Physiological Disposition and Therapeutic Consequences of Adenine Administered via the Gastrointestinal Tract in Normal and Tumor-bearing Mice*

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SUMMARY

Each of five ascites-cell neoplasms utilized gastrically administered adenine-8-C14 as a source of purine nucleotides. The disposition of the adenine-8-C14 among selected tissues of normal and tumor-bearing animals was examined. The percentage of the administered isotope that was retained by tumor-bearing animals was greater than that of normal animals, and in the mice with neoplasms the radioactivity of carcass was greater than normal in all but 63HED lymphosarcoma-bearing animals. Addition of 0.2 per cent adenine to a partially purified diet prior to the injection of 4-amino-pyrazolo(3,4-d)pyrimidine, 8-azaadenine, or 6-hydroxylaminopurine into mice bearing implants of Sarcoma 180 ascites cells resulted in a decrease in the inhibition produced by these agents.

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Mammalian cells, in general, possess a potentiality for the formation of purine nucleotides by two alternate routes. One involves the stepwise formation of such nucleotides from relatively small molecules, whereas the other involves the conversion of preformed purines to the nucleotide level (19). The presence of these alternate metabolic routes in tumor cells is of great significance to the effective utilization of inhibitors of purine metabolism as chemotherapeutic agents and prompted an earlier study of the relative capacities of several mouse ascitic neoplasms to utilize these two alternate routes (20). Henderson and LePage (15) investigated the utilization of host purines by transplanted neoplasms and showed that the host tissues are capable of supplying tumors with purines. Dietary adenine has been reported to serve as a precursor of nucleic acids in rodent tissues (5, 9), even though it is not required for growth (4). It has also been assumed that dietary purines would be available to neoplastic cells. Since such a supply might minimize the chemotherapeutic effectiveness of inhibitors of purine nucleotide biosynthesis, it was of importance to determine the extent to which orally ingested purines were utilized by tumor cells.

A number of investigations have shown that purine nucleotide metabolism of certain rodent tissues is altered by the presence of a tumor. Cerecedo et al. (6) reported an increase in nucleic acid adenine and guanine as well as in total DNA and RNA in the liver and lung of mice bearing subcutaneous implants of Sarcoma 180. An increased rate of incorporation of phosphate-P32 into the DNA of liver (16, 29) and into the DNA of spleen and kidney (16) of tumor-bearing rodents has been documented. In addition, an increased incorporation of C14-labeled precursors of purine biosynthesis de novo (7, 22, 29) and of adenine-8-C14 (30) into liver and spleen nucleic acids of tumor-bearing rodents has been observed, whereas the incorporation of precursors of purines into the DNA of intestine was lower in the tumor-bearing animals (22). The present report has extended these findings by measuring the disposition, among a number of tissues from normal and tumor-bearing mice, of adenine-8-C14 absorbed from the gastrointestinal tract.
MATERIALS AND METHODS

Female C3H mice (agent-free), 20–25 gm., bred in our laboratories, were used in these experiments. The tumors employed were ascites-cell forms of Sarcoma 180, Ehrlich carcinoma, Hepatoma 134, Hepatoma 129, and 6C3HED lymphosarcoma. Tumor transplantation was carried out by withdrawing ascites fluid from a donor mouse bearing a 7-day tumor growth. The fluid was centrifuged for 2 minutes in a clinical centrifuge (1600 × g), and the supernatant peritoneal fluid was decanted; the cells were diluted tenfold with isotonic saline, and 0.1 ml of the cell suspension was inoculated into each animal. Mice were used for experimentation 5 days after tumor implantation. In some instances a single intraperitoneal injection of 50 mg of toxohormone per mouse, prepared from the Walker carcinosarcoma 256 by the method of Nakagawa et al. (21), was administered to normal animals 5 days prior to isotope.

