The Effect of Tumor Fractions on the Uptake of Carbon-14-Labeled Adenine into the Deoxyribonucleic Acids of Mouse Tissues*

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Kelly and associates (8, 10, 20) have demonstrated that phosphorus-32 is incorporated to a greater extent into the liver, kidney, and spleen deoxyribonucleic acid in mice bearing mammary carcinomas than into those of normal control mice. Comparable results were also obtained by those investigators (19) when tumor-bearing mice were given injections of either formate-carbon-14 (C14) or glycine-2-C14. More recently, Kelly and Jones (9) observed that repeated injections of tissue mashes bring about changes in the nucleic acid turnover of spleen and liver in normal animals. Tyner, Heidelberger, and LePage (24) have found that the specific activity of P32 in the deoxyribonucleotides was lower in normal liver than in the liver of rats bearing Flexner-Jobling carcinoma at 12 and 48 hours following administration of the isotope. The specific activity of adenine-C14 and guanine-C14 followed the same trend following administration of glycine-2-C14. The present paper reports observations which establish a stimulation of adenine-8-C14 uptake into the deoxyribonucleic acid (DNA) of various tissues of tumor-bearing mice. In addition, a similar stimulation of adenine uptake was observed when normal mice were given single injections of tumor homogenate.

MATERIALS AND METHODS

Female mice (C57BL)1 were given injections intraperitoneally (I.P.) of 0.1–0.2 ml. of a saline homogenate of freshly excised mammary carcinoma, E 0771. This tumor is palpable within 10 days and lethal within 30 days. Thirteen of these mice with noticeably developed mammary tumors and an equal number of normal mice were given intraperitoneal injections of 2 μc. of adenine-8-C14. Approximately half of these tumor-bearing and normal mice were sacrificed after 3 hours and the remaining animals, 24 hours following the adenine injection. The spleen, liver, intestine, kidney, and tumor were immediately removed from each animal and cooled to 0° C. Subsequent steps were also carried out in a cold room maintained at 0°–5° C.

The organs from each of the 3- and 24-hour control and tumor groups were pooled separately. The tissues were homogenized in either a Waring Blender or a glass pestle homogenizer, usually at a ratio of 100 mg of tissue/ml of distilled water. Aliquots of this homogenate were taken and the proteins precipitated by the addition of cold 10 per cent trichloroacetic acid. The remainder of the fractionation procedure was carried out as described by Schmidt and Thannhauser (23). The following fractions were thus obtained for each tissue: total homogenate, acid-soluble, lipid-soluble, ribonucleic acid (RNA) and DNA. C14 determinations were carried out on each fraction with an automatic, windowless gas flow counter developed in this laboratory (18). Total phosphorus concentration of each tissue fraction was also carried out according to the method of King (11).

In expanding this study, the effect of certain experimental variables was investigated. The effect of the stage of tumor development on the uptake of adenine-8-C14 into the DNA of lung, muscle, and thymus was determined. A tumor cell suspension, prepared by partial homogenization of actively growing tumor tissue, was injected subcutaneously into normal female mice (C57BL). On the 8th, 10th, 12th, and 14th days after implantation,
groups of these mice were sacrificed. Twenty-four hours prior to sacrifice, each mouse was given injections of a solution of radioactive adenine in distilled water. The DNA of lung, muscle, and thymus was isolated and the C\textsuperscript{14} activity measured as described above.

To determine whether tumor homogenates would duplicate the stimulating effect of tumors \textit{in situ}, tumors were excised from the host, trimmed of surrounding tissues, weighed and homogenized for 5 minutes in Ten Broeck homogenizers, care being taken to keep the tissues cold. The homogenates were centrifuged at slow speeds and the cell debris discarded.

