Biochemical Events Associated with Regression of 7,12-Dimethylbenz(a)anthracene-induced Mammary Carcinomas after Ovariectomy

Russell Hilf, Harold Goldenberg, Inge Michel, Margot Gruenstein, David R. Meranze, and Michael B. Shimkin

SUMMARY

Mammary adenocarcinomas of the rat induced by gastric intubation of 7,12-dimethylbenz(a)anthracene will regress after ovariectomy. The activities of enzymes and the levels of nucleic acids and lipids were measured in tumors of animals sacrificed 1, 5, and 14 days after ovariectomy. Palpation of the tumors showed that no significant decreases in tumor size or volume occurred until 14 days after ovariectomy, at which time tumor size and volume had decreased 27 and 62%, respectively. Significant changes in biochemical parameters were first observed as follows: Day 1, glutamate dehydrogenase, hexokinase, aspartate aminotransferase, and cholesterol; Day 5, pyruvate kinase, glucose-6-phosphate dehydrogenase, NADP-malate dehydrogenase, phosphoglucomutase, RNA, and RNA/DNA ratios; and Day 14, NADP-isocitrate dehydrogenase and glucosephosphate isomerase. The activities of α-glycerophosphate dehydrogenase and glucokinase, as well as the levels of DNA, free fatty acids, and triglycerides, were not significantly altered at any time. Similar results were obtained in animals ovariectomized when the first tumor that appeared approximated 2 x 2 cm and in others ovariectomized 35 days after the first tumor had reached 0.5 x 0.5 cm, and the first three tumors were analyzed. These data indicate that certain specific biochemical changes precede measurable changes in tumor size and are implicated as essential components for continued neoplastic growth.

INTRODUCTION

Mammary carcinomas induced by the gastric instillation of DMBA have been shown to regress after removal of the ovaries of pituitary of the host or in response to the administration of exogenous hormones (5, 11, 13, 23, 30, 33, 36, 37). Few studies exist relevant to biochemical changes in the DMBA-induced neoplasms as influenced by alterations in the hormonal milieu of the tumor-bearing host.

In an earlier study, Hilf et al. (20) reported that, 3 weeks after ovariectomy, the remaining DMBA-induced carcinomas showed decreases in the level of RNA and in RNA/DNA ratios, as well as significantly reduced activities of glucose-6-phosphate dehydrogenase, NADP-isocitrate dehydrogenase, glucosephosphate isomerase, and phosphoglucomutase. These responses resembled those in neoplasms of androgen-treated animals. It seemed important to extend these studies to measure any biochemical changes in the neoplasms that occurred shortly after removal of the ovaries of the tumor-bearing host. The data reported here indicate that numerous alterations occur in several enzymes prior to significant changes in tumor size.

MATERIALS AND METHODS

Induction of Tumors with DMBA. Female rats weighing between 110 and 130 g were purchased from Sprague-Dawley, Madison, Wis. The animals were housed in groups of 2 to 4 animals in plastic shoebox cages and fed Rockland complete rat/mouse diet and water ad libitum. DMBA was dissolved in sesame oil. A solution containing 5 mg DMBA/ml of sesame oil was given through a No. 8 French stomach catheter, starting when the rats were 50 days old. The treatment was repeated weekly for 5 weeks (total dose of DMBA was 25 mg).

Treatment of Animals. All animals were palpated for tumors at weekly intervals. Ovariectomy was accomplished under ether anesthesia by incisions through the lumbar region of the back. Removal of the ovaries from tumor-bearing hosts was performed at either of two different times after tumor induction with DMBA. In Experiment 1, ovariectomy was done after the 1st tumor to appear had reached approximately 2.0 x 2.0 cm, and animals were sacrificed 1, 5, or 14 days after ovariectomy. At sacrifice, only the 1st tumor to appear was excised for analysis. In Experiment 2, the rats were ovariectomized 35 days after the 1st tumor had reached 0.5 x 0.5 cm. Only animals with 3 or more tumors, all at least 0.5 x 0.5 cm at the time of ovariectomy, were used; and the animals were sacrificed 1, 5, and 14 days after ovariectomy. Palpation of the tumors occurred until 14 days after ovariectomy, at which time tumor size and volume had decreased 27 and 62%, respectively. Significant changes in biochemical parameters were first observed as follows: Day 1, glutamate dehydrogenase, hexokinase, aspartate aminotransferase, and cholesterol; Day 5, pyruvate kinase, glucose-6-phosphate dehydrogenase, NADP-malate dehydrogenase, phosphoglucomutase, RNA, and RNA/DNA ratios; and Day 14, NADP-isocitrate dehydrogenase and glucosephosphate isomerase. The activities of α-glycerophosphate dehydrogenase and glucokinase, as well as the levels of DNA, free fatty acids, and triglycerides, were not significantly altered at any time. Similar results were obtained in animals ovariectomized when the first tumor that appeared approximated 2 x 2 cm and in others ovariectomized 35 days after the first tumor had reached 0.5 x 0.5 cm, and the first three tumors were analyzed. These data indicate that certain specific biochemical changes precede measurable changes in tumor size and are implicated as essential components for continued neoplastic growth.

