Utilization of Host Purines by Transplanted Tumors*

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Tumors derive their nutrients from the host. These materials may come directly from the diet via the blood, from labile pools which may exist in host tissues, or from the substance of host tissues through their breakdown and transport to the tumor. This has been well studied in the case of tumor-host protein relationships and has resulted in the concept of the tumor as a nitrogen trap, since it is in positive nitrogen balance even when the host is starving (7, 9, 12). There are indications that this situation exists with respect to other essential dietary components also (11).

A similar phenomenon may exist with respect to nucleic acid precursors, but this has received less study. Purines are not required in the diet, and it is well known that both normal tissues and tumors are able to synthesize them from glycine and other precursors via the so-called de novo pathway (5, 6, 12). However, when presented with preformed purines, tumors and normal tissues utilize these and incorporate them into cellular nucleic acids (1, 2, 8). Information regarding the extent of utilization of preformed purines by tumors has great relevance to cancer chemotherapy, particularly that involving antimetabolites.

The relative importance of the two modes of synthesis of purine nucleotides in tumors was measured in several mouse ascites tumors by LePage and Sartorelli (7). Dancis and Balis (4) investigated the utilization of host nucleic acid purines by, among other systems, transplanted solid tumors; and, while some labeled purine was found in the tumor, it was a small amount, and these investigators considered it insignificant. In the present paper the problem has been reinvestigated, and the utilization of host purines by tumors has been measured. The results indicate that significant utilization of host purines by tumors does occur.

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MATERIALS AND METHODS

Female Swiss mice, 25–30 gm., and male CAF1 mice, 20–25 gm., were used in these experiments. Swiss mice given injections intraperitoneally (IP) of 5 × 10⁶ Ehrlich ascites tumor cells or Sarcoma 180 ascites cells were used as a source of tumor cells at 6–7 days. CAF1 mice were given injections IP of 5 × 10⁶ TA3 ascites cells and used as a source of this tumor at 6–7 days.

To obtain acid-soluble adenine, normal tissues and tumors were homogenized in cold 0.2 M perchloric acid (PCA) and washed to obtain the acid-soluble fraction. Purine nucleotides were hydrolyzed at 120°C in an autoclave for 45 minutes with 0.3 M PCA. The purines were chromatographed on Dowex-50 columns, then on paper, and the radiocarbon was either counted directly with circles cut from the paper chromatograms or eluted, plated on aluminum discs, counted.

The mice were fed Purina Laboratory Chow pellets ad libitum, except for those in some experiments in which a purified diet which contained no purines was fed ad libitum. This diet contained 78 per cent cereose, 16 per cent casein, 2 per cent corn oil (fortified with shark liver oil), 4 per cent salts (10), and the following vitamins (in mg/kg of diet): thiamin, 3.0; riboflavin, 3.0; pyridoxine, 3.0; niacin, 5.0; calcium pantothenate, 20.0; biotin, 0.10; pteroylglutamic acid, 1.0; paraaminobenzoic acid, 1000; inositol, 1000; choline, 2000; and vitamin B₃₅. 0.010.

RESULTS AND DISCUSSION

One of the experiments of Dancis and Balis was repeated in a slightly modified fashion. Swiss
mice received 750 µg of adenine-8-C14,2 divided into two equal doses given at a 1-hour interval. Forty-eight hours later each mouse received approximately 130 mg wet weight of Sarcoma 180 ascites cells subcutaneously, divided among three to four sites. After 7 days the animals were killed by exsanguination. Acid-soluble and nucleic acid purines were isolated as described above. The tumors weighed 0.25–0.4 gm/animal at this time. The specific activities of the acid-soluble and nucleic acid adenine are presented in Table 1. The ratio of the specific activity of acid-soluble adenine of tumor to that of liver was about the same as in the experiments of Dancis and Balis. The specific activities of the tumor purines compared favorably with those of the other tissues, especially when it is remembered that the host tissues were exposed to labeled adenine for 2 days longer than was tumor. Radioactivity must therefore have been transferred from the tissues of the animal to the tumor in some manner.

No unchanged adenine was detected in the plasma of these animals. Bennett (1) has reported that less than 2 per cent of injected adenine was present unchanged in mouse tissues 2 hours after the injection of 1.2 mg/mouse. Throughout the course of all these experiments no purine with the properties of either adenine or guanine was found in the mouse plasma. Plasma radioactivity was associated with an unknown acidic purine compound which, after hydrolysis, was eluted from Dowex-30 columns with 6 N HCl (as adenine is) but not with 3 N HCl (as guanine is); but on paper chromatography in an isoamyl alcohol-disodium phosphate system (3), it had an Rr of zero (adenine Rr = 0.45, guanine Rr = 0.00). Spectrophotometric analysis showed a strong peak at about 255 µm in acid, but its spectrum did not correspond with known purines. The charac-

terization of this compound is presently being attempted.