The supernatant liquid was injected I.P. into normal mice. This was followed immediately by an injection of 0.5-1 \textmu c. of an adenine-8-C\textsuperscript{14} solution. Twelve to 24 hours later, the mice were sacrificed, DNA was extracted from the excised lung, and C\textsuperscript{14} activity of the DNA was measured by the above procedures. Experience with tumor homogenates gained concurrently with the lung, muscle, and thymus incorporation study described above established the lung as a convenient and typical assay tissue.

Various means of fractionating the low-speed centrifugal supernate of tumor homogenate were now applied. High-speed centrifugal fractions were obtained by subjecting the tumor preparation to 40,000 r.p.m. for 2 or 24 hours with a Spinco Model L refrigerated centrifuge fitted with the No. 40 head. The residual fractions resuspended in water and the supernatant liquid were assayed for adenine uptake-stimulating ability by I.P. injection into normal mice with the simultaneous injection of a solution of the adenine-8-C\textsuperscript{14} to establish the normal incorporation rate. After a suitable incorporation time, the mice were sacrificed, and the DNA was extracted as discussed above, by the Schmidt and Thannhauser procedure. The adenine-8-C\textsuperscript{14} was synthesized in this laboratory by the method of Clark and Kalckar (3). It possessed an activity of 1.75 \textmu c./mg of dry crystalline adenine.

**RESULTS AND DISCUSSION**

Since the method of Schmidt and Thannhauser has been used throughout the experimental series for the extraction of DNA, it must be kept in mind that the values given for C\textsuperscript{14} activity of extracted DNA refer to DNA of Schmidt-Thannhauser purity. However, it must also be emphasized that all values reported are relative values obtained by comparison with values measured from control animals subjected to identical inoculation, extraction, and counting procedures for each experimental series. The duplicability of the results under the same experimental conditions makes unlikely the possibility that any given result was affected by a spurious contaminant of the DNA.

The DNA of several tissues of tumor-bearing mice exhibited a considerably higher uptake of C\textsuperscript{14} 24 hours after administration of the labeled adenine than was observed in the control animals (Table 1). This was evident for the DNA of liver and spleen, each exhibiting a higher uptake by a factor of from two to four, if tumors were present in the animal. The DNA of intestinal tissue, on the other hand, was not influenced by the presence of tumors. The kidney showed a decrease. These findings are essentially in agreement with those re-
ported by Kelly et al. (8, 10) from experiments with P32 and with either formate-C14 or glycine-2-C14 (19). In the present studies, we did not observe any appreciable difference between the tissues of normal and tumor-bearing mice with respect to the C14 incorporation into the total tissue fraction, the acid-soluble fractions, or the ribonucleic acid fraction.

Results comparable to those reported in Table 1 were obtained from experiments performed to investigate the effect of the stage of tumor development on the uptake of adenine into lung, muscle, and thymus. The DNA from these tissues all exhibited from two- to eightfold increases in C14 incorporation when tumors were present in the body. DNA of thymus and kidney was less active in this respect. We were unable to obtain any consistent results as to the effect of tumor size on the incorporation of adenine-8-C14 by the normal tissue DNA fraction. The tissue DNA from mice that had been given the tumor transplants 8-10 days prior to sacrifice exhibited approximately the same C14 incorporation as that observed in mice bearing 12-14-day transplants.

The next phase of this study was to establish whether the administration of tumor homogenate or fractions thereof would alter the DNA metabolism of certain normal tissues as noted above for tumors in situ (Tables 2-4). Values for these studies are expressed in terms of N-fold change from normal; this refers all values to the experimental values for normal control mice injected with the same dose of adenine but not given any of the tumor preparations. Direct experimental values were expressed in terms of disintegrations/second of the DNA fraction obtained from a gram of wet weight of tissue, or as disintegrations/second/mg DNA phosphorus. These values were then divided by the same expression for animals with no injections of tumor fraction to arrive at the N-fold expression as presented in the tables. Compared with control mice injected only with C14-labeled adenine, the administration of the tumor homogenate did result in an increased uptake of C14 in the DNA fraction of the lung, muscle, and thymus. The magnitude of this N-fold increase was from 1.5 to 3 and was especially evident in the DNA obtained from lung tissue (fractions XI and XIII, Table 3).

