Searches for Exploitable Biochemical Differences between Normal and Cancer Cells

I. Nucleic Acid Purine Metabolism in Animal Neoplasms*

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Many types of experimental investigations have implicated nucleic acid metabolism as a possible site for the biochemical lesions recognized as cancer (14). The fact that a number of the known temporarily effective anticancer agents affect nucleotide metabolism (17) suggests that the cytotoxic specificity exhibited by these agents may result from quantitative or qualitative differences between normal and neoplastic cells having to do with biosynthesis of polynucleotides. With this concept in view, the following studies on the uptake of labeled precursors of nucleic acids by a large variety of animal tumors and certain normal tissues were undertaken.

The specific objective of this program was to search for chemical events vital to the existence of cancer cells but less important to normal cells, the blocking of which might be expected to damage cancer cells preferentially.

It is known that formate (80), adenine (8), 2,6-diaminopurine (4), and guanine (1, 3, 6, 7, 12) are incorporated into the nucleic acids of mammalian tissues; however, few data have been available for comparing the relative utilization of these precursors by a wide variety of tumors and certain normal tissues.

EXPERIMENTAL

To move rapidly in search of leads, the incorporation of labeled compounds into combined nucleic acids (CNA) of certain normal and cancer tissues were measured first, and these pilot studies were followed by more exact and comprehensive studies in which adenine and guanine of deoxy-ribonucleic acid (DNA) and ribonucleic acid (RNA) were isolated for activity assay.

The procedure employed for the isolation of crude CNA has already been described (19). To further test the reliability of these CNA values, an experiment was carried out in which two groups of ten mice each, one bearing Sarcoma 180 and the other bearing Adenocarcinoma 755, were given intraperitoneal (IP) injections of formate-C\textsuperscript{14} at a level of 2 \textmu C/mouse. After 6 hours all mice were sacrificed, and tumors, intestines, and livers were pooled separately and homogenized. From portions of the homogenates CNA and CNA silver purins were isolated (19); both were oxidized and counted as infinitely thick samples of barium carbonate in a windowless proportional counter. The specific activities of the CNA and CNA silver purins are presented in Table 1.

The results presented in Table 1 indicate that the CNA are of sufficient purity to permit rough comparison of the incorporation of labeled compounds by different tissues.

Preliminary to the present studies, it was demonstrated that guanine-C\textsuperscript{14}, when injected into mice, is incorporated into the nucleic acids. That the activity isolated as CNA is not due to free guanine was demonstrated by experiments in which CNA silver purines were isolated from (a) tissues 6 hours after injection of the labeled compound and (b) excised inactive tissues to which labeled guanine had been added. The results of these experiments are summarized in Table 2. In this and following experiments the lower specific activities (<4 \textmu C/M C) were determined in an internal gas phase Geiger counter (18).

Similar experiments have been carried out which demonstrate that free 2,6-diaminopurine is not isolated along with CNA.

Guanine-2-C\textsuperscript{14} was then injected into mice, and

* This work was made possible by grants from the American Cancer Society, the Alfred P. Sloan Foundation, Inc., the C. F. Kettering Foundation, and the Biology and Medicine Division, U.S. Atomic Energy Commission.

Received for publication March 28, 1955.
the viscera DNA and RNA were isolated, hydrolyzed, and the adenine and guanine from each separated on ion exchange columns (9) and assayed for activity. These studies, which are summarized in Table 3, show that the activity appeared largely in the guanine of both DNA and RNA.

TABLE 1
COMPARISON OF THE SPECIFIC ACTIVITIES OF CNA AND CNA SILVER PURINES FOLLOWING INJECTION OF FORMATE-C"14

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumor</th>
<th>Intestine</th>
<th>CNA</th>
<th>Ag purines</th>
<th>Ratio*</th>
<th>CNA</th>
<th>Ag purines</th>
<th>Ratio*</th>
<th>Liver</th>
<th>Ag purines</th>
<th>Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-180</td>
<td>67</td>
<td>226</td>
<td>3.4</td>
<td>69</td>
<td>170</td>
<td>2.4</td>
<td>4.3</td>
<td>9.7</td>
<td>2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad. 755</td>
<td>64</td>
<td>208</td>
<td>3.2</td>
<td>72</td>
<td>224</td>
<td>3.5</td>
<td>5.1</td>
<td>15.3</td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Formate-C"14 was injected at a level of 8 µc/mouse, and animals were sacrificed 6 hours later.

