DNA Synthesis in Autonomous and Hormone-responsive Mammary Tumors

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SUMMARY

Hormone responsiveness of autonomous and diversely hormone-responsive mammary tumors of the rat was assayed in organ cultures by measuring DNA synthesis. Rat sera rich in hormones secreted by mammotropic pituitary cells were compared with sera of normal female and male and of ovariectomized and hypophysectomized rats. Mammotropic sera invariably stimulated DNA synthesis of hormone-responsive tumors and normal mammary glands but not of autonomous tumors.

INTRODUCTION

The prime objective of this study is to distinguish between hormone-responsive and autonomous mammary tumors in organ cultures on basis of stimulation of nucleic acid synthesis by homologous mammotropic hormones. The diverse types of mammary tumors studied were characterized by transplantation assays (13). The method of organ cultures used and mammotropic hormone and somatotropic hormone values in the type of sera added to the cultures were described (13). Although several variables remain to be controlled, the technique used enables us to differentiate between hormone-responsive and non-responsive tumors on basis of their responsiveness to sera of rats bearing mammotropic pituitary tumors by stimulation of DNA synthesis.

MATERIALS AND METHODS

The materials used, methods of organ cultures, radioautography, histology, and control of certain variables are described in the preceding article (13). In the studies here described all media contained 5 µg insulin/1 ml medium. All analyses were made in duplicate. All sera unless otherwise stated were from female rats.

RESULTS AND DISCUSSION

Comparison of Nucleic Acid Synthesis in Organ Cultures of Autonomous, Hormone-responsive, and Nonresponsive Tumors. The body of this paper is limited to DNA synthesis. The introductory Table 1 gives data also on RNA synthesis...
done in several earlier experiments because they were made simultaneously with DNA synthesis and served as background for further studies on RNA synthesis to be reported.

Under the conditions tested, DNA synthesis was considerably greater in the autonomous than in the responsive tumors.

The values for the autonomous, rapidly growing tumor (9D) in normal sera were 9,990 and 11,670 cpm/mg protein, and in the slowly growing autonomous tumor (9B) they were 7,000 and 15,800 cpm/mg protein. In contrast the corresponding values in the responsive tumor (9A) ranged from 800 to 2,880 cpm/mg protein.

MtT serum markedly stimulated DNA synthesis of the hormone responsive but not of the autonomous mammary tumors. The increase of incorporation into DNA in cultures containing MtT versus normal female serum in the rapidly growing autonomous MT9D was -9% and -19%; in the slowly growing autonomous MT9B it was +3%; and in the slowly growing responsive MT9A it was 310, 147, and 115% (Table 1).

RNA synthesis of the hormone-responsive tumor (9A) was also stimulated during the 12-hr pulse period by MtT serum in all 3 experiments but not as much as DNA synthesis. The increase of RNA synthesis in the autonomous tumors was variable. It was stimulated in 2 and was about unchanged in 1 experiment.

Our recently isolated hormone-dependent MT449 was assayed for DNA synthesis in 3 experiments (Table 2).

Both MtT and DES sera markedly stimulated DNA synthesis (Table 2). It should be recalled that DES sera are related to MtT sera, DES being the inducer of MtT. The donors of these DES sera had enlarged pituitaries due to hyperplasia of mammotropes resulting from sustained stimulation by pellets of this estrogen. However, they may also have had increased levels of estrogen.

Our standard base for comparison was normal female serum. Without any serum added to the medium DNA synthesis by the tumors was low (Table 2). Since all these culture dishes contained insulin, stimulation of DNA synthesis by normal serum was due in part to the unknown growth-stimulating "serum factor," in part to physiological levels of mammary-gland-stimulating hormones in female sera.