Since it was possible to effect an increase in the

### TABLE 1

**THE EFFECT OF MAMMARY CARCINOMA* ON THE INCORPORATION OF ADENINE-8-C14 BY THE DEOXYRIBONUCLEIC ACID OF MOUSE TISSUES**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Time after administration of 8.0 μc.</th>
<th>Adenine-8-C14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (control)</td>
<td>144</td>
<td>12</td>
</tr>
<tr>
<td>Liver (tumor-bearing)</td>
<td>120</td>
<td>45</td>
</tr>
<tr>
<td>Kidney (control)</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>Kidney (tumor-bearing)</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Spleen (control)</td>
<td>605</td>
<td>277</td>
</tr>
<tr>
<td>Spleen (tumor-bearing)</td>
<td>1,303</td>
<td>355</td>
</tr>
<tr>
<td>Intestine (control)</td>
<td>570</td>
<td>35</td>
</tr>
<tr>
<td>Intestine (tumor-bearing)</td>
<td>402</td>
<td>88</td>
</tr>
<tr>
<td>Tumor</td>
<td>55</td>
<td>219</td>
</tr>
</tbody>
</table>

* C57BL mice bearing mammary carcinoma E 0771, obtained from Roscoe B. Jackson Memorial Laboratories, Bar Harbor, Maine.

† Values are reported as disintegrations/second/DNA obtained from 1 gm. fresh tissue. Each value obtained from the pooled tissue of five to seven mice.

The next phase of this study was to establish whether the administration of tumor homogenate or fractions thereof would alter the DNA metabolism of certain normal tissues as noted above for tumors in situ (Tables 2-4). Values for these studies are expressed in terms of N-fold change from normal; this refers all values to the experimental values for normal control mice injected with the same dose of adenine but not given any of the tumor preparations. Direct experimental values were expressed in terms of disintegrations/second of the DNA fraction obtained from a gram of wet weight of tissue, or as disintegrations/second/mg DNA phosphorus. These values were then divided by the same expression for animals with no injections of tumor fraction to arrive at the N-fold expression as presented in the tables. Compared with control mice injected only with C14-labeled adenine, the administration of the tumor homogenate did result in an increased uptake of C14 in the DNA fraction of the lung, muscle, and thymus. The magnitude of this N-fold increase was from 1.5 to 3 and was especially evident in the DNA obtained from lung tissue (fractions XI and XIII, Table 3).