Since it was thought that acid-soluble pools of adenine in host tissues were a more probable source of tumor purines than the tissue nucleic acids, further experiments were carried out at shorter intervals after adenine injection, when the specific activity in the acid-soluble fraction was quite high. The procedure was as follows: 250 µg of adenine-8-C14 was injected IP per mouse in two doses at a 1-hour interval. Three, 24, and 48 hours later, 5 × 10⁶ ascites tumor cells (0.5 ml packed cells) were injected IP per mouse in ascitic fluid, the total volume being 1.5 ml. Within each of these groups, animals were sacrificed 3 and 24 hours after tumor implantation and tumor and various normal tissues analyzed as before. Thus, the amount of radioactive adenine present in various forms and the amount that the tumor took up could both be measured over a time course. Experiments were done in this manner, both with Ehrlich ascites carcinoma in Swiss mice (nine mice per point in three experiments) and TA3 ascites carcinoma in CAP; mice (four mice per point in one experiment). The results were similar in all these experiments, although variation in absolute amounts did occur. Since the results were so similar and more time intervals were studied, only data for the experiment with TA3 cells will be reported.

The adenine uptake by TA3 cells is plotted in Chart 1. It is apparent that labeled adenine was available to and taken up by the tumor in these short intervals, and that the tumor had become appreciably labeled after 3 hours' exposure even when the cells were implanted 48 hours after adenine administration. The fact that the specific activity was increasing during the 24 hours in vivo even when de novo purine synthesis was also taking place to dilute the specific radioactivity speaks for an active and continuous process of adenine transfer from host tissues to tumor. The decrease in specific activity which occurred when cells were introduced 3 hours after adenine administration is unexplained, but is quite reproducible.

To gain some insight into the possible source or sources of this transferred adenine and its mode of transfer to the tumor, different host tissues were analyzed at the same times and in the same manner as was the tumor. Specific activities of acid-soluble and nucleic acid adenine from several tissues and from the tumor are given in Chart 2, and the total radioactivities are given in Chart 3. (Total radioactivity was not determined for spleen.) Liver, spleen, small intestine,

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TABLE 1

SPECIFIC ACTIVITY OF ADENINE IN TISSUES OF MICE BEARING SARCOMA 180

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Acid-soluble adenine (counts/min/mg)</th>
<th>Nucleic acid adenine (counts/min/µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>Liver</td>
<td>94</td>
<td>120</td>
</tr>
<tr>
<td>Intestine</td>
<td>60</td>
<td>45</td>
</tr>
<tr>
<td>Muscle</td>
<td>20</td>
<td>34</td>
</tr>
<tr>
<td>Blood cells</td>
<td>114</td>
<td>49</td>
</tr>
</tbody>
</table>

These values were determined 9 days after injection of adenine-8-C14 and 7 days after tumor implantation.

*Obtained from California Foundation for Biochemical Research on allocation from the U.S. Atomic Energy Commission. Specific activity was 5.4 mc/millimole.
muscle, and blood cells have acid-soluble adenine of high specific activity for at least 48–72 hours after injection of isotope, thus indicating an active purine metabolism. In considering a source for the tumor adenine, these tissues are probably the best possibilities. From the data available, it is not possible to single out one tissue as sole or principal donor. The radioactivity present in each of the tissues just listed is many times that of the tumor. While the time course of the specific and total activities for these tissues may point to a single tissue, the relative amount of adenine involved in this transfer is such that it could be overshadowed by larger fluctuations due to other processes.

The mechanism by which adenine was transferred from host to tumor (and perhaps among the host tissues also) is unclear. The plasma purine mentioned earlier was present in low amounts and had a relatively low specific activity at all time periods. For instance, 6 hours after adenine injection, there was 12 μg/ml at a specific activity of 6 counts/min/μg (based on the molar absorbance of adenine). Unless it has an extremely high turnover it does not seem likely that this is the mode of transport. The blood cells, however, do have a high specific activity within 6 hours after isotope has been injected and maintain this at an almost constant level for several days (see Table 1 and Chart 2). It would appear that the adenine of the blood cells is in a state of rapid turnover and that its radioactivity may be kept at a high level by replacement from one or more of the normal tissues. That this adenine could be deposited for use by tumor or other tissues seems not unlikely. However, Bennett (2) decided, on the basis of kinetic studies, that interchange among tissues did not occur. He did not study the blood
cells. Further experiments are in progress on this point.