Table 2 also shows stimulation of DNA synthesis of a spontaneous milk-secreting adenocarcinoma (MT.WB3) and of 2 hyperstimulated mammary glands. One was obtained from an MtT (MtH)-stimulated rat (Experiment 19); the other came from a pregnant rat (Experiment 20). In considering the stimulation of these mammary glands by MtT sera it should be remembered that they were already in a highly stimulated state when cultured. [The microscopic appearance of MT.WB3 is illustrated in Fig. 7 and that of the stimulated mammary gland used in Experiment 19 is shown in Fig. 8 of the preceding paper (13).]

Fibroadenoma. DNA synthesis of 2 spontaneous fibroadenomas was tested under identical conditions as were the various carcinomas. Both gave exceedingly low values for DNA synthesis. One was a tough fibrous tumor with no grossly detected glandular elements. It gave average values ranging from 25 to 33 cpm/mg protein. The other was also fibrous but softer (it might be termed adenofibroma) and gave values ranging from 440 to 870 cpm/mg protein.

These values reflect the biological behavior of these common tumors: exceedingly slow growth, lack of invasive.
ness, failure to undergo malignant transformation, and having normal-appearing connective tissue as major component. Pursuance of these data may clarify the unique biological behavior of these most common tumors of the rat.

DNA Synthesis of Various Types of Mammary Tumors and Mammary Gland in Media Containing Serum from Ovariectomized versus Normal and MtT Serum. Routinely, stimulation of DNA synthesis in organ cultures of mammary tumors by MtT sera was compared with DNA synthesis in the presence of normal sera. The possibility that sera of ovariectomized rats may form a better base was tested in several experiments (Table 3). The results suggest that sera of ovariectomized rats may form a better base than normal sera. However, the MtH levels during estrus are high (8, 13) and there may be some estrogen production by the adrenals following ovariectomy. Thus sera of ovariectomized rats verified for lack (or near zero levels) of mammotropic hormones may form a better base for assessment of responsiveness to MtH sera than randomly obtained normal rat sera. If the latter is used the sera should be obtained during

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### Table 2

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Experiment No.</th>
<th>No serum supplement (cpm/mg protein)</th>
<th>Female serum added to media</th>
<th>Increase vs. normal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT449, dependent, slowly growing</td>
<td>14a</td>
<td>16,070</td>
<td>MtT 40,700</td>
<td>154</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>3,370</td>
<td>MtT 4,190</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>2,970</td>
<td>MtT 9,270</td>
<td>212</td>
</tr>
<tr>
<td>MT.WB3b, spontaneous</td>
<td>18</td>
<td>3,380</td>
<td>MtT 6,780</td>
<td>100</td>
</tr>
</tbody>
</table>

In this experiment 10% serum was used. TdR-3H uptake in the cultures containing 1% MtT serum was 28,600 cpm/mg, and in that containing 5% MtT serum 31,700 cpm/mg respectively.

### Table 3

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Experiment No.</th>
<th>Ovary (cpm/mg protein)</th>
<th>Normal (cpm/mg protein)</th>
<th>Increase by normal vs. ovex (%)</th>
<th>MtT (cpm/mg protein)</th>
<th>Increase by MtT vs. (%) ovex</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT9D autonomous</td>
<td>26</td>
<td>5,940</td>
<td>9,990</td>
<td>68</td>
<td>9,110</td>
<td>53</td>
</tr>
<tr>
<td>MT9A responsive</td>
<td>10</td>
<td>650</td>
<td>1,720</td>
<td>165</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1,840</td>
<td>13,590</td>
<td>7,370a</td>
<td>35,600b</td>
<td>152</td>
</tr>
<tr>
<td>MT.WB3 spontaneous</td>
<td>18</td>
<td>2,180</td>
<td>3,380</td>
<td>55</td>
<td>6,780</td>
<td>211</td>
</tr>
<tr>
<td>Mammary glands</td>
<td>19</td>
<td>12,700</td>
<td>10,700</td>
<td>18</td>
<td>45</td>
<td>23,500</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>8,800</td>
<td>9,280</td>
<td>-5</td>
<td>34,200</td>
<td>288</td>
</tr>
</tbody>
</table>

*The media also contained 5% serum of ovariectomized rats.

bThe media contained 20% serum; all other media contained 5% serum.
Nucleic Acid Synthesis in Cultured Mammary Tumors

Diestrus; radioimmunoassays for pituitary hormone levels are in any event desirable.