### TABLE 2

**THE EFFECT OF VARIOUS TUMOR FRACTIONS ON THE INCORPORATION OF ADENINE-8-C14 BY DEOXYRIBONUCLEIC ACID OF MOUSE LUNG**

<table>
<thead>
<tr>
<th>Fraction no.</th>
<th>Fraction injected</th>
<th>Adenine-8-C14 injected (μc/mouse)*</th>
<th>N-fold change in lung DNA†</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Resuspended precipitate obtained from low-speed centrifugation of tumor homogenate adjusted to pH 4.8</td>
<td>44</td>
<td>0.4</td>
</tr>
<tr>
<td>II</td>
<td>Supernatant fraction from Fraction I</td>
<td>44</td>
<td>0.4</td>
</tr>
<tr>
<td>III</td>
<td>Resuspended precipitate obtained from low-speed centrifugation of tumor homogenate previously adjusted to pH 5.0</td>
<td>50</td>
<td>0.3</td>
</tr>
<tr>
<td>IV</td>
<td>Same as Fraction I, except tumor homogenate adjusted to pH 5.2</td>
<td>50</td>
<td>0.3</td>
</tr>
<tr>
<td>V</td>
<td>Same as Fraction I, except tumor homogenate adjusted to pH 5.0</td>
<td>50</td>
<td>0.3</td>
</tr>
<tr>
<td>VI</td>
<td>Resuspended sediment obtained from centrifugation of tumor homogenate at 40,000 r.p.m. for 2 hours</td>
<td>55</td>
<td>0.4</td>
</tr>
<tr>
<td>VII</td>
<td>Supernatant fraction from Fraction VI</td>
<td>55</td>
<td>0.4</td>
</tr>
<tr>
<td>VIII</td>
<td>Same as Fraction VI but centrifuged for 24 hours at 40,000 r.p.m.</td>
<td>100</td>
<td>0.4</td>
</tr>
<tr>
<td>IX</td>
<td>Supernatant fraction from Fraction VIII</td>
<td>100</td>
<td>0.4</td>
</tr>
<tr>
<td>X</td>
<td>Resuspended precipitate from addition of acetone to tumor homogenate</td>
<td>100</td>
<td>0.4</td>
</tr>
</tbody>
</table>

* Mice injected with Fractions I, II, and III were sacrificed 18 hours after injection of labeled adenine. All other groups were sacrificed 24 hours after administration of labeled adenine.

† N-fold change obtained by disintegrations/sec of the DNA fraction obtained from 1 gm. of lung from mice injected with tumor fraction divided by same expression for control mice.
DNA incorporation of C14 following the injection of mammary tumor homogenate and labeled adenine, an attempt was made to fractionate the tumor tissue and possibly to isolate the stimulatory components. The clear supernatant fraction obtained from the centrifugation of a water homogenate of tumor tissue (2 hours at 20,000 r.p.m., Spinco Model L, 40 head) did stimulate up to a threefold intake of the adenine C14 into the DNA of the liver, lung, and muscle. This would indicate that the stimulating activity was present in a soluble and perhaps relatively low molecular weight form. To facilitate the study of this DNA-stimulatory factor of tumor tissue, all subsequent tests, including various tumor fractions, were restricted to a single tissue, the lungs.

### TABLE 3

**INCORPORATION OF ADENINE-8-C14 BY DEOXYRIBONUCLEAR ACID OF LUNGS FROM MICE GIVEN INJECTIONS OF MAMMARY TUMOR**

<table>
<thead>
<tr>
<th>Fraction no.</th>
<th>Treatment of tumor tissue represented in injection</th>
<th>Adenine-8-C14 injected (µc/mouse)*</th>
<th>N-fold change in lung DNA†</th>
</tr>
</thead>
<tbody>
<tr>
<td>XI</td>
<td>None</td>
<td>190</td>
<td>0.96</td>
</tr>
<tr>
<td>XII</td>
<td>Boiled for 8 minutes</td>
<td>190</td>
<td>0.96</td>
</tr>
<tr>
<td>XIII</td>
<td>None</td>
<td>1,500</td>
<td>0.4</td>
</tr>
<tr>
<td>XIV</td>
<td>Boiled for 8 minutes</td>
<td>1,500</td>
<td>0.4</td>
</tr>
<tr>
<td>XV</td>
<td>Room temperature for 20 minutes</td>
<td>80</td>
<td>0.4</td>
</tr>
<tr>
<td>XVI</td>
<td>Room temperature for 2 hours</td>
<td>80</td>
<td>0.4</td>
</tr>
<tr>
<td>XVII</td>
<td>Room temperature for 24 hours</td>
<td>80</td>
<td>0.4</td>
</tr>
</tbody>
</table>

* Mice injected with Fractions XI and XII were sacrificed 12 hours after injections of labeled adenine. All other groups sacrificed 48 hours after administration of labeled adenine.
† N-fold change obtained by disintegrations/sec of the DNA fraction obtained from 1 mg of lung from mice injected with tumor fraction divided by same expression for control mice.

