Effect of Actinomycin D on Estrogen-induced Changes in Enzymes and Nucleic Acids of R3230AC Mammary Tumors, Uteri, and Mammary Glands

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SUMMARY

The R3230AC mammary adenocarcinoma responds to estrogen treatment with lactation accompanied by marked increases in glucose-6-phosphate dehydrogenase and NADP-malic enzyme activities. The effect of actinomycin D on these hormone-induced responses was studied. NADP-isocitric dehydrogenase, phosphoglucoisomerase, and phosphoglucomutase activities were also measured in the tumor as well as the RNA and DNA contents. Similar measurements were made on uteri of these animals.

Actinomycin D prevented the estrogen-induced increase in glucose-6-phosphate dehydrogenase, NADP-malic enzyme, and phosphoglucomutase activities in the tumor. The antibiotic likewise prevented the elevation of uterine glucose-6-phosphate dehydrogenase and NADP-malic enzyme activities; the prevention of these hormone responses occurred at lower doses in the uterus compared with the tumor. There was a significant decrease in RNA in the neoplasm and uterus following treatment with actinomycin D. Treatment with the antibiotic alone caused a decrease in the activities of all of the enzymes studied in the tumor and the uterus, with the exception of phosphoglucoisomerase in the neoplasm. These data indicate that the estrogen-induced enzyme activity increases resulted from DNA-directed RNA synthesis.

An unusual and interesting result was the effect of actinomycin D and estradiol valerate on some of these enzymes in normal mammary tissue. Under the conditions of these experiments, an increase in the activities of glucose-6-phosphate dehydrogenase, NADP-malic enzyme, and NADP-isocitric dehydrogenase occurred following treatment with actinomycin D in combination with estrogen, an increase that was not obtained by hormonal treatment alone. These data are discussed in light of recent data on the effects of actinomycin D on other enzymes.

Hilf et al. (13) recently reported the characteristics of a transplantable mammary carcinoma with certain unusual biochemical properties. This tumor, R3230AC, did not exhibit anaerobic utilization of glucose substrate in vitro and responded to estrogen treatment with lactation accompanied by marked increases in glucose-6-phosphate dehydrogenase and NADP-malic enzyme activities. This tumor thus appeared to be particularly suitable for the study of the nature of these hormone-induced enzyme responses. The availability of actinomycin D and puromycin, whose modes of action are well documented (1, 8, 19), prompted us to investigate the effects of these antibiotic agents on the enzyme changes following in vivo treatment with estrogen.

Such an approach has been utilized in the study of hepatomas of different rates of growth. In an attempt to determine the extent to which the Morris Hepatoma 5123 differs from normal liver, Pitot and Morris (18) were unable to induce a significant elevation in tryptophan pyrrolase activity in that tumor following parenteral administration of the inducing agent, tryptophan. These investigators concluded that the failure of Hepatoma 5123 to show enzyme adaptation was due to some intrinsic defect in the neoplastic cell. However, since their report, Dyer et al. (5) and Cho et al. (3) have found several hepatomas that were capable of responding to enzyme induction by tryptophan. Dyer et al. (5) also reported...
preliminary findings that the enzyme induction was prevented by actinomycin D, a further indication of the biochemical similarity of the tumor to normal tissue.

The data presented here indicate that the estrogen-induced increases in glucose-6-phosphate dehydrogenase and NADP-malic enzyme activities in the R3230AC tumor were completely prevented by certain doses of actinomycin D. These estrogen-induced enzyme changes were similarly blocked in uteri of the test animals. In addition to the above enzymes, the activities of NADP-isocitric dehydrogenase, phosphoglucose isomerase, and phosphoglucomutase were measured in neoplasms and uteri. The last 2 enzymes were selected because of their relationship to the metabolism of glucose-6-phosphate, a key intermediate in both anaerobic and aerobic pathways.

A rather surprising result was the ability of actinomycin D to augment, rather than to inhibit, the effect of estrogen on certain enzymes in normal mammary tissue. Actinomycin D produced the anticipated interference with DNA-mediated RNA synthesis, as shown by measurements of RNA and DNA in tumors and uteri. These data are discussed with reference to some of the more recent results in the literature concerning this antibiotic.

