Incorporation of $^{32}$P Into Nucleic Acids in Mammary Tissue of Mice Susceptible and Resistant to Breast Cancer

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SUMMARY—Incorporation of $^{32}$P into RNA and DNA fractions of mammary and tumor tissue was studied in virgin C3H mice, which are susceptible to the development of breast cancer. Virgin C57BL mice, which are resistant to the development of breast cancer, were the controls. Virgin C3H and C57BL mice, 4 and 8 months old, and tumor-bearing C3H mice were used as the experimental groups. The incorporation of $^{32}$P into RNA and DNA fractions did not differ significantly among the 4- and 8-month-old, virgin C3H mice. The tumor tissue had the highest rate of uptake of $^{32}$P into both nucleic acids. In castrated C3H mice and C57BL females, the incorporation of $^{32}$P into nucleic acids in the mammary tissue of the C57BL females was significantly lowered, whereas in the C3H mice it remained comparable with that observed in the corresponding control groups.—J Nat Cancer Inst 46: 731-734, 1971.

PROGRESSIVE metabolic alterations in spontaneous mammary carcinogenesis have been studied in this laboratory for several years. These studies essentially concerned the levels of nucleic acids (1) and the enzymes involved in the catabolism of nucleic acids such as ribonuclease, xanthine oxidase, adenosine deaminase, and adenylic acid deaminase (2, 3). We observed that important changes in the levels of nucleic acid and activity of xanthine oxidase occurred in mice at the age of 8 months, when precancerous hyperplastic nodules appeared in breast tissue of C3H virgins which are susceptible to the development of breast cancer. Thus we thought it worthwhile to study the rate of biosynthesis of nucleic acids in spontaneous mammary carcinogenesis. The present paper deals with the incorporation of $^{32}$P into nucleic acids in mammary tissue of the susceptible virgin C3H mice and of virgin C57BL mice, which are resistant to the development of breast cancer. It further reports the incorporation of $^{32}$P into nucleic acids in the breast tissue of castrated mice of both strains.

MATERIALS AND METHODS

Animals.—Virgin C3H and C57BL mice were from the animal colony of the Cancer Research Institute, Bombay.

Chemicals.—$^{32}$P-labeled sodium phosphate was from the Bhabha Atomic Research Centre, Bombay. The other chemicals were of an analytical grade.

Experimental procedures.—For age-group studies 4- and 8-month-old virgin mice of strains C3H and

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C57BL and spontaneous tumor-bearing, virgin C3H mice were used. For studies on castrated mice, 3-week-old females of strains C3H and C57BL were gonadectomized and killed when 8 and 12 months old.

Each experimental group consisted of 6 mice. Intact and castrated virgin mice of different ages were given intraperitoneal injections of 15 μc 32P-labeled sodium-phosphate solution 18 hours before they were killed. Eighteen hours was selected to obtain an optimum uptake of labeled 32P into nucleic acid fractions of mammary tissue and of the tumor as well. Bresciani (4), from the results obtained in his autoradiographic studies, reported that the mean DNA synthesis period in mammary alveolar cells was 20 hours, the range being from 14–30 hours. In contrast, Barnum et al. (5) observed that in the mammary tumor the incorporation of 32P into nucleic acids rose steadily up to 16 hours; they did not study the tumor beyond 16 hours. We therefore thought that 18 hours was a suitable mean period for the study of both mammary tissue and tumor tissue.

Mice were killed by cervical dislocation, and mammary tissue was dissected from the lymph nodes and mixed in a Potter Elvehjem homogenizer with a solution (6) containing sodium-p-aminosalicylate (6%), sodium chloride (1%), sodium triisopropyl naphthalene sulfonate (1%), and sec-butanol (6%). The homogenate was then centrifuged at 0°C at 2500 × g. The fat accumulating at the top was carefully removed. The supernatant was set aside. The sediment was again homogenized in 2 ml of solution and added to the supernatant. The combined solution was then used for the extraction of RNA and DNA by the method of Diamond et al. (7).

The breast tissue from 3 castrated mice was pooled, and the sodium salts of the nucleic acids extracted were dissolved in 0.5 ml distilled water. Then 0.1 ml of the solution was used for the measurement of optical density, and 0.25 ml was plated on planchets and counted in a gas-flow counter (Nuclear Chicago model). Specific activity of a given sample was expressed in terms of counts/minute/mg DNA or RNA.

