In view of the interest in the tumor growth-inhibiting property of 8-azaguanine (5-amino-7-hydroxy-1H-1,2,4-triazolo[d]pyrimidine [4, 5, 10]), it became of interest to learn more about the mechanism of its action. Kidder and Dewey (6) reported that Tetrahymena geleii requires guanine in its diet and that 8-azaguanine is a powerful inhibitor (7), which was thought to produce its effect by being incorporated in the nucleoprotein. It has been shown (2) that rats can synthesize guanine from adenine but that they do not extensively make use of dietary guanine. Kidder et al. (5) theorized that, since certain tumors (Eo771) were inhibited in growth by 8-azaguanine, perhaps cancer cells had lost the ability to synthesize guanine and had gained the ability to metabolize it.

We have synthesized 8-azaguanine (1) labeled with carbon 14 in the 2-position and have followed its incorporation (1) into the principal tissues of the mouse (CFW and C57 black with Eo771 tumors). The jejunum was shown to be highest in radioactivity of all tissues studied, including the tumors.

In this paper we present evidence to show that the nucleic acid fractions isolated from normal viscera and from Eo771 tissue of mice injected with carbon 14-labeled 8-azaguanine contain small amounts of 8-azaguanine.

EXPERIMENTAL

Injection of radioactive 8-azaguanine.—CFW mice were injected with 2 mg. each of 8-azaguanine labeled with carbon 14 in the 2-position and with an activity of 0.97 μc. per milligram. C57 black mice were injected with the same amount of carbon 14-labeled 8-azaguanine 8 days after subcutaneous implantations of small pieces of Eo771 tumor tissue. Groups of four mice each were utilized in each experiment.

Isolation of nucleic acids and purines.—Twenty-four hours after injection of the radioactive compound, the animals were killed, and liver, spleen, thymus, gonads, kidneys, and washed intestine were combined for isolation of the nucleic acids of the viscera. The tissue was disintegrated in a Waring Blender and dehydrated with several portions of absolute ethanol and ethyl ether. The dehydrated tissue was extracted with 10 per cent NaCl, and the sodium salts of the nucleic acids were precipitated with alcohol, dissolved in water, and the nucleic acids precipitated with HC1, washed with water, and dried with alcohol and ether (8). To obtain DNA, the nucleic acids were twice hydrolyzed for about 8 hours at room temperature in a solution of approximately 2 N NaOH and reprecipitated in acid alcohol (3).

Analyses of the nucleic acid fractions by the orcinol and diphenylamine color reactions showed that the combined nucleic acids and DNA were more than 90 per cent pure.

The nucleic acids were hydrolyzed in 0.5 N HCl for 1 hour, and the purines were precipitated in acid solution with AgNO3. The purines were redissolved in 0.5 N HCl and reprecipitated with AgNO3 in acid solution.

Chromatographic separation of purines.—The purines were extracted from their silver compounds with 6 cc. of 0.5 N HCl, and 10 mg. of

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inactive 8-azaguanine was added to the solution as a carrier for radioactive 8-azaguanine which might be present. This solution was applied as a series of drops from a 1-ml hypodermic syringe to the bottom of a sheet of 18×18-inch Schleicher and Schüll No. 597 filter paper. The drops were so spaced as to form a continuous band across the paper.

The solvent system used for separation of the purines (11) consisted of 4 parts butanol, 1 part diethylene glycol, and 1 part aqueous 0.1 N HCl, and the separation was achieved in 18 or 20 hours by the ascending method. After drying in the air for several hours and in an oven at 90° C. for 30 minutes, the adenine, guanine, and 8-azaguanine strips were cut from the paper in the light of a Mineralight ultraviolet lamp. This light is strongly absorbed by the above compounds, making the location of each easily discernible. The \( R_f \) values for guanine, adenine, and 8-azaguanine in this solvent system were 0.12, 0.21, and 0.45, respectively. These values agree well with those obtained by Bendich.²

The 8-azaguanine strip and adenine strip were eluted separately with 0.1 N NaOH, while the guanine strip was eluted with 0.1 N KOH. Adenine was precipitated from solution as the picrate by adding an equal volume of saturated picric acid. To the 8-azaguanine and guanine solutions were added 10 mg. of nonradioactive 8-azaguanine and guanine, respectively, and these purines were then precipitated as their silver salts.

**Determination of radioactivity.**—Measurements for radioactivity were made as previously described (9).

**RESULTS AND DISCUSSION**

Table 1 shows the radioactivity of the original tissue (initial homogenate), the combined nucleic acid fraction, DNA, and silver purines from mice 24 hours after each animal was injected with 2 mg. of 8-azaguanine labeled at the 2-position with carbon 14 and with an activity of 0.97μc. per milligram.