MATERIALS AND METHODS

The R3230AC mammary adenocarcinoma, maintained in female Fischer rats, which weighed 90–110 gm at the time of transplanting, was used throughout these studies. The tumor was allowed to become established during a period of 3 weeks following implantation by sterile trochar technic (11, 12). Animals were paired according to weight and tumor dimensions, with 10 animals/treatment group. A single s.c. injection of estradiol valerate, 10 mg/kg, was given to all animals receiving the hormone treatment. This was preceded 1 hr by an i.p. injection of actinomycin D, given at various doses as listed in the tables. A 2nd dose of the antibiotic was administered in the afternoon (about 4 P.M.): this schedule of 2 daily injections of the antibiotic was continued for 2 more days. The last dose of antibiotic was administered approximately 1 hr before necropsy and removal of tissue from the animals. Each animal received 7 i.p. injections of antibiotic. The groups receiving actinomycin D at 300 μg/kg (total dose of 1.5 mg/kg) were an exception to this schedule, as they were sacrificed after receiving only 5 injections of the antibiotic. At necropsy, R3230AC tumor, uterus, and mammary glands were removed, trimmed of adhering fat and hemorrhagic tissue, and quick-frozen in an acetone-Dry Ice freezing mixture. Tissues were stored at −20°C until assayed.

All enzyme assays were conducted under identical conditions by determination of the production of NADPH by following the spectrophotometric absorbancy at 340 μm. Assay conditions were selected to ensure zero-order kinetics. Since no significant differences in nitrogen content of these tissues occurred under these experimental conditions, the enzyme values are comparable to one another and are expressed in terms of μmoles of NADPH produced/min/100 mg of tissue wet weight. The enzymes were measured by the following procedures: glucose-6-phosphate dehydrogenase (n-glucose-6-phosphate : NADP oxidoreductase, EC 1.1.1.49), Glock and McLean (7); NADP-malic enzyme (L-malate : NADP oxidoreductase, decarboxylating, EC 1.1.1.40), Ochoa et al. (16); NADP-isocitric dehydrogenase (L-isocitrate: NADP oxidoreductase, decarboxylating, EC 1.1.1.42), Ochoa method (15); phosphoglucose isomerase (n-glucose-6-phosphate ketol-isomerase, EC 5.3.1.9), Shonk and Boxer (23); and phosphoglucomutase (α-d-glucose-1,6-diphosphate; α-d-glucose-1-phosphate phosphotransferase, EC 2.7.5.1), adaptation according to Shonk and Boxer (22).

Nucleic acid concentrations were measured by the Schneider method (21), with the use of the orcinol reaction for determination of ribose according to Ceriotti (2) and the diphenylamine reaction for deoxyribose determination according to Dische (4).

All the data are presented as the mean ± S.E. Since these data were obtained from several experiments, the values for the control and the estradiol valerate-treated animals were pooled and the mean represents 40 animals. The mean of all of the other treatment groups represents values from 10 animals. Significance was determined by the Student "t" test; a probability (P) value of 0.05 or less was considered to be significant. In determining significant changes, values obtained in tissues from animals receiving estrogen plus actinomycin D were compared with the value obtained in the group receiving estrogen treatment alone. All other treatment groups were statistically paired with the diluent-injected control group.

RESULTS AND DISCUSSION

Effect of estrogen, alone and in combination with actinomycin D, on tumor, carcass, and uterus weights.—Table 1

The enzyme nomenclature and numbering system are according to the Report of The Commission on Enzymes of The International Union of Biochemistry (Oxford: Pergamon Press, Ltd. 1961).
contains a summary of the effects of estradiol valerate and actinomycin D on the weights of the neoplasms and uteri removed for biochemical assays. The weight of the R3230AC carcinoma was significantly reduced by the highest dose of the antibiotic when given alone or in combination with estrogen treatment. Estradiol valerate, at the dose regimen used in these studies, had no effect on the growth of the neoplasm. While one cannot rule out the possibility that the effect of the antibiotic on tumor growth was due in part to the effect of the antibiotic on nutritional and/or hormonal alterations of the host (stress), the changes in carcass weight of the animals receiving both hormone and antibiotic were identical to the changes in carcass weight of animals receiving estrogen alone. Nevertheless, tumor weight was reduced by the antibiotic at the highest dose employed in these studies. A typical uterotrophic response to estrogen was obtained; this uterine response was inhibited by actinomycin D at the 2 highest doses of the antibiotic.

