The Effect of Mammary Tumors on the Glucuronidase and Esterase Activities in a Number of Mouse Strains*

SAUL L. COHEN AND JOHN J. BITTNER

(Department of Physiological Chemistry, the Max H. Hoffman Endocrinological Research Laboratory, and the Division of Cancer Biology, Department of Physiology, University of Minnesota Medical School, Minneapolis, Minn.)

The reports of Fishman and Anlyan (4) and of Odell and Burt (12) indicating a high β-glucuronidase activity in a number of human cancerous tissues have elicited considerable interest in the possibility of a relationship between this enzyme and neoplasms in general. The lack of species specificity for such a relationship is indicated in the observation by Kerr et al. (9) of a higher glucuronidase concentration in mammary tumors than in the normal tissue of mice (of undesignated strains).

Harris and Cohen (8) observed an inverse relationship between changes in β-glucuronidase and esterase activity levels for a number of tissues of mice subjected to variations in the amount of circulating sex hormones. Similar inverse changes between these enzymes have also been reported (2) as occurring in the sera of patients with breast cancer on estrogen therapy. The investigations reported in this paper were undertaken in part to determine whether a similar inverse relationship might occur in the mammary tumors of inbred mice. Such a possibility seemed supported by the report of Greenstein in 1944 that tumors of the liver, lymph node, and intestine in a number of strains of mice possessed a considerably lower esterase concentration than the corresponding normal tissue.

A second aspect of the studies reported herein was to determine whether the glucuronidase and esterase activities in the mammary tissues varied for different strains of mice. A number of reports have appeared within the last decade on variations in enzyme patterns in a variety of inbred mouse strains. In 1942 Khanolkar and Chitre reported that the serum esterase activity of C57 mice was lower than for the C3H and A strains. Subsequently, Shimkin, Greenstein, and Andervont (13) noted a lack of correlation between serum esterase and susceptibility to mammary tumors. Thus, they observed that the C and I strains showed serum esterase activities which were, respectively, the same as and greater than those found in the mice of the C3H strain. Many studies have also been carried out on the livers of inbred mice with tumors, and certain strain variations have been reported for the enzymes xanthine dehydrogenase (5, 6), liver catalase (5), and glucuronidase (11). No strain differences have been observed for a large number of other hepatic enzymes studied (for references see 11).

These two problems are answered, at least in part, by the data reported in this paper, which is based on studies on the glucuronidase and esterase activities of the tumorous and nontumor mammary tissue of a number of strains of mice.

METHODS

Mouse strains.—A total of 69 mice was used in these experiments. Animals with mammary cancer were from the following stocks or F1 generations: the Andervont subline of the C3H stock; the author's (J. J. B.) line of the CSH stock referred to as the Z strain; D8 subline of the D or dilute brown stock; ZDxF1 hybrids (Z♀ × D♂); A; AJKF1 (A♀ × JK♂); and C stock. Animals of five strains, Ax, Zb, JK, C57 black (sublines 1 and 4), and C, were without the milk agent and therefore without mammary tumors (1). Of these latter groups, the Ax, Zb, and C mice were susceptible to the development of spontaneous mammary cancer. All the females employed in these studies were nonpregnant and averaged 18 (8–17) months of age. For each strain three to twelve mice were used.

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Preparation of tissues.—Most of the animals were killed with ether; those animals on which blood enzyme assays were also carried out were killed by cutting the jugular veins. The mammary tissue was immediately removed, the tumorous and nontumorous tissue separately pooled and chilled. The tissues were weighed and prepared for assay by procedures already described (8).

Enzyme assays.—The glucuronidase and esterase (butyrate) activities of the tissues were determined by the methods outlined by Harris and Cohen (8). The serum enzymes were determined by procedures slightly modified from those of Cohen and Huseby (8). A unit of enzyme is defined as the amount of enzyme which liberates 1 \( \mu \)g per hour of phenolphthalein and butyric acid from their corresponding substrates.

To obviate the diluting effects of water and of lipids, nitrogen determinations were carried out on aliquots of each homogenate, and the tissue enzyme activities reported in this paper are expressed in terms of the nitrogen content of the tissue, rather than on a basis of tissue wet weight.