S This is the ratio of the specific activity of Ag purines to specific activity of CNA. On the assumption that all the radioactivity is in the purines and thymine of CNA, the theoretical value is calculated to be about 3.3.

TABLE 2
SPECIFIC ACTIVITIES OF NUCLEIC ACID PURINES ISOLATED FROM INTESTINES OF ANIMALS RECEIVING INJECTIONS OF GUANINE-2-C"14 AND FROM INACTIVE INTESTINE HOMOGENIZED WITH GUANINE-2-C"14

<table>
<thead>
<tr>
<th>EXP.</th>
<th>Guanine-2-C&quot;14</th>
<th>Purines</th>
<th>Intestine</th>
<th>Silver</th>
<th>CNA</th>
<th>RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 µc. *</td>
<td>1.1</td>
<td>41</td>
<td>67</td>
<td>226</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2 µc. *</td>
<td>0.7</td>
<td>22</td>
<td>69</td>
<td>170</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>None†</td>
<td>4.6</td>
<td>0.1</td>
<td>64</td>
<td>208</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>None†</td>
<td>4.9</td>
<td>0.1</td>
<td>72</td>
<td>224</td>
<td></td>
</tr>
</tbody>
</table>

* IP injection of 8 µc of guanine-2-C"14 (sp. activity, 8.4 mc/mc); animals sacrificed at 6 hours.
† Guanine-2-C"14 homogenized with inactive intestine.

An investigation of the incorporation of guanine-2-C"14, 2,6-diaminopurine-2-C"14, adenine-8-C"14, and formate-C"14 by a large number of tumors was then undertaken. The specific activities of the labeled compounds were as follows: guanine, 2.4 mc/mc; 2,6-diaminopurine, 3.2 mc/mc; adenine, 1 or 1.5 mc/mc; and formate, 1 or 2 mc/mc. The guanine-2-C"14 and 2,6-diaminopurine-2-C"14 were synthesized (5) at Southern Research Institute.

TABLE 3
SPECIFIC ACTIVITIES OF DNA AND RNA PURINES OF MOUSE VISCERA FOLLOWING INJECTION OF GUANINE-2-C"14

<table>
<thead>
<tr>
<th>EXP.</th>
<th>DNA</th>
<th>RNA</th>
<th>Adenine</th>
<th>Guanine</th>
<th>Adenine</th>
<th>Guanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.5</td>
<td>35</td>
<td>1.7</td>
<td>65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3.0</td>
<td>33</td>
<td>0.7</td>
<td>46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.0</td>
<td>33</td>
<td>2.8</td>
<td>57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Guanine-2-C"14 administered IP at a level of 8 µc/mouse. Experiments were terminated at 6 hours. Pooled tissues of 20—50 mice were used in each experiment.

Data from experiments which demonstrate that guanine-2-C"14 is incorporated into the nucleic acids of a number of tissues of the normal mouse are given in Table 4.

With this background of information, several groups of animals bearing subcutaneous implants of Leukemia L1210, Sarcoma 180, and Adenocarcinoma 755 were used in experiments in which the incorporation of guanine into the DNA of the neoplastic tissue was compared with that of intestine and liver. In all instances it was observed that guanine was poorly incorporated by neoplastic tissues. While these studies were in progress, Mandel and Carlo (19) reported that guanine was poorly incorporated into the nucleic acids of Sarcoma 87 when compared with liver, spleen, or intestine.
tumor, intestine, and liver, which had been separately pooled. The specific activity of each isolated CNA was determined. The ratios of the CNA specific activities of tumor to host intestine and tumor to host liver are presented in Table 5.