An attempt was made to alter purine metabolism by placing mice on a diet which was purine-free. (The animals on the purine-free diet will be called "dieted" in subsequent discussion, and those on chow diet called "normally fed" or "normal.") Animals analyzed after 5 weeks on this diet showed no significant difference in the acid-soluble adenine content of liver and blood cells compared with mice receiving Purina Laboratory Chow pellets. As a further stress on the tissue purine pools, two such mice were given injections of $10^7$ Ehrlich ascites tumor cells after 9 weeks on this diet. Seven days later, these and two tumor-bearing mice fed chow diet were each injected IP with 100 μg of glycine-2-C$^{14}$, s After 1 hour the animals were killed, and tumor, blood cells, and liver of all animals, the eviscerated carcass (skin, bones, and muscle only) of the normally fed animals, and the body pools of adenine are diminished by the absence of dietary purine, and no great increase in the rate of de novo purine synthesis is evident.

In conclusion, it seems clear that significant amounts of purines are transferred from host tissues to ascites and solid mouse tumors. Its exact mode of transport is not entirely clear, although there are indications that the blood cells participate in this process. The importance of this method of acquisition of purines in the economy of the tumor cell is difficult to assay, since both normal and malignant cells make purines de novo, and tumors are known to vary widely in their use of preformed purines (8). The importance of this process to different tumors and possibly species differences among hosts in their capacity to carry out this process may be a partial explanation for the variation in effectiveness of various purine antimetabolites as chemotherapeutic agents.

**TABLE 2**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>&quot;Purine-free&quot; Diet (10 weeks)*</th>
<th>Controls*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific activity (counts/min/μg)</td>
<td>Specific activity (counts/min/μg)</td>
</tr>
<tr>
<td></td>
<td>(μg.)</td>
<td>(μg.)</td>
</tr>
<tr>
<td>Intestine</td>
<td>1.5†</td>
<td>1.79</td>
</tr>
<tr>
<td>Liver</td>
<td>0.59</td>
<td>0.53</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Blood cells</td>
<td>0.7</td>
<td>0.90</td>
</tr>
<tr>
<td>Tumor</td>
<td>31.0</td>
<td>27.7</td>
</tr>
<tr>
<td>Carcass</td>
<td>0.59</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>520</td>
<td>0.47</td>
</tr>
<tr>
<td>Liver</td>
<td>1.0</td>
<td>0.47</td>
</tr>
<tr>
<td>Muscle</td>
<td>3.2</td>
<td>0.47</td>
</tr>
<tr>
<td>Tumor</td>
<td>2.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Carcass</td>
<td>0.06</td>
<td>2.00</td>
</tr>
</tbody>
</table>

* Dieted mice averaged 21 gm.; controls averaged 26 gm.
† Each figure is an average of analyses on two mice.

**SUMMARY**

The role of host tissue purines in the nutrition of tumor cells has been investigated. When Sarcoma 180 was implanted into mice 48 hours after injection of adenine-8-C$^{14}$ and allowed to grow for 7 days, the tumor had acquired significant radioactivity in the acid-soluble and nucleic acid purines.

At intervals of 9, 24, and 48 hours after the injection of adenine-8-C$^{14}$ into CAF1 mice, $5 \times 10^8$ T3A ascites tumor cells were injected intraperitoneally into each mouse. Three and 24 hours later the tumor and host tissues were analyzed for acid-soluble and nucleic acid adenine and their specific activities determined. At every time interval, significant amounts of radioactivity had been taken up and utilized by the tumor, indicating the presence of an active mechanism for transfer of purines from host tissues to tumor. It is suggested that the blood cells may be implicated in this process.

Mice placed on a diet very low in purines for up to 10 weeks showed little change in the tissue content of acid-soluble or nucleic acid adenine, except for the blood cells, where the acid-soluble adenine pool had decreased. No difference was found in the rate of de novo purine synthesis of tissues of these animals, as measured by incorporation of glycine-2-C$^{14}$ into adenine, even when the stress of ascites tumor growth was imposed.

The implications of this transfer of purine from
host tissues to tumor are discussed in relation to chemotherapy with purine antimetabolites.

REFERENCES

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