RESULTS AND DISCUSSION

The results of the enzyme determinations for mouse mammary tissues are summarized in Table 1. The following observations may be made:

A. \( \beta \)-Glucuronidase activities

1. Marked variations occur in the glucuronidase activity of the mammary tissue of different strains of mice. The lowest nontumorous value for the mice with the milk agent was found for the Andervont C3H mice (4.0 ± 0.47), while the highest values were observed for the AJKF1 (112 ± 18.1) and for the C (119 ± 7.9) strains. The Z mice (Bittner C3H line) were also found to possess a low mammary glucuronidase level (25 ± 3.4). A significant difference for the two sublines of the C3H stock (Andervont and Bittner sublines) is, however, to be noted. No significant differences were observed for ZD\(_{F1}\), D\(_{b}\), and A mice (average glucuronidase activity about 88 units).

2. Similar strain differences were also observed for the glucuronidase activities of mice without the milk agent and without spontaneous mammary tumors. Of the five such strains examined, an increasing order of enzyme activity was found for the Zb (48 ± 4.5), JK, C, and Ax (125 ± 7.8) mice. It is also to be noted that for strains A and Z the presence of the milk agent results in a considerable decrease in the glucuronidase activity of the nontumorous mammary tissue, while for the C strain the mammary tissue glucuronidase level was higher than that in animals possessing the milk agent. It is possible that this difference is due to the fact that the C mice received the milk agent relatively late in life.

3. There are indications that the glucuronidase activity levels for the different strains of animals are genetically determined. This is particularly indicated in the mammary tissue values obtained for the ZD\(_{F1}\) strain of mice in which the glucuronidase levels are about the same as for the D\(_{b}\) mice but about 3–4 times as great as for the Z mice. Further support for the hypothesis of genetic control of glucuronidase levels is seen in the data (summarized in Table 2) obtained for enzyme assays on blood sera. It is seen that the serum glucuronidase of the Z mice is only about 20 per cent as high as that of the A strain, while the glucuronidase activity for the C strain is about 50 per cent as high as that of the Zb strain.
dase activity of the nontumorous mammary tissue for the Z strain was about 28 per cent of that of the A strain. In this connection it might also be pointed out that Morrow et al. (11) reported the liver glucuronidase activity of Andervont CSH mice to be only about 10 per cent of that of A mice, which is comparable to our findings of about 5 per cent for nontumorous mammary tissue. The final proof of a possible genetic determination of the glucuronidase activity in mice must await assays on a large number of mice in experiments specifically designed for this purpose.

4. An increased $\beta$-glucuronidase activity of the malignant mammary tissue, as compared to that of the nonmalignant mammary tissue, was observed for all strains of mice examined. Indeed, no exception to this relationship has been found in any of the 42 mice with mammary tumors thus examined in our laboratory. It can also be seen that a similar species difference is demonstrated for the cancerous mammary tissue, as for the corresponding noncancerous tissues. The percentage increase in glucuronidase activity, however (see Table 1, column c), varies inversely with the relative levels in the noncancerous tissue. Thus, in mice of three strains, the cancer tissue in the CSH strain showed 1500 per cent as much glucuronidase as the noncancer tissue, while corresponding values of nearly 300 per cent for the Z stock and of only 160 per cent for the C mice were shown.

B. Esterase (butyrate) activities

1. A much smaller percentage variation in the esterase content of nontumorous tissues was noted among different mouse strains than was found for the $\beta$-glucuronidase activities. A maximum spread of about 2.5-fold was found for the strains examined (the C animals with the milk agent showed the lowest values, while the ZD1F1 mice with the agent and JK group without the agent had the highest esterase activities). There seems to be no uniform relationship between the relative glucuronidase and esterase activity levels in the nontumorous mammary tissue of different strains of mice.

A lack of relationship between the relative glucuronidase and esterase levels of different mouse strains is also to be seen in the serum assays (Table 2). Thus, while the Z and C57 mice showed approximately the same serum esterase levels, the serum glucuronidase of the former strain was about $\frac{1}{4}$ that of the latter group of mice. These data also confirm the previous reports of others (10, 13) that the C57 mice have a lower serum esterase activity than that of the A strain.

2. The presence of the milk agent had no significant effect on the esterase content of nontumorous mammary tissue of the A or Z strains but was associated with a marked decrease (about 50 per cent) for the C strain.