After a 12-hour fast, 400 μg of adenine-8-C\(^{14}\) (California Corp. for Biochemical Research or Volk Radiochemical Co.) (0.6–1.0 × 10\(^8\) counts/min/μg) in 0.25 ml water was intubated into the stomach of each experimental animal. At various times after intubation mice were ether-anesthetized, and ascites tumor cells were harvested quantitatively. Blood was collected by severing the vena cava and was removed from the thoracic cavity with a capillary pipette into heparinized tubes. Liver, spleen, kidney, and the complete digestive tract from pharynx to anus were rapidly removed, chilled, blotted, and weighed. The gastrointestinal tract was cut into segments, and the contents were collected by rinsing with several volumes of distilled water. Tissues were homogenized in water at 4° C.—liver, spleen, and kidney in Potter-Elvehjem homogenizers, and gastrointestinal tract and skinned carcass (tissues remaining after removal of blood, liver, kidney, spleen, and gastrointestinal tract) in Waring Blenders. Aliquots of tissue homogenates were plated in duplicate on tared aluminum planchets for radioactivity determination. Concentrated perchloric acid was added to the remaining portion to yield a final concentration of 0.4 M, and mixed nucleic acid and acid-soluble purines were isolated and analyzed as described by LePage (18). Radioactivity was measured with a Nuclear-Chicago model D47 gas-flow counter, and corrections were made for self-absorption.

Total blood volume per mouse was estimated from body weight by assuming 5.6 ml. of blood per 100 gm. (11). Total radioactivity present in tissues was calculated by measurement of the total volume of the homogenate and determination of the counts/min/ml of homogenate. Drugs were injected intraperitoneally in isotonic saline once daily for 3–4 consecutive days. All agents were soluble except 4-aminopyrazolo(3,4-d)pyrimidine, which was dissolved with the aid of sodium hydroxide, neutralized to pH 7–8 with hydrochloric acid, and injected as a suspension of the resulting fine precipitate. Tumor-bearing mice were fed a partially purified complete diet (Nutritional Biochemicals Corp.) for two 30-min. periods daily; immediately prior to the injection of drugs one-half of the animals were given a diet containing 0.2 per cent adenine for one of the feeding periods. Total packed cell volume, determined as previously described (24), was used as the criterion of tumor inhibition. Animals were housed on wire screens throughout the experiments to prevent coprophagy.

RESULTS AND DISCUSSION

The percentage of adenine-8-C\(^{14}\) administered by intubation, present in the tissues of normal, tumor-bearing, and toxohormone-treated mice 1 hour after the isotope, is shown in Table 1. The table also shows that intraperitoneal injection of adenine gave results that were similar to those obtained with gastric intubation; this suggests that in both instances the isotopic precursor entered the same body pools from which allocation to the various tissues occurred. The presence of detectable counts in the gastrointestinal contents of animals receiving an intraperitoneal injection of adenine would suggest that secretion into the alimentary tract took place, or that diffusion of isotope from serosal to mucosal side occurred, or that the washing procedure dislodged radioactive mucosal cells. Microscopic examination of the wash fluid showed the presence of a few islands of mucosal cells; however, a choice among these three alternatives was not possible. The results obtained with normal C3H mice were similar to those reported by Bennett (1), who measured the tissue distribution of carbon-14 in male C57 mice after the injection of adenine-4,6-C\(^{14}\).

The presence of a neoplasm or treatment with a toxohormone preparation, an experiment prompted by the report that injection of certain tumor fractions caused an increase in the uptake of adenine-8-C\(^{14}\) into the DNA of liver, spleen, and lung (8), resulted in alterations in the distribution of adenine in the various tissues. In these animals the radioactivity present in carcass was greater than normal, as was that in the spleen of all but 6C3HED lymphosarcoma-bearing mice. Other changes were not common to all tumor-
bearing animals but appeared to depend upon the particular neoplasm.

All the tumors were capable of utilizing absorbed adenine, although the total quantity present in the tumor was dependent to some extent upon cell mass. The average quantities (ml. packed cell volume) of ascites cells obtained after 5 days growth were as follows: Sarcoma 180, 1.6 ml.; Ehrlich carcinoma, 1.4 ml.; Hepatoma 184, 1.8 ml.; Hepatoma 129, 1.6 ml.; and 6C3HED lymphosarcoma, 0.4 ml. The per cent radioactivity retained by tissues of tumor-bearing animals was slightly greater than that of normal mice and may be the expression of an attempt by the host to replenish metabolic pools depleted by the competitive demand imposed by neoplastic growth. Approximately 30 per cent of the administered radioactivity from a normal mouse intubated with adenine-8-C14 could be accounted for in the urine during a 3-hour period; therefore, radioactivity not accounted for in analyzed tissues was assumed to have been excreted in the urine. It should be stressed that the experiments did not measure potential differences between normal and tumor-bearing animals in the rate of absorption of adenine from the gastrointestinal tract, since, although 15-55 µg. of adenine-C14 was recovered in the digestive tract of the various groups, it is not certain that this was available for utilization. The quantity of isotope administered was limited by its solubility in a volume which could be intubated without regurgitation. A time study was carried out with normal mice intubated with 400 µg. of adenine-8-C14. Fifteen minutes after isotope intubation the tissues contained only slightly less radioactivity than did those obtained from animals 30 or 60 minutes after adenine, indicating that the label was rapidly absorbed from the gastrointestinal tract.