**Effect of actinomycin D on enzyme activities in R3230AC tumor.**—The effect of different doses of actinomycin D, alone or with a single injection of estradiol valerate, on the 5 enzymes studied in the R3230AC carcinoma is recorded in Table 2. In comparison with the enzyme activities of tumors from the diluent-injected control group, significant reductions in the activities of glucose-6-phosphate dehydrogenase, NADP-malic enzyme, NADP-isocitric dehydrogenase, and phosphoglucomutase were obtained in neoplasms of animals receiving the high dose of actinomycin D. There was a suggestion that these decreases were dose-related. Phosphoglucomutase activity was not altered by actinomycin D treatment.

The responses of the enzymes in the tumor to estrogen treatment were similar to those reported previously (10, 13), namely, an increase in glucose-6-phosphate dehydrogenase and NADP-malic enzyme and a decrease in NADP-isocitric dehydrogenase activities. The effect of hormonal treatment was extended in these studies to include 2 additional enzymes associated with glucose-6-phosphate metabolism; phosphoglucomutase activity was elevated in response to estrogen treatment, whereas phosphoglucomutase activity was not altered. Thus, the lactational response to estrogen was accompanied by marked stimulation of 2 enzymes that are involved in the production of NADPH. This response may reflect an increased demand for NADPH, a cofactor that has been shown to be involved in lipogenesis (17, 27). Data have been obtained in our laboratory that demonstrate an increased rate of synthesis and accumulation of free fatty acids and triglycerides; labeled acetate incorporation techniques were used in neoplasms of animals treated in vivo with estrogen. Although studies have not yet been performed to determine if there is a concomitant increase in protein synthesis, for which NADPH has also been implicated as a cofactor (26), the similarities of morphologic changes in the tumor to those seen in the lactating mammary gland are suggestive of the active synthesis of milk proteins.

**Effect of actinomycin D on estrogen-induced enzyme changes in the R3230AC tumor**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total dose of actinomycin D (mg/kg)</th>
<th>Glucose-6-phosphate dehydrogenase&lt;sup&gt;a&lt;/sup&gt;</th>
<th>NADP-malic enzyme&lt;sup&gt;a&lt;/sup&gt;</th>
<th>NADP-isocitric dehydrogenase&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Phosphoglucomutase&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Phosphoglucomutase&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluent</td>
<td></td>
<td>0.576 ± 0.021&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.426 ± 0.020</td>
<td>0.462 ± 0.009</td>
<td>0.150 ± 0.089</td>
<td>0.058 ± 0.003</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>1.050</td>
<td>0.473 ± 0.048&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.375 ± 0.048</td>
<td>0.431 ± 0.042</td>
<td>2.226 ± 0.314</td>
<td>0.049 ± 0.008</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>1.500</td>
<td>0.367 ± 0.033&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.250 ± 0.033&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.404 ± 0.033</td>
<td>2.280 ± 0.156</td>
<td>0.038 ± 0.002</td>
</tr>
<tr>
<td>Estrogen treatment (E)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.042</td>
<td>1.103 ± 0.080&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.615 ± 0.045</td>
<td>0.379 ± 0.011</td>
<td>2.054 ± 0.137</td>
<td>0.078 ± 0.009</td>
</tr>
<tr>
<td>E + Actinomycin D</td>
<td>0.210</td>
<td>1.128 ± 0.065&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.642 ± 0.038</td>
<td>0.368 ± 0.008</td>
<td>2.137 ± 0.136</td>
<td>0.077 ± 0.006</td>
</tr>
<tr>
<td>E + Actinomycin D</td>
<td>1.050</td>
<td>1.098 ± 0.154&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.599 ± 0.057</td>
<td>0.437 ± 0.010</td>
<td>1.587 ± 0.177</td>
<td>0.086 ± 0.004</td>
</tr>
<tr>
<td>E + Actinomycin D</td>
<td>1.500</td>
<td>0.428 ± 0.041&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.248 ± 0.029&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.383 ± 0.013</td>
<td>1.986 ± 0.108</td>
<td>0.038 ± 0.002</td>
</tr>
</tbody>
</table>

<sup>a</sup> Enzyme activity expressed as μmoles of NADPH produced/min/100 mg of tissue.
<sup>b</sup> Mean ± S.E.
<sup>c</sup> Differ significantly from diluent-injected control animals, P < 0.01.
<sup>d</sup> Animals received 1 injection s.c. of estradiol valerate, 10 mg/kg.
<sup>e</sup> Differ significantly from diluent-injected control animals, P < 0.05.
<sup>f</sup> Differ significantly from estrogen-treated animals, P < 0.01.