3. In all the mice examined, the neoplastic mammary tissue had a markedly lower esterase activity than did the noninvolved mammary tissue. It is also of interest that, with the exception of the A mice, there seems to be an inverse relationship between the esterase level of the mammary cancer tissue and an approximately direct one for the ratio of noncancer tissue to cancer tissue esterase activities (see Table 1, column f), and the relative glucuronidase levels for the different strains of mice.

### Table 2

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>NO. OF MICE</th>
<th>GLUCURONIDASE LEVELS</th>
<th>ESTERASE LEVELS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td>4</td>
<td>368±111</td>
<td>28,700±5,900</td>
</tr>
<tr>
<td>CSH</td>
<td>1</td>
<td>718</td>
<td>92,100</td>
</tr>
<tr>
<td>C57, line 1</td>
<td>3</td>
<td>1,150±88.5</td>
<td>25,600±2,830</td>
</tr>
<tr>
<td>C57, line 4</td>
<td>3</td>
<td>1,150±44.2</td>
<td>27,100±1,490</td>
</tr>
<tr>
<td>A</td>
<td>8</td>
<td>1,250±185</td>
<td>37,700±2,620</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>55,900</td>
<td></td>
</tr>
</tbody>
</table>

All values are expressed as averages ± the standard error.

4. While no proof for the discrepancy shown by the A strain in the above relationships is at present available, it is possible that this may be related to the lipid content of the mammary tissue. Thus, in a preliminary experiment the lipid (the petroleum ether-soluble fraction) content of the mammary tissue has been observed to be about $\frac{1}{4}$ as great for cancerous as for noninvolved tissue for mice of both the A and CSH strains. For both the involved and noninvolved mammary tissues, however, the lipid content was about 3 times as high for the A strain (0.67 ± 0.36 per cent and 3.1 ± 1.3 per cent, respectively) than for the CSH strain (0.27 ± 0.06 per cent and 1.3 per cent, respectively). It is entirely possible that some direct relationship exists between the lipid content and esterase activity of a tissue. Thus, a relatively low esterase level effected by one set of circumstances might be masked by a high esterase activity related to another set of circumstances. Such an explanation might also explain the apparent lack of consistency for the esterase levels referred to in sections B, 1 and 2, above.

5. An increased glucuronidase and decreased esterase have been observed for all neoplastic mammary tissues, as compared to the corresponding uninvolved tissues examined. This inverse relationship between glucuronidase and esterase changes is similar to that previously reported for other experiments (2, 8).
There appears to be no relationship between mammary glucuronidase and esterase activities and tumor incidence in the mice studied. Thus, for example, both the glucuronidase and esterase activities of the C3H strain (possessing high tumor incidence) are lower than those of the C and JK strains, but the C mice are also a strain of high tumor incidence when they possess the milk agent, while the JK mice are a strain showing a low tumor incidence under similar circumstances.

SUMMARY
1. The results of $\beta$-glucuronidase and esterase determinations for the cancerous and uninvolved mammary tissue of 39 mice of 7 different strains with the milk agent and of 30 mice of 5 strains without the agent are reported.
2. Considerable variation in the $\beta$-glucuronidase levels was found for the various strains of mice both with and without the milk agent. Of the animals possessing tumors, the C3H strain of mice showed only 3.3 per cent as much glucuronidase activity as did the C and AJKF1 strains, with the Z, ZDxF1, D8, and A strains possessing values between these extremes.
3. Evidence of a genetic influence on the glucuronidase activity levels of different mouse strains is presented and discussed.
4. Variations in the esterase levels of the cancerous mammary tissues were found in most instances to vary inversely with the relative mammary glucuronidase activities of the various strains of mice examined. A possible explanation for the one exception (A mice) in this group, as well as the apparent small and unrelated variations in the esterase levels of the uninvolved mammary tissues, is discussed.
5. In all the animals examined, tumors of the mammary glands were associated with increased $\beta$-glucuronidase and decreased esterase activities, as compared to those of uninvolved mammary tissue. In most cases the per cent difference between the involved and uninvolved tissue varied inversely for the glucuronidase and directly for the esterase activities with the relative glucuronidase levels of the different strains of mice.
6. The variations in $\beta$-glucuronidase and esterase activity levels are apparently not related to the tumor incidence in the various strains of mice.

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REFERENCES
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