ±0.0</td>
<td>36</td>
</tr>
</tbody>
</table>

* The mean with average mean deviation.

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variability in the first experiment indicated that smaller groups would yield significant results. Sixty female rats of the same specifications described earlier were selected into ten groups of six each, with identical average weights for each group. Six of these groups (36 rats) were inoculated subcutaneously with Flexner-Jobling carcinoma, each at four sites. After 91 days each of the 60 rats was injected intraperitoneally with 200 μg of glycine-2-C\textsuperscript{14} (2,900,000 counts/min).\textsuperscript{1}

After a lapse of 12 hours, at which interval it was indicated in earlier data that the proteins would have reached maximum radioactivity (8), one group of six normal rats and one of six tumor-bearing rats were sacrificed and tissue weights obtained. The other groups were now put on fasting or feeding regimes as indicated. As each group was sacrificed, it was divided into two pools, each pool consisting of the tissues from three rats. Each liver, kidney, and tumor pool was dispersed in 9 volumes of water in the Waring Blender, and protein isolated as described for the first experiment. The isolated proteins were finely ground in a mortar with 25 per cent ethanol-75 per cent water and collected on duplicate paper discs by filtration. They were dried \textit{in vacuo}, weighed, and counted in internal flow counters. The radioactivity was corrected for self-absorption and expressed as counts/min/mg of protein. All samples were counted for a sufficiently long period to obtain a 5 per cent statistical accuracy.

The results of this experiment are presented in

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\textbf{Chart 1.} — O — O fed control rats; • — • fasted control rats; □ — □ fed tumor-bearing rats; ■ — ■ fasted tumor-bearing rats.

Top row: changes in body and tissue weights with feeding and fasting.
Center row: changes in specific radioactivity with feeding and fasting.
Bottom row: changes in total radioactivity per organ with feeding and fasting.

\textsuperscript{1}Obtained from Tracerlab, Inc., on allocation by the United States Atomic Energy Commission.
Chart 1. The top row in Chart 1 gives the weight changes in body (including the tumors), tumors, liver, kidney, and spleen. In this experiment the increase in tumor weight to 4 days was rapid in both fed and fasted groups, with less difference between the two than at 5 days in the earlier experiment. McEwen and Haven (2) noted an increase in liver weight in tumor-bearing animals. With the much smaller tumors in these experiments one can still observe an increase of liver weight in the tumor-bearing group. A much more pronounced increase is notable in the spleens of the tumor-bearing groups, which even fasting does not completely suppress. In the center row of Chart 1 are expressed the changes in specific radioactivity of the liver, kidney, and tumor proteins. The individual plots of the two sub-groups were averaged, in each case, since it was confusing to have eight points in juxtaposition at some places in the charts. The two sub-groups gave closely agreeing results with the exception of one case (tumors of fed rats at 2 days), and here the average fitted into position with the trend of the other points. The specific radioactivity of all three tissues is declining. However, since the radioactivity of tumor protein declines because of dilution by further growth and the specific radioactivity of the precursor glycine declines to a low level very early after a single dose of glycine-2-C\textsuperscript{14} (8), the more significant plot is presented in the bottom row of Chart 1. Here it can readily be seen that in all groups the total radioactivity of liver and kidney proteins, presumably representative of the normal body proteins, declines considerably. In contrast, the total radioactivity of tumor shows a significant increase, whether the animals are fed or fasted.

Since glycine is a precursor of both proteins and nucleic acid purines and both are included in the material referred to here as "protein," it was appropriate to separate the two and to determine the relative proportions of the radioactivity present in each. Six samples, representative of different experimental times and of liver and tumors from the same animal groups, were used. Each was extracted with sodium chloride according to the method of Hurlbert and Potter (1) to separate nucleic acid from protein. After washing and drying, the proteins and nucleic acids were plated directly and radioactivity measured as before. The results are given in Table 3. The proportion of the radioactivity in the nucleic acids is sufficiently small and regular that routine extraction of the samples did not seem justified, since the interpretation of results would be unchanged. Apparently nucleic acid and protein activities are changing in a parallel manner.

**TABLE 3**

**Radioactivity Distribution Between Protein and Nucleic Acids of Liver and Tumor "Proteins"**

Each figure represents duplicate counts on one of the protein samples obtained from a pool of the tissues of three rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Tissue</th>
<th>Protein</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats sacrificed initially</td>
<td>liver</td>
<td>14,100*</td>
<td>12,100*</td>
<td></td>
</tr>
<tr>
<td>Rats fasted 4 days</td>
<td>liver</td>
<td>6,100</td>
<td>5,900</td>
<td>415</td>
</tr>
<tr>
<td>Rats sacrificed initially</td>
<td>tumor</td>
<td>17,000</td>
<td>14,000</td>
<td>360</td>
</tr>
<tr>
<td>Rats fasted 4 days</td>
<td>tumor</td>
<td>9,300</td>
<td>7,000</td>
<td>1,690</td>
</tr>
<tr>
<td>Rats sacrificed initially</td>
<td>tumor</td>
<td>12,900</td>
<td>14,000</td>
<td>1,900</td>
</tr>
</tbody>
</table>

*Counts/min.

**DISCUSSION**

It is apparent from these data that the nitrogen metabolism of the Flexner-Jobling carcinoma is largely, if not entirely, a "one-way passage." The tumor is able to obtain all its requirements from the blood and to grow rapidly when the host is fasted and forced to maintain the blood constituents by catabolism of normal tissues. The tumor protein is not available to the body for conversion to fuel even under the stress of starvation. Since the tumors at the final time studied in these experiments were necrotic, it seems likely that radioactivity, if released by the autolysis of central portions of these tumors, must be efficiently reincorporated by the growing portions to permit the observed rapid net increase of radioactivity in both fasted and fed rats.

It is probable that some of the more slowly growing tumors, especially those which have retained some of the specialized functions of the tissue of origin, will not show as clear a result and will permit greater exchange of amino acids with the body pool. The experiments described here must be repeated with tumors of various types and with normal growing tissues before it can be
concluded that uncontrolled growth is inversely correlated with the rate of return of amino acids to the body pool.

SUMMARY
Control rats and rats bearing multiple implants of Flexner-Jobling carcinoma were fed or fasted and the weight changes determined for tumors and normal tissues. Glycine-2-C\(^14\) was used to label the tissue proteins and to permit a study of amino acid exchange. Animals fasted 5 days lost 31 per cent of their body weight and 39 per cent of their liver protein. Tumors on such animals grew almost as rapidly as on fed rats. It was observed that in either fed or fasted rats, the specific radioactivity of the proteins and nucleic acids of normal tissues declined with time, as did the total radioactivity per organ. In contrast, the total radioactivity of the tumor proteins increased rapidly. It was concluded that protein metabolism in the Flexner-Jobling carcinoma is essentially, if not completely, a “one-way passage,” and that the proteins of this tumor are not available to the host for fuel during starvation. It is suggested that the type of experiment described herein be used to survey host-tumor relationships for a variety of tumors, including some which grow more slowly and are less anaplastic than the Flexner-Jobling carcinoma.

REFERENCES
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