As has been pointed out, in most cases these are the results of single experiments in which isolations were administered to mice bearing Sarcoma 180 or Adenocarcinoma 755, and the specific activities of the silver purines from the combined nucleic acids were determined. The results of these experiments are given in Table 7.

As a further extension of these observations, more refined experiments were carried out in which...
were checked for reproducibility. For this purpose, intestines from a large group of mice which had received formate-\textsuperscript{14}C were pooled and homogenized. Nucleic acid purines were then isolated from separate portions of this homogenate by two different procedures.

**Procedure 1.**—Each of two 3-gm. samples of the homogenate was extracted successively with three 5-ml. portions of cold 5 per cent trichloroacetic acid (TCA) solution, two 5-ml. portions of a 3:1 alcohol-ether solution, and, finally, with 5 ml. of ether. The residue was then incubated overnight with 6 ml. of 0.5 N sodium hydroxide solution (15), after which period any insoluble residue was removed by centrifugation and washed with 0.5 N sodium hydroxide solution. The combined supernatant and washings were acidified with 5 N sulfuric acid, and sufficient 20 per cent TCA solution was added to make the solution 3 per cent with respect to TCA. The precipitate formed (DNA plus protein) was washed with 3 per cent sodium chloride solution and precipitated as the sodium salts by the addition of 3 volumes of ethanol to the extract (16). The sodium nucleates were then subjected to a Schmidt-Thannhauser hydrolysate, the DNA from the DNA-protein mixture resulting from acidification of the Schmidt-Thannhauser hydrolysate. This was accomplished by heating the DNA-protein precipitate with 5 ml. of 5 per cent TCA at 90° C. for 15 minutes (16); the extract was then made 1 N with sulfuric acid and hydrolyzed, after which procedure the purines were isolated as described above.

The following tumors were selected for confirmatory experiments in which individual purines were isolated according to Procedure 1: Sarcoma 180, Adenocarcinoma 755, RC Carcinoma, Harding-Passey Melanoma, and Walker Carcinosarcoma.

### TABLE 7

**INTEGRATION OF CERTAIN LABELED PURINES INTO DNA PURINES OF TUMOR, LIVER, AND INTESTINE**

<table>
<thead>
<tr>
<th>LABEL</th>
<th>COMPOUND</th>
<th>Adenine</th>
<th>Guanine</th>
<th>Adenine</th>
<th>Guanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanine-\textsuperscript{2-14}C</td>
<td>2</td>
<td>S-180</td>
<td>4</td>
<td>87</td>
<td>21</td>
</tr>
<tr>
<td>2,6-DAP-\textsuperscript{2-14}C</td>
<td>2</td>
<td>S-180</td>
<td>403</td>
<td>194</td>
<td>91</td>
</tr>
<tr>
<td>Adenine-\textsuperscript{8-14}C</td>
<td>0.5</td>
<td>S-180</td>
<td>41</td>
<td>94</td>
<td>111</td>
</tr>
<tr>
<td>0.5</td>
<td>Ad. 755</td>
<td>45</td>
<td>102</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: All experiments were terminated at 6 hours. Pooled tissues from ten to twelve mice were used in each experiment.

### TABLE 8

**EVALUATION OF ISOLATION PROCEDURES**

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>ISOLATION PROCEDURE</th>
<th>Specific activities (cpm/\mu g of purine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.52</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.52</td>
</tr>
<tr>
<td>3</td>
<td>0.52</td>
<td>0.48</td>
</tr>
<tr>
<td>4</td>
<td>0.52</td>
<td>0.50</td>
</tr>
</tbody>
</table>

* Each sample was taken from the homogenate of pooled intestines of mice which had received formate-\textsuperscript{14}C.