DNA Synthesis in Serum of Hypophysectomized Rats. In one experiment (Table 4) serum of hypophysectomized rats was used as base. DNA synthesis was less in the sera of hypophysectomized rats than in normal female sera. The degree of stimulation of DNA synthesis was greater by MtT.W15 than by MtT.F4 as might be expected on basis of higher MtH content of MtT sera. The use of sera of hypophysectomized rats was not further pursued because interpretation of the data calls for the determination of the quantity of 2 nonspecific anabolic pituitary hormones, thyrotropins and somatotropins, also absent in these sera. The data of Table 4 suggest that nonspecific stimulation by physiological levels of thyrotropins and somatotropins is slight, if any. However, the range of DNA synthesis of this tumor in the 4 types of sera parallels the MtH concentration of these as indicated by radioimmunoassays (13).

Table 4
Increase of TdR-3H uptake of MT449 in various sera with serum of hypophysectomized (hypox) rats as a base (Experiment 16)

<table>
<thead>
<tr>
<th>Serum supplement to 5% hypox serum</th>
<th>cpm/mg protein</th>
<th>Increase by MtT.W15 serum (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% hypox female</td>
<td>4,110</td>
<td>112</td>
</tr>
<tr>
<td>5% normal female</td>
<td>5,930</td>
<td>47</td>
</tr>
<tr>
<td>5% MtT.F4 female</td>
<td>6,430</td>
<td>36</td>
</tr>
<tr>
<td>5% MtT.W15 female</td>
<td>8,710</td>
<td></td>
</tr>
</tbody>
</table>

The Effect of Male Sera on DNA Synthesis. The effect of male sera on DNA synthesis was tested in several experiments (Table 5). In contrast to clear-cut data presented in the preceding tables with respect to the stimulating effects of MtT versus normal female sera and sera of ovariectomized and hypophysectomized rats, the data on male sera show a perplexing inconsistency when compared with female sera. While MtT sera in these as in all other experiments gave uniformly distinctly greater stimulation than normal sera of either sex, in 3 of the 6 tests male sera stimulated DNA synthesis more than female sera. Male sera were anticipated to inhibit synthesis of DNA of both mammary epithelium and mammary tumors. There are reasons to suppose that this divergence is not due to technical variations and that the riddle will be clarified by hormone assays of the individual male sera. The mammary gland of the normal male rat is often hyperplastic. Spontaneous pituitary tumors are common in both male and female rats and are readily induced by estrogens in both sexes; most of these tumors are mammotropic even in males (personal observation).

Unfortunately, most male sera were pooled from males over 1 year old, some of the age when hyperplasia and "pre-tumors" of mammotropin- and somatotropin-secreting cells are common. Further, many autonomous mammary tumors grow equally well in males as in females and transplantable mammary tumors are known which are "versely responsive," growing better in males than in females.

Individual sera used in such studies will have to be assayed for both protein and steroid hormones. Tests with sera of castrated rats may help to clarify this situation.

Table 5
Effect of male sera on TdR-3H uptake in various mammary tumors and stimulated mammary glands, pulsed during 12 to 24 hr of incubation

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Experiment No.</th>
<th>Male serum (cpm/mg protein)</th>
<th>Female vs. male</th>
<th>MtT vs. male</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT9A, autonomous responsive</td>
<td>6</td>
<td>4,320</td>
<td>-33</td>
<td>+173</td>
</tr>
<tr>
<td>MT449 dependent</td>
<td>8</td>
<td>3,100</td>
<td>-45</td>
<td>+37</td>
</tr>
<tr>
<td>MT.WB3 spontaneous adenocarcinoma</td>
<td>22</td>
<td>1,890</td>
<td>+57</td>
<td>+390</td>
</tr>
<tr>
<td>Mammary glands</td>
<td></td>
<td></td>
<td>+37</td>
<td>+166</td>
</tr>
<tr>
<td>stimulated</td>
<td>19</td>
<td>7,140</td>
<td>+50</td>
<td>+229</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>12,500</td>
<td>-26</td>
<td>+174</td>
</tr>
</tbody>
</table>

For absolute values in simultaneous cultures containing MtT or normal female sera see Tables 1 and 2.