* R. Hilf and C. Gibbs, unpublished observations.
the anticipated responses of the enzymes to estrogen treatment.5

Effect of actinomycin D on enzymes in the uterus.—To clarify the nature of the enzyme response to estrogen in the tumor, the uterus was studied as an example of an estrogen target organ. The effects of the antibiotic, alone and given with estrogen, on the enzyme activities of the uterus from the same tumor-bearing animals are presented in Table 3. Actinomycin D caused a decrease in the activities of glucose-6-phosphat dehydrogenase, NADP-malic enzyme and NADP-isocitric dehydrogenase at both levels studied and also resulted in a decrease in phosphoglucone isomerase and phosphoglucomutase activities at the highest dose (1.5 mg/kg, total dose).

Estrogen treatment resulted in a significant increase in glucose-6-phosphat dehydrogenase and NADP-malic enzyme activities; these increases were accompanied by a decrease in the activity of NADP-isocitric dehydrogenase. Neither phosphoglucone isomerase nor phosphoglucomutase were altered by treatment with estradiol valerate. Actinomycin D prevented the estrogen-induced increase in glucose-6-phosphat dehydrogenase and NADP-malic enzyme; a significant inhibition of the response of NADP-malic enzyme occurred with as little as 0.042 mg/kg as the total dose of the antibiotic. Thus, the uterus appeared to be more sensitive to the inhibition by actinomycin D of the hormone-induced enzyme changes. On the other hand, the estrogen-induced decrease in NADP-isocitric dehydrogenase activity was enhanced by concomitant treatment with this antibiotic.

Effect of estrogen and actinomycin D in mammary glands.—Table 4 illustrates the data obtained by analyses of mammary tissue from the same animals. Actinomycin D caused a decrease, not necessarily dose-related, in the activities of all of the enzymes with the exception of phosphoglucomutase. A single injection of estradiol valerate did not cause an increase in the activity of any of the enzymes studied and actually caused a decrease in both NADP-malic enzyme and NADP-isocitric dehydrogenase activities. An unexpected result was obtained when actinomycin D was given along with estradiol valerate, since the combination (particularly at the highest dose of the antibiotic) resulted in an increase in the activities of glucose-6-phosphate dehydrogenase as well as of NADP-isocitric dehydrogenase (compared with the diluent-treated controls). Actinomycin D reversed the estrogen-induced decrease in NADP-malic enzyme, its activity returning to control levels. The combined hormone-antibiotic treatment had no different effect than actinomycin D alone on the activities of phosphoglucone isomerase and phosphoglucomutase. The increase in enzyme activity obtained by the combination treatment may be related to recent observations of stimulatory effects of this antibiotic reported with several different experimental systems (6, 14). Rosen et al. (20) reported induction of several adaptive enzymes by this antibiotic, using an experimental design and dose of actinomycin D that permitted survival of animals for several days. Schwartz et al. (22) have found complete recovery of RNA synthesis in rat liver 16 hr after a dose of actinomycin D that produced a 90% inhibition within 30 min after exposure to the antibiotic. Certainly it seems warranted to investigate further the effects of chronic administration of actinomycin D and to determine whether these effects are direct or indirect through nutritional and/or hormonal alterations. Studies in our laboratory have shown that a high carbohydrate diet can increase the activity of certain of the enzymes studied here.4

Effects of actinomycin D on nucleic acid concentrations in R3230AC tumors and uteri.—Since the antibiotic used in these studies interferes with protein synthesis by inhibiting certain metabolic sequences of nucleic acids, the RNA and DNA levels were determined in the neoplasm. Table 5 contains data indicating that actinomycin D caused a decrease in RNA concentration, which was dose-related.
The high dose of actinomycin D also produced a decrease in DNA/mg of tumor. Estrogen treatment resulted in a decrease in DNA concentration, as reported earlier (10, 13), with little or no effect on RNA. The antibiotic, given with estradiol valerate, caused a decrease in RNA (at certain dose levels) and reversed the estrogen-induced decrease in DNA/mg of neoplastic tissue as the dose of antibiotic was increased. The 2 lower doses of actinomycin D appeared to augment the estrogen-induced reduction in DNA concentration. The resulting RNA/DNA ratios reflected the effect of actinomycin D in reducing RNA, the ratios being significantly lower at the higher doses of the antibiotic.