Changes in the size of some organs accompany tumor growth; therefore, the specific activities of some tissues were calculated to correct for differences in mass (Table 2). The results showed that the per cent of administered adenine per unit volume of whole blood was higher in tumor-bearing animals. Since adenine is transported among tissues by blood cells (14), these results might suggest an increased mobilization and transfer of purines among the tissues of tumor-bearing mice.

### Table 1

**Per Cent of Administered Adenine-8-C14 in Tissues of Normal and Tumor-bearing C57 Mice**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Normal (6-7)</th>
<th>Normal (5)</th>
<th>Sarcoma (5)</th>
<th>Ehrlich carcinoma (5)</th>
<th>Hepatoma 184 (4-6)</th>
<th>Hepatoma 129 (5)</th>
<th>6C3HED lymphosarcoma (5)</th>
<th>Toxohormone (5)</th>
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</thead>
<tbody>
<tr>
<td>Blood</td>
<td>1.7±0.11</td>
<td>1.0±0.1</td>
<td>3.1±0.4</td>
<td>2.2±0.07</td>
<td>4.9±0.5</td>
<td>2.8±0.3</td>
<td>1.8±0.1</td>
<td>1.4±0.05</td>
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<td>Liver</td>
<td>11.4±0.6</td>
<td>13.1±0.4</td>
<td>16.5±1.4</td>
<td>12.5±0.9</td>
<td>15.0±2.0</td>
<td>13.1±0.5</td>
<td>11.8±0.8</td>
<td>13.4±1.3</td>
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<tr>
<td>Spleen</td>
<td>1.0±0.1</td>
<td>0.9±0.08</td>
<td>4.6±0.9</td>
<td>2.3±0.1</td>
<td>2.0±0.3</td>
<td>3.4±0.2</td>
<td>1.2±0.05</td>
<td>3.8±0.3</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.7±0.4</td>
<td>3.1±0.5</td>
<td>4.1±0.4</td>
<td>3.5±0.2</td>
<td>4.3±0.5</td>
<td>3.3±0.5</td>
<td>3.9±0.4</td>
<td>3.7±0.02</td>
</tr>
<tr>
<td>GI tract</td>
<td>17.1±1.8</td>
<td>17.4±1.7</td>
<td>13.8±0.7</td>
<td>12.2±0.3</td>
<td>6.9±1.0</td>
<td>10.0±1.3</td>
<td>16.2±0.7</td>
<td>13.8±0.7</td>
</tr>
<tr>
<td>Carcass</td>
<td>16.6±0.9</td>
<td>19.4±0.6</td>
<td>23.1±1.4</td>
<td>23.0±0.6</td>
<td>25.4±0.9</td>
<td>25.3±0.8</td>
<td>28.4±1.0</td>
<td>23.9±1.2</td>
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<tr>
<td>GI contents</td>
<td>10.5±0.9</td>
<td>5.0±0.6</td>
<td>6.6±2.1</td>
<td>6.5±0.4</td>
<td>6.5±1.2</td>
<td>15.8±0.8</td>
<td>3.7±0.7</td>
<td>3.3±0.6</td>
</tr>
<tr>
<td>Tumor</td>
<td>10.5±0.9</td>
<td>5.0±0.6</td>
<td>6.6±2.1</td>
<td>6.5±0.4</td>
<td>6.5±1.2</td>
<td>15.8±0.8</td>
<td>3.7±0.7</td>
<td>3.3±0.6</td>
</tr>
<tr>
<td>Per cent radioactivity</td>
<td>62.0</td>
<td>60.8</td>
<td>78.1</td>
<td>66.9</td>
<td>69.8</td>
<td>76.6</td>
<td>69.2</td>
<td>73.7</td>
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<td>recovered from tissues</td>
<td></td>
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</table>

* Results are expressed as per cent of the adenine-8-C14 dose present in the tissue after a 1-hour incorporation period.
† Values in parentheses indicate numbers of mice per group.
‡ Mean ± standard error.
The live properties of the intestine of tumor-bearing animals are not decreased when tumor masses are relatively small (less than 10 per cent of body weight).