The combined nucleic acid fractions have several times more activity than the viscera from which they were isolated, while the DNA is low in activity. The silver purines have an activity about 4 times higher than the combined nucleic acid fraction from which they were isolated.

The nucleic acid fractions isolated from Eo771 tumor tissue were somewhat lower in radioactive carbon than the visceral nucleic acid fractions from the same animals.

² A. Bendich, Sloan-Kettering Institute for Cancer Research. Private communication.

Inasmuch as it was desirable to determine whether or not the method for nucleic acid isolation also resulted in concentrating any "free" 8-azaguanine which might be present, an amount of this compound was added to viscera from injected animals. The nucleic acids isolated from this preparation were at least 20 times lower in radioactivity than nucleic acids from injected animals (Table 1), thus showing that the method used for isolating the nucleic acid fractions does not concentrate "free" 8-azaguanine.

**Table 1**

<table>
<thead>
<tr>
<th>EXPER. NO.</th>
<th>SAMPLE DESIGNATION</th>
<th>Initial Combined</th>
<th>Silver</th>
<th>Specific activity, μc. per mole of carbon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Black mice with Eo771 Tumor: 0.12</td>
<td>1.06</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Black mice with Eo771 Tumor: 0.12</td>
<td>1.10</td>
<td>3.98</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>CFW mice 0.14</td>
<td>0.12</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>CFW mice 0.18</td>
<td>0.85</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>CFW mice 0.29</td>
<td>0.68</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>CFW mice 0.37</td>
<td>0.43</td>
<td>0.20</td>
<td></td>
</tr>
</tbody>
</table>

* All animals were sacrificed 24 hours following injection. Each experiment represents values obtained on pooled organs or tissues from four mice.

**Table 2**

<table>
<thead>
<tr>
<th>EXPER. NO.</th>
<th>SAMPLE DESIGNATION</th>
<th>Initial Combined</th>
<th>Adenine</th>
<th>Guanine</th>
<th>8-Azaguanine</th>
<th>Specific activity, μc. per mole of carbon</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>CFW mice 0.29</td>
<td>0.96</td>
<td>0.12</td>
<td>0.09</td>
<td>1.34 (x)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>CFW mice 0.08</td>
<td>0.72</td>
<td>0.26</td>
<td>0.59</td>
<td>2.16 (x)</td>
<td></td>
</tr>
</tbody>
</table>

* Animals were sacrificed 6 hours following injection.

**NOTE:** The adenine and guanine specific activities have been corrected for dilution with inactive carrier; the 8-azaguanine values have not been corrected. A correction factor (x) in excess of 15,000 is estimated.

Table 2 shows the radioactivity of the purines obtained from combined nucleic acid fractions. The values for adenine and guanine have been corrected for dilution, while the values for 8-azaguanine must be multiplied by a large factor (x = at least 15,000), since the dilution with inactive 8-azaguanine is great. These results indicate that a small amount of the injected 8-azaguanine is combined in the nucleic acid structure as suggested by Kidder et al. (5), or that it is isolated along with the nucleic acid fraction in some other
fashion, possibly as an enzyme-8-azaguanine complex.

Assuming for the purpose of calculation that all the radioactivity of the combined nucleic acids is due to 8-azaguanine, it is possible to estimate that the maximum quantity of 8-azaguanine contained in the visceral nucleic acid fraction isolated from one mouse is about 0.5 μg.

It would appear that the presence of radioactive carbon (from 2-labeled 8-azaguanine) in the adenine and guanine of visceral nucleic acids (Table 2) could only be accounted for by utilization of degradation products containing the active atom in synthesis of the naturally occurring purines.

**SUMMARY**

1. Radioactive carbon from carbon-labeled 8-azaguanine, at 6 and 24 hours after injection, is found in higher concentrations in the nucleic acid fractions of normal viscera and of Eo771 tumor tissue than in the tissues from which the nucleic acids were isolated.

2. The nucleic acid fraction from Eo771 tumor tissue is no higher in radioactivity than the nucleic acid fraction from normal visceral tissue.

3. 8-Azaguanine has been shown to be present in the purines isolated from the ribonucleic acid fraction of visceral tissue of animals injected with radioactive 8-azaguanine.

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Investigation of the Nucleic Acids of Viscera and Tumor Tissue from Animals Injected with Radioactive 8-Azaguanine

Jack H. Mitchell, Jr., Howard E. Skipper and Leonard L. Bennett, Jr.


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