The gland in Experiment 19 was from an MtT rat; that in Experiment 20 was from a late-pregnant rat.

COMMENTS

Measurement of the growth of the mammary gland and mammary tumors by assaying DNA synthesis has been used by several investigators but by somewhat different techniques with steroids and heterologous pituitary hormones, and the tumors studied in vitro were rarely characterized in vivo. Pharmacological and physiological effects, the biphasic and indirect effect of estrogens, were not considered or not adequately considered (6, 7, 12).
Turkington and Topper (16, 17) noted inhibition of DNA synthesis of the mammary gland by androgens and stimulation by human placental lactogen. The latter was noted in our Experiment 8 (with the hormone-responsive MT9A) while our results with male sera were variable. Turkington and Hilf (15) and Hilf et al. (4) found that estrogen inhibits DNA synthesis in a slowly growing, responsive mouse mammary carcinoma but not in an autonomous rat carcinoma.

Insulin has been reported to stimulate DNA synthesis in normal mouse mammary gland (9, 15) as well as in a virus-induced responsive mouse mammary carcinoma, but not in an autonomous rat mammary carcinoma (15). In our studies it did. Lockwood et al. (10, 11) found that insulin initiates DNA synthesis in the epithelial cells of mammary explants in presence of hydrocortisone and MTH, and that this effect is elicited at least in part by the insulin-dependent emergence of DNA polymerase activity in these cells. Insulin and somatotrophic hormone augment initiation of DNA synthesis but do not alter the rate of DNA replication (14).

In studies of Heuson et al. (2) stimulation of thymidine incorporation became evident on the second day in 5 of 12 dimethylbenzanthracene-induced rat tumors. The insulin-nonresponsive tumors had a higher rate of cell proliferation. [According to data of Huggins (5) and Kim (6) most if not all such tumors are highly hormone sensitive.] No morphological basis was found to account for this difference. The growth-promoting effect was accomplished with little or no effect on glucose consumption. However, at low concentration in the presence of insulin glucose became a rate-limiting factor (3).

Studies with human tissues made by J. Fabricant (personal communication) have shown that neoplastic tissues can synthesize DNA in normal sera longer than normal tissues can. It is well known that it is easier to grow tumor cells in vitro than normal cells and that, upon addition of fresh serum, plateaued growth of normal cells will resume. Fabricant found that insulin can substitute for the unknown growth factor present in normal sera. Their observations show a growth advantage of neoplastic tissue over normal tissue in vivo.

The addition of MHT sera invariably stimulated DNA synthesis in cultures of in vivo hormone-responsive tumors and of normal mammary glands. In contrast tumors which do not respond to these hormones in vivo showed little or no effect in vitro on addition of serum containing these hormones.

While the present studies clearly indicate a characteristic difference in behavior between fully autonomous and hormone-responsive tumors, the components of MHT sera, the associated RNA synthesis, and the many variables and unknown factors discussed in these 2 papers remain to be analyzed. Hopefully, this may lead to a practical procedure of distinguishing responsive from autonomous tumors in vitro, to a technique of predictive value for chemotherapeutic agents, and to a better understanding of the biochemical defects of diverse types of neoplasms. Quantitative determination of all growth factors in the sera of tumor hosts and in sera (media) of organ cultures used for stimulation or inhibition of mammary tumor is not a remote possibility in this age of automation; neither is the interpretation of such multifactorial events by computerization.

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REFERENCES


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