The distribution of radioactivity among the tissues of normal and Hepatoma 134-bearing animals was determined at various times after the intubation of adenine, and the results are depicted in Chart 1. At all the measured times, carcass, blood, and spleen from Hepatoma 134-bearing mice contained more radioactivity than did these organs from normal mice, whereas the liver from mice with neoplastic growths initially contained slightly more radioactivity. The per cent of the administered adenine dose present in kidney was essentially the same for the two groups. The quantity of isotope present in the digestive tract of tumor-bearers was initially lower, and from 3 to 12 hours after intubation the isotope content remained relatively constant in the gastrointestinal tract from Hepatoma 134-bearing mice; during this time period the radioactivity in the gastrointestinal tract from normal animals decreased with time. Radioactivity in whole blood from mice with tumors decreased with time, whereas the activity in spleen increased in these animals. In contrast, the per cent of the administered dose in blood and spleen from normal animals remained constant. Measurement of the total radioactivity in Hepatoma 134 ascites cells at these times indicated that the isotope content was relatively constant.

Table 3 shows the relative specific activities of mixed nucleic acid purines and acid-soluble adenine from selected tissues of normal, tumor-bearing, and toxohormone-treated animals 1 hour after intubation of adenine-8-C14. In general, the relative specific activities of the purines isolated from whole blood of tumor-bearing and toxohormone-treated animals were higher than those obtained from normal animals. Other differences between normal and tumor-bearers were not common to all the neoplasms employed. The specific activity of the acid-soluble adenine of the kidneys from individuals in the same experimental group was not consistent and may be related to individual differences in the rate of urinary excretion of the molecules. It is interesting to note that the specific activities of purines isolated from Sarcoma 180 ascites cells are from three- to five-fold higher than those obtained from Hepatoma 134. An earlier report from this laboratory (25) showed that intraperitoneal injection of a tracer dose of adenine-8-C14 into C3H mice bearing either Sarcoma 180 or Hepatoma 134 ascites cells resulted in cellular purines whose specific radioactivities were not markedly different. Assuming the quantity of intubated adenine available to both cell lines to be similar, the observed difference between the two routes of administration may be

**TABLE 2**

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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>1.4±0.12</td>
<td>1.6±0.2</td>
<td>2.4±0.4</td>
<td>2.0±0.1</td>
<td>3.3±0.4</td>
<td>2.1±0.2</td>
<td>9.8±0.3</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>Liver</td>
<td>9.0±0.7</td>
<td>11.7±0.4</td>
<td>18.4±0.6</td>
<td>12.3±0.3</td>
<td>12.2±1.4</td>
<td>11.1±0.6</td>
<td>9.4±0.6</td>
<td>12.3±1.3</td>
</tr>
<tr>
<td>Spleen</td>
<td>9.5±0.6</td>
<td>8.6±0.2</td>
<td>14.4±0.7</td>
<td>12.5±0.7</td>
<td>7.0±1.6</td>
<td>9.8±0.3</td>
<td>7.8±0.3</td>
<td>14.4±1.0</td>
</tr>
<tr>
<td>Kidney</td>
<td>14.8±2.0</td>
<td>11.9±1.7</td>
<td>17.9±3.1</td>
<td>15.6±2.6</td>
<td>14.2±2.3</td>
<td>14.2±1.4</td>
<td>15.0±1.6</td>
<td>14.0±1.7</td>
</tr>
<tr>
<td>GI tract</td>
<td>11.2±0.4</td>
<td>11.0±1.0</td>
<td>8.4±0.4</td>
<td>9.6±0.8</td>
<td>8.1±0.6</td>
<td>8.6±0.8</td>
<td>9.4±0.3</td>
<td>9.4±0.3</td>
</tr>
<tr>
<td>Tumor</td>
<td>4.4±0.4</td>
<td>14.1±0.0</td>
<td>5.1±0.3</td>
<td>1.6±0.05</td>
<td>2.0±0.4</td>
<td>5.1±0.4</td>
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</tr>
</tbody>
</table>