1. Procedure 1: isolation of purines without preliminary extraction of sodium nucleates.
2. Procedure 2: preliminary extraction of sodium nucleates followed by the usual Schmidt-Thannhauser hydrolysate. For details, see text.
3. Each set of three values represents triplicate elutions and assays from a single chromatogram.

ma 256. The tumor-bearing animals were given intraperitoneal injections of guanine-\textsuperscript{2-14}C, 2,6-diaminopurine-\textsuperscript{2-14}C, adenine-\textsuperscript{8-14}C, and formate-\textsuperscript{14}C. After 6 hours the animals were sacrificed and the excised tissues pooled and homogenized for purine isolations as described above. The results are presented in Table 9. It will be noted that many of the values in this table are preceded by "less than" signs. These indicate that the activities of these isolated adenine or guanine samples were too low to be counted and, therefore, could be calculated only as less than a certain value.
DISCUSSION

Results reported by Mandel and Carló (12) indicate that guanine-4-C14 is incorporated into the DNA guanine of liver, kidney, and spleen of CAF1 mice at levels approximately 10-15 times that of Sarcoma 37 and that these normal tissues incorporate labeled guanine into RNA guanine 15-80 times as well as does Sarcoma 37 (24 hours after the last of three daily injections). Balis et al. (3) have shown that incorporation of guanine-8-C14 into polynucleotide guanine of Sherman rat tissues, although significant, is considerably lower than that observed in tissues of C57BL mice. In the present studies we have noted that guanine-2-guanine by two different human tumors growing in cortisonized hamsters and rats has been observed repeatedly. The fact that guanine incorporation into polynucleotides of every tumor studied to date has been quite low when compared with that in normal tissues raises the question whether this quantitative difference is associated with the primary biochemical lesion of neoplasia.

Most of the tumors studied (fifteen of nineteen) incorporated 2,6-diaminopurine into combined nucleic acids (as guanine) as well as or better than intestine or liver (average tumor:intestine ratio, 1.4; average tumor:liver ratio, 1.9). This observation was confirmed in the additional studies in which DNA and RNA purines were isolated. It has previously been observed that 2,6-diaminopurine, when injected into the intact mouse, is rapidly converted to 2,6-diaminopurine ribose phosphate (21).

A consideration of the biochemical events depicted in Chart 1 suggests as possible explanations of these observations that (a) tumors contain relatively high levels of guanase and therefore rapidly

\[
\text{Uric acid} \xleftarrow{\text{Xanthine oxidase}} \text{Xanthine} \xrightarrow{\text{Guanase}} \text{Guanine} \xrightarrow{\text{Nucleic acid}}
\]

CHART 1.—Guanine metabolism

\[C^4\text{ is incorporated into the polynucleotides of a number of mouse tissues (intestine, liver, spleen, muscle, kidney, testis) in readily detectable amounts. Guanine was incorporated into brain nucleic acids at a very low level (Table 4). In an extensive pilot studies it was first observed that guanine-2-C14 is poorly incorporated into the polynucleotides of twenty different animal tumors (Table 5). Under the conditions of these experiments, tumor incorporation of guanine was 0.5-19 per cent that of intestine (average, 7 per cent) and 0.5-94 per cent that of liver (average, 7 per cent). Further studies (Table 9) with representative animal tumors have confirmed these initial observations and have demonstrated that guanine is poorly incorporated into both DNA and RNA of animal tumors when compared with intestine or liver. Similarly poor incorporation of

TABLE 9

Incorporation of Labeled Compounds into Nucleic Acid Purines of Tumors, Intestines, and Livers