The nucleic acid contents of the uteri from these animals also presented in Table 5. As with the tumor, actinomycin D caused a dose-related decrease in RNA levels of the uterus. The increase in RNA resulting from estrogen treatment was effectively inhibited by the antibiotic. Although actinomycin D treatment alone had no effect on uterine DNA, it did prevent the estrogen-induced decrease in DNA content. It is interesting that the lower doses of the antibiotic potentiated the estrogen-induced decrease (similar to the results in the tumor), whereas the higher doses inhibited the estrogen effect completely. The alterations in the RNA/DNA ratios were a result of the effect of actinomycin D in reducing RNA.

Thus, the nucleic acid responses to actinomycin D are rather clear. Since the antibiotic interferes with DNA-directed RNA synthesis, one would anticipate a decrease in RNA concentration, and a dose-related decrease in RNA in both neoplasm and uterus was obtained. It is also of interest that the antibiotic reversed the estrogen-induced decrease in DNA concentration in both tissues. In view of these results, calculation of enzyme activities in terms of DNA concentration produced the same over-all pattern as seen in Tables 2 and 3. Actinomycin D caused a 13% reduction in DNA levels only in the tumor. Since antibiotic treatment reduced the enzyme activities in the neo-

### TABLE 4

**Effect of Actinomycin D upon Estrogen-induced Enzyme Changes in the Mammary Gland**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total dose of actinomycin D (mg/kg)</th>
<th>Glucose-6-phosphate dehydrogenase</th>
<th>NADP-malic enzyme</th>
<th>NADP-isocitric dehydrogenase</th>
<th>Phosphoglucomutase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluent</td>
<td></td>
<td>0.037 ± 0.003 b</td>
<td>0.050 ± 0.004</td>
<td>0.085 ± 0.004</td>
<td>0.455 ± 0.011</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>1.050</td>
<td>0.027 ± 0.003 c</td>
<td>0.026 ± 0.003</td>
<td>0.066 ± 0.006</td>
<td>0.401 ± 0.028</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>1.500</td>
<td>0.035 ± 0.007</td>
<td>0.036 ± 0.011</td>
<td>0.070 ± 0.005</td>
<td>0.380 ± 0.017</td>
</tr>
<tr>
<td>Estrogen treatment (E)</td>
<td></td>
<td>0.038 ± 0.004</td>
<td>0.033 ± 0.004</td>
<td>0.067 ± 0.005</td>
<td>0.503 ± 0.039</td>
</tr>
<tr>
<td>E + Actinomycin D</td>
<td>0.042</td>
<td>0.035 ± 0.005</td>
<td>0.036 ± 0.004</td>
<td>0.059 ± 0.004</td>
<td>0.534 ± 0.032</td>
</tr>
<tr>
<td>E + Actinomycin D</td>
<td>0.210</td>
<td>0.038 ± 0.005</td>
<td>0.033 ± 0.003</td>
<td>0.066 ± 0.004</td>
<td>0.521 ± 0.031</td>
</tr>
<tr>
<td>E + Actinomycin D</td>
<td>1.050</td>
<td>0.045 ± 0.005</td>
<td>0.064 ± 0.011</td>
<td>0.066 ± 0.010</td>
<td>0.545 ± 0.031</td>
</tr>
<tr>
<td>E + Actinomycin D</td>
<td>1.500</td>
<td>0.066 ± 0.005</td>
<td>0.047 ± 0.002</td>
<td>0.121 ± 0.012</td>
<td>0.381 ± 0.017</td>
</tr>
</tbody>
</table>

* Enzyme activity expressed as µmoles of NADPH produced/min/100 mg of tissue.

* Mean ± S.E.

* differs significantly from the diluent-injected control animals, P < 0.01.

* Animals received 1 injection s.c. of estradiol valerate, 10 mg/kg.

* differs significantly from the estrogen-treated animals, P < 0.01.

* Differs significantly from the estrogen-treated animals, P < 0.05.