* Blood is expressed as per cent of the adenine-8-C14 dose per ml.; other tissues are expressed as per cent of the adenine-8-C14 dose per gm. wet weight.
† Values in parentheses indicate numbers of mice per group.
‡ Mean ± standard error.
the result of the metabolic form of the adenine made available to the tumor cells. It might be expected that intraperitoneally injected adenine would be available to the cells as the free base, a form apparently utilized well by both neoplasms, whereas orally administered adenine might become available to the neoplastic cells in metabolic form(s) necessary for transport that are better utilized by Sarcoma 180 cells.

To ascertain the potential role of dietary purines in lessening the chemotherapeutic effectiveness of purine antimetabolites and other inhibitors of purine biosynthesis, the effect of azaserine on the uptake of orally administered adenine by Hepatoma 134 ascites cells was determined (Table 4). The level of azaserine employed (0.2 mg/kg) caused essentially complete inhibition of purine nucleotide biosynthesis de novo for the duration of the experiment (23), and the intracellular pool of acid-soluble adenine-containing compounds was maximally depressed to one-half the normal quantity in a similar ascites cell neoplasms at 12 hours after the drug (26); this was the time elapsed in these experiments prior to intubation of adenine. Azaserine did not significantly increase the total quantity of adenine-8-C14 taken up by the tumor; this may be attributable either to a limited quantity of the oral dose reaching the tumor or to an increased uptake by the ascites cells of purines released from normal tissues which dilute the adenine-8-C14 made available from gastrointestinal absorption. Nevertheless, the specific activities of cellular purines from neoplastic cells was increased; this is indicative of the decrease in metabolic pools created by the azaserine treatment.

The data in Table 5 show the effect of an adenine-containing diet on the chemotherapeutic efficacy of some potential adenine antimetabolites.
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with antineoplastic properties. Previous work has shown that 4-aminopyrazolo(3,4-d)pyrimidine (13, 28) possesses moderate carcinostatic activity, whereas 8-azaadenine (17) and 6-hydroxylaminopurine (10) have been reported to be essentially noninhibitory to transplanted neoplasms in vivo. 4-Aminopyrazolo(3, 4-d)pyrimidine caused 74 per cent inhibition of the Ehrlich ascites carcinoma, and addition of adenine to the diet resulted in a 20 per cent lessening of the tumor inhibition. This partial reversal of the inhibitory properties by adenine is in accord with the finding that this agent did not antagonize the uptake of adenine-8-

<table>
<thead>
<tr>
<th>TABLE 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EFFECT OF DIETARY ADENINE ON THE TUMOR-INHIBITORY PROPERTIES OF SOME POTENTIAL ADENINE ANTAGONISTS</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>TOTAL PACKED CELL VOLUME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Complete diet</td>
</tr>
<tr>
<td>Control</td>
<td>0.66 ± 0.03</td>
</tr>
<tr>
<td>4-Aminopyrazolo(3,4-d)pyrimidine</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>8-Azaadenine</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>6-Hydroxylaminopurine</td>
<td>0.32 ± 0.05</td>
</tr>
</tbody>
</table>

Ehrlich ascites tumor-bearing mice were treated daily immediately after a 30-min. feeding period. Treatments were begun 24 hours after tumor implantation; 6-hydroxylaminopurine was injected for 4 consecutive days, and 8-azaadenine and 4-aminopyrazolo(3,4-d)pyrimidine were injected for 3 consecutive days. The total packed cell volume was determined on the 5th day following tumor implantation. Each value represents the mean ± standard error of results from eight to twelve mice.

C14 by these neoplastic cells (3). The production of fatty livers by 4-aminopyrazolo(3, 4-d)pyrimidine (12, 27) was also found to be reversed by the adenine-containing diet. In addition, the inhibitory effects of 8-azaadenine and 6-hydroxylaminopurine are also lessened (i.e., 31 and 25 per cent less inhibition, respectively) by the adenine-containing diet. These results indicate that dietary purines are capable of decreasing the effectiveness of purine analogs.

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REFERENCES


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