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>DNA Tumor</th>
<th>RNA Tumor</th>
<th>DNA Intestine</th>
<th>RNA Intestine</th>
<th>DNA Liver</th>
<th>RNA Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanine</td>
<td>S-180</td>
<td>&lt;0.04</td>
<td>&lt;0.03</td>
<td>&lt;0.04</td>
<td>&lt;0.04</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td></td>
<td>W-556</td>
<td>&lt;0.02</td>
<td>&lt;0.04</td>
<td>&lt;0.07</td>
<td>&lt;0.04</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Formate</td>
<td>S-180</td>
<td>0.01</td>
<td>0.57</td>
<td>0.57</td>
<td>0.57</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>W-556</td>
<td>0.01</td>
<td>0.57</td>
<td>0.57</td>
<td>0.57</td>
<td>0.57</td>
</tr>
<tr>
<td>2,6-Diamo-</td>
<td>S-180</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>no-purine</td>
<td>W-556</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Adenine</td>
<td>S-180</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>W-556</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Note: All experiments were terminated at 6 hours following injection of labeled compounds. The specific activities of the labeled compounds employed were as follows: guanine-4-C14, 5.4 mc/mg; formate-C14, 5 mc/mg; 2,6-diaminopurine-4-C14, 3 mc/mg; adenine-8-C14, 1 mc/mg.

* For complete names of tumors and hosts, see Table 5.

† 179 mc/µg.
‡ 900 mc/µg.
degrade guanine before it can be incorporated into nucleic acids or (b) tumors are deficient in the catalysts responsible for the conversion of guanine to nucleic acid guanine. Differential cell permeability represents a third possibility.

The data of Greenstein et al. (10) suggest that in vitro mouse Hepatoma 587 does not deaminate guanine more rapidly than liver, kidney, spleen, or brain (pancreas and muscle failed to deaminate guanine). Likewise, normal tissues deaminated guanosine and dephosphorylated guanylic acid at rates not grossly dissimilar to those for Hepatoma 587. Hirschberg et al. (11) have reported that the livers and intestines of tumor-bearing mice are more active than most tumors studied in the deamination of 8-azaguanine.

The data presented in Table 6 suggest that it is not a failure of guanine or its derivatives to get to tumor tissue which is responsible for its relatively low incorporation into tumor polynucleotides.

If cancer cells are deficient in the enzymes responsible for conversion of guanine to polynucleotide guanine, then it is perhaps reasonable to postulate that the reverse reaction, degradation of polynucleotides or guanylic acid (or some guanine derivative), is slower in cancer cells. Such reasoning raises the question of the possibility that "loss of control" of growth in certain cancer cells may be associated with loss of the ability to degrade certain oligonucleotides or nucleotides, with a resulting recycling of such materials and a shift in reaction equilibrium toward polynucleotide formation and cell division. This "recycling hypothesis" is now under investigation.

In most of the tumors studied it has been observed that de novo synthesis of DNA and RNA adenine and guanine proceeds relatively efficiently, although the specific activities of the tumor nucleic acids and individual purines were somewhat less than those of the nucleic acids and purines of intestine (Tables 5 and 9). As has been observed previously, liver incorporated formate-C14 into polynucleotide purines poorly when compared with an actively growing tissue such as intestine (6).

The specific activities of the nucleic acids of most tumors were somewhat less than those of intestine 6 hours following administration of adenine-C14. The relative incorporation of adenine by tumors and liver was variable (Tables 5 and 9).

The preference by most of the tumors studied for dianminopurine rather than guanine as a nucleic acid guanine source is rather profound. These results suggest that important differences between normal and cancer cells may exist in the chemical events leading to formation of nucleic acids.

**SUMMARY**

The relative incorporation into polynucleotides of guanine-2-C14, 2,6-diaminopurine-2-C14, adenine-8-C14, and formate-C14 by a wide variety of animal tumors and host intestine and liver has been determined at 6 hours following a single injection of the labeled compounds.

Relative to the control tissues, all tumors incorporated guanine poorly. Most tumor tissues utilized 2,6-diaminopurine as a polynucleotide guanine source as well as or better than did intestine or liver. The specific activities of intestinal polynucleotides 6 hours after the administration of labeled formate and adenine was somewhat higher than those of most tumors. Utilization of formate by liver was much lower than that by intestine or that by any of the tumors studied.

**ACKNOWLEDGMENTS**

The authors wish to thank the following for technical assistance: Miss Martelia Bell, Mrs. Sally Johnson, Mr. Donald Smithers, Miss Linda Simpson, Miss Doris Adamson, and Miss Patricia Morgan.

**REFERENCES**