### TABLE 5

**Effect of Actinomycin D on Estrogen-induced Alterations in Nucleic Acid Contents in R3230AC Tumors and Uteri**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total dose of actinomycin D (mg/kg)</th>
<th>RNA (µg/mg)</th>
<th>DNA (µg/mg)</th>
<th>RNA/DNA ratio</th>
<th>RNA (µg/mg)</th>
<th>DNA (µg/mg)</th>
<th>RNA/DNA ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluent</td>
<td></td>
<td>7.71 ± 0.14</td>
<td>5.40 ± 0.08</td>
<td>1.43 ± 0.03</td>
<td>4.09 ± 0.08</td>
<td>4.89 ± 0.12</td>
<td>0.86 ± 0.03</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>1.050</td>
<td>6.31 ± 0.56</td>
<td>5.88 ± 0.50</td>
<td>1.29 ± 0.02</td>
<td>3.12 ± 0.13</td>
<td>5.24 ± 0.21</td>
<td>0.60 ± 0.03</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>1.500</td>
<td>5.24 ± 0.01</td>
<td>4.70 ± 0.20</td>
<td>1.21 ± 0.05</td>
<td>1.87 ± 0.47</td>
<td>4.94 ± 0.75</td>
<td>0.37 ± 0.05</td>
</tr>
<tr>
<td>Estrogen treatment (E)</td>
<td></td>
<td>7.39 ± 0.15</td>
<td>4.94 ± 0.01</td>
<td>1.50 ± 0.02</td>
<td>4.74 ± 0.08</td>
<td>5.59 ± 0.10</td>
<td>0.85 ± 0.03</td>
</tr>
<tr>
<td>E + Actinomycin D</td>
<td>0.042</td>
<td>7.03 ± 0.19</td>
<td>4.46 ± 0.08</td>
<td>1.57 ± 0.04</td>
<td>4.10 ± 0.14</td>
<td>3.27 ± 0.07</td>
<td>1.25 ± 0.03</td>
</tr>
<tr>
<td>E + Actinomycin D</td>
<td>0.210</td>
<td>6.72 ± 0.16</td>
<td>4.62 ± 0.10</td>
<td>1.45 ± 0.03</td>
<td>4.30 ± 0.30</td>
<td>3.33 ± 0.04</td>
<td>1.29 ± 0.02</td>
</tr>
<tr>
<td>E + Actinomycin D</td>
<td>1.050</td>
<td>7.91 ± 0.23</td>
<td>5.08 ± 0.13</td>
<td>1.56 ± 0.04</td>
<td>3.08 ± 0.07</td>
<td>4.36 ± 0.35</td>
<td>0.73 ± 0.07</td>
</tr>
<tr>
<td>E + Actinomycin D</td>
<td>1.500</td>
<td>4.82 ± 0.59</td>
<td>4.67 ± 0.23</td>
<td>1.03 ± 0.07</td>
<td>2.45 ± 0.22</td>
<td>4.92 ± 0.19</td>
<td>0.50 ± 0.05</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

* differs significantly from the diluent-injected control animals, P < 0.05.

* Differs significantly from the diluent-injected control animals, P < 0.01.

* Animals received 1 injection s.c. of estradiol valerate, 10 mg/kg.

* differs significantly from the estrogen-treated animals, P < 0.01.

* Differs significantly from the estrogen-treated animals, P < 0.05.
plasm by 36, 41, and 36% for glucose-6-phosphate dehydrogenase, NADP-malic enzyme, and phosphoglucomutase, respectively, the inhibition of enzyme activity by actinomycin D was not due only to reduced tumor weight. Obviously, the elevated enzyme activities resulting from estrogen treatment are even more striking when expressed in terms of RNA, since estrogen treatment produced a marked decrease in RNA concentration.

Effects of puromycin on estrogen-induced enzyme changes in tumors and uteri.—Administration of puromycin at the same dose regimen (up to a total of 1.225 mg/kg) was ineffective in preventing the enzyme changes produced by estrogen. When puromycin was given hourly (7 daily injections i.p. for 2 consecutive days at a total dose of 140 mg/kg) a slight inhibition was obtained of the estrogen-induced increase in NADP-malic enzyme activity in the neoplasm and in both glucose-6-phosphate dehydrogenase, NADP-malic enzyme, and phosphoglucomutase, respectively, the inhibition of enzyme activity being 36, 41, and 36% for glucose-6-phosphate dehydrogenase, NADP-malic enzyme, and phosphoglucomutase, respectively, the inhibition of enzyme activity by puromycin at the same dose regimen (up to a total of 1.225 mg/kg) was ineffective in preventing the enzyme changes produced by estrogen. When puromycin was given hourly (7 daily injections i.p. for 2 consecutive days at a total dose of 140 mg/kg) a slight inhibition was obtained of the estrogen-induced increase in NADP-malic enzyme activity in the neoplasm and in both glucose-6-phosphate dehydrogenase, NADP-malic enzyme, and phosphoglucomutase, respectively, the inhibition of enzyme activity

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