RESULTS

Table 1 summarizes the results. In the incorporation of labeled phosphate into RNA fractions of mammary tissue and tumor, the uptake of 32P in 4- and 8-month-old groups of both strains was comparable. The data show that the tumor tissue had the highest rate of incorporation.

In the 32P incorporation into DNA fraction of both strains, the rate of DNA biosynthesis in 4- and 8-month-old C3H and C57BL virgins was comparable and was highest in the tumor tissue. Further, 32P uptake into DNA fractions of 4- and 8-month-old C57BL virgins was higher than that observed in the corresponding C3H groups.

The uptake of 32P in both DNA and RNA did not differ remarkably in 8-month-old, castrated C3H mice and their corresponding controls. Further, the rate of nucleic acid biosynthesis in 8-month-old castrated mice was comparable to that in the 12-month-old, castrated C3H group.

The pattern of incorporation into nucleic acids was different in castrated C57BL mice. The uptake

<table>
<thead>
<tr>
<th>Nucleic acid fraction</th>
<th>Strain</th>
<th>Specific activity in each group</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>4 months</td>
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<tr>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>DNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3H</td>
<td>1785 ± 7</td>
<td>1927 ± 11</td>
</tr>
<tr>
<td>C57BL</td>
<td>3214 ± 11</td>
<td>3800 ± 7</td>
</tr>
<tr>
<td>C3H</td>
<td>8500 ± 10</td>
<td>7700 ± 18</td>
</tr>
<tr>
<td>C57BL</td>
<td>10,000 ± 17</td>
<td>8700 ± 10</td>
</tr>
</tbody>
</table>

*Results are expressed as the mean of three experimental values. Specific activity is expressed as counts/minute/mg nucleic acid: ± SE.
of \(^{32}P\) into DNA fraction of 8-month-old castrated mice was remarkably lower than that in their controls and continued to be low even in the 12-month-old castrated group. With RNA, the incorporation in 8-month-old, castrated C57BL mice was comparable to that in the corresponding controls but fell significantly in 12-month-old C57BL mice.

**DISCUSSION**

These results clearly illustrate that there is no significant alteration in the rate of incorporation of \(^{32}P\) into nucleic acids of mammary tissue of C3H mice at the age of 8 months when precanerous hyperplastic nodules begin to appear. We have observed a very high uptake of \(^{32}P\) into the nucleic acids of mammary tumors; conceivably, with the increase in cell division and growth rate, the tumor should have a high rate of nucleic acid biosynthesis. Other investigators \((8-10)\) have reported an increased rate of \(^{32}P\) incorporation into various types of malignant tumors.

The present studies also show that 4-month-old, virgin C57BL mice have a higher rate of incorporation of \(^{32}P\) into DNA than do C3H mice of the same age. We observed previously that DNA in mammary tissue of 4-month-old C57BL virgins is higher than that of C3H virgins of the same age \((2)\). However, this initial high content of DNA and its initial high rate of biosynthesis do not change appreciably in the older age groups and remain comparable.

The pattern of nucleic acid biosynthesis also differs considerably in castrated females of the susceptible (C3H) and resistant (C57BL) strains. In castrated C3H mice, the uptake of \(^{32}P\) into RNA and DNA fractions at the age of 8 months is comparable with that in the corresponding control group and does not change appreciably in the 12-month-old castrated group. In castrated C57BL females, the uptake of \(^{32}P\) into DNA is considerably low in both age groups. Even RNA biosynthesis, comparable in 8-month-old castrated and control groups, decreases considerably in 12-month-old, castrated C57BL females. Thus the rate of biosynthesis of nucleic acids in the mammary tissue of castrated virgins of susceptible strains is apparently not affected, whereas it is significantly reduced in mammary tissue of the castrated females of the resistant strain. Biological studies on castrated females of these two strains support this contention. Ranadive reported that the secondary sex organs (e.g., mammary gland, uterus, etc.) of the castrated C3H females are fairly well developed and mammary tumors are even observed in old castrated females \((11)\). She proposed that hyperplastic adrenal nodules secrete steroid hormones responsible for the development of the mammary gland. Unlike castrated C3H mice, the castrated C57BL females have rudimentary growth of mammary gland and other secondary sex organs and have adrenals which do not show any hyperplasia, as was observed with castrated C3H females.

To summarize, the females of the two strains, one susceptible and the other resistant to development of mammary cancer, show specificity in the nucleic acid biosynthesis of the mammary gland in the normal and castrated females. Exploration of such inherent metabolic differences between these two strains could reveal the mechanism by which spontaneous mammary carcinogenesis occurs in susceptible strains.

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