By adjusting the pH of the tumor homogenate it was possible to precipitate a fraction that would stimulate the incorporation of adenine by the normal mouse lung DNA (Groups I, II, III, and IV, Table 2). When the pH was adjusted to 3.0, however, no such stimulatory effect was present in the precipitated fraction. Comparison of the values obtained by the injection with the high-speed centrifuge fractions of tumor tissue points up a wide variation in the total response, e.g., compare VI and VII with VIII and IX. The reason for this is not entirely clear but could have resulted from extensive autolysis of the fractions centrifuged for 24 hours. It is also of interest to point out that an acetone-precipitated fraction of tumor tissue did not influence the lung DNA incorporation of the labeled adenine (Group X, Table 2).

Heating of the tumor homogenate or prolonged exposure to room temperature inactivated the stimulatory activity (Table 3). To ascertain if tissues other than tumor would affect the metabolism of lung DNA, we injected homogenates of liver or lung into normal mice, also injected with labeled adenine (Table 4). Injection of liver homogenates, and of lung homogenate to a lesser degree, did result in an increased uptake of the isotope by the lung DNA. From these findings, we conclude that this stimulatory activity is not restricted to tumors; however, the N-fold change obtained from tumor homogenate was usually greater than that obtained from other tissues. Kelly and Jones (9) also examined the effect of tissue mashes on the P32 turnover in the nuclei of various normal tissues. They found no effect produced by one injection but could demonstrate changes if the particular mash was injected intraperitoneally in eight daily doses. The DNA of both liver and spleen showed increased specific activity of P32 when mashes of liver, spleen, embryo, and tumor were so injected. An alcohol precipitate of liver and spleen homogenates and a crude salt-extracted nucleoprotein fraction from liver, according to Kelly and Jones, were also active.

From the results of the current investigation and from the results of other investigators we conclude that tumor tissues and, to a lesser extent, some normal tissues elaborate a factor(s) which seems to induce the DNA of normal tissues to increase their incorporation of labeled adenine or other precursors of nucleoprotein. The factor(s) has the following properties:

1. It is completely or at least partially inactivated by heat.
2. It is inactivated or solubilized in solutions of low pH.
3. It is rapidly inactivated in tumor homogenate left at room temperature.
4. It is either inactivated or not precipitated by...
There is increasing evidence that tumors alter the metabolism and composition of other tissues and organs of the body. In addition to the work of Kelly, Jones, and associates and of Tyner et al., we may refer to the interesting observations of Reddy, Cerecedo et al. (1, 2, 12-14, 21, 22) that the nucleic acid content of many normal organs is increased in an animal undergoing the stress of tumor or embryonic growth. Greenstein and Andervont (7) and Greenfield et al. (4–6) have noted that the liver catalase content is lowered in the tumor-bearing animal. These workers have concentrated and partially characterized this inhibitory component present in tumor tissues. Nakahara and Fukoka (15–17) also isolated a possible polypeptide substance termed “toxohormone” from tumor tissue which inhibited liver catalase. While the present studies are obviously preliminary, the results indicate the presence of a factor(s) in tumor tissue that alters the DNA metabolism of certain normal tissues. The isolation, purification, and the full characterization of this stimulatory factor should add considerably to our knowledge of tumor-host relation and to the over-all mechanism of tumor development.

SUMMARY

1. C57BL line mice were given injections of homogenates of a transplantable mammary tumor, and the change in adenine-8-C14 uptake by the deoxyribonucleic acid of several normal tissues was observed.

2. An increase in adenine-8-C14 uptake into the DNA from liver, spleen, and lung was noted when tumor homogenates were injected.

3. Certain properties of the factor(s) responsible for this increased nucleic acid activity were determined with regard to its behavior toward heat, acid, acetone, and centripetal force.

REFERENCES


The Effect of Tumor Fractions on the Uptake of Carbon-14-Labeled Adenine into the Deoxyribonucleic Acids of Mouse Tissues


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