Received July 14, 1970; accepted September 18, 1970.
sacrificed 1, 5, or 14 days after ovariectomy. The 1st, 2nd, and 3rd tumors to appear were excised for analysis. Control animals (intact) were sacrificed at various times 13 to 17 weeks after initiation of carcinogen feeding. All tissues were quick-frozen in a mixture of Dry Ice-acetone and stored at −20° until assayed.

Biochemical Assays. Tissues were thawed and separated into portions approximating 200 to 250 mg, wet weight. Each portion was homogenized in cold 0.05 M Tris buffer, pH 7.4, with the volume of diluent added to produce either a 10 or 5% homogenate (w/v). Aliquots of the homogenate were taken for determinations of nucleic acids and lipids; the remainder of the homogenate was used for enzyme assays.

Enzyme assays were performed on the supernatant of the homogenate after centrifugation at 20,000 X g for 20 min. Each of the enzyme assays was performed under identical conditions by measurement of the absorbance changes at 340 nm due to the production of NADPH or the oxidation of NADH. Conditions of each assay were established to ensure optimum (zero order) kinetics for substrate and cofactor requirements on as small a sample as feasible. Under these conditions, the enzyme values are comparable and are expressed as μmoles of NADPH produced per min per 100 mg of tissue weight or per mg of DNA or as μmoles of NADH oxidized per min per 100 mg of tissue weight or per mg of DNA. The enzymes were measured, with minor modifications, by the following procedures: glucose-6-phosphate dehydrogenase (D-glucose 6-phosphate:NADP oxidoreductase, EC 1.1.1.49), a method of Glock and McLean (9), isocitrate dehydrogenase, decarboxylating (L-isocitrate: NADP oxidoreductase, decarboxylating EC 1.1.1.42) method of Ochoa (25); malate dehydrogenase, decarboxylating (L-malate:NADP oxidoreductase, decarboxylating, EC 1.1.1.40), method of Ochoa et al. (26); glucose-6-phosphate isomerase (D-glucose 6-phosphate ketol isomerase, EC 5.3.1.9), method of Shonk and Boxer (31); phosphoglucomutase (α-D-glucose 1,6-diphosphate; α-D-glucose-1-phosphate phosphotransferase, EC 2.7.5.1), enzyme activation according to the method of Harshman et al. (12) and assay as outlined by Shonk and Boxer (31); α-glycerophosphate dehydrogenase (L-glyceraldehyde 3-phosphate:NAD oxidoreductase, EC 1.1.1.8), method of Beisenherz et al. (3), modified by using dihydroxyacetone phosphate as substrate and measuring the oxidation of NADH; glutamate dehydrogenase (L-glutamate-NAD oxidoreductase, deaminating, EC 1.4.1.2) with the use of the reverse reaction and oxidation of NADH described by Hogeboom and Schneider (22); aspartate aminotransferase (L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1), modified from the assay procedure of Karmen (24); pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.40), method of Susor and Rutter (35); hexokinase (ATP:D-hexose-6-phosphotransferase, EC 2.7.1.1), method of Sharma et al. (29); and glucokinase (ATP:D-glucose-6-phosphotransferase, EC 2.7.1.2), method of Sharma et al. (29).

Nucleic acid concentrations were measured by a modifica-


Ovariectomy and DMBA-induced Mammary Tumors

RESULTS

Effect of Ovariectomy on Tumor Growth. Two separate experiments were performed to ascertain the effect of ablation of the ovaries on subsequent growth of the DMBA-induced mammary carcinomas, and the results are summarized in Table 1. In the 1st experiment, ovariectomy was performed on the day that the 1st tumor to appear reached 2.0 x 2.0 cm, as measured with calipers. No significant alteration in tumor size (mean diameter) or tumor volume (4/3πr³) was observed within 14 days after removal of the ovaries. In the 2nd experiment, only animals with 3 or more tumors were used, and ovariectomy was delayed until 35 days after the 1st tumor had reached 0.5 x 0.5 cm. At sacrifice, the 1st, 2nd, and 3rd tumors to appear were measured. A significant reduction of the tumor diameter and volume was found at 14 days after ovariectomy. Thus, the better-established tumors showed a greater response to ovariectomy.

Effect of Early Ovariectomy on the Biochemistry of the Mammary Carcinomas. Table 2 summarizes the data obtained from analysis of nucleic acids, lipids, and certain enzymes in neoplasms of tumor-bearing animals sacrificed 1, 5, or 14 days after ovariectomy. Compared with control or intact tumor-bearing rats, some biochemical values were significantly altered soon after removal of the ovaries. Tumors obtained 1 day after ovariectomy demonstrated significant decreases in glucose-6-phosphate dehydrogenase activity (48%) and cholesterol content (34%), an elevation in aspartate aminotransferase activity (72%), and a striking increase in the activity of hexokinase (340%). By the 5th day after ovariectomy, a decrease in the RNA/DNA ratio (32%) was found in these DMBA-induced neoplasms. Also occurring for the 1st time at 5 days were significant decreases in the activity of phosphoglucomutase (49%), glutamate dehydrogenase (61%), and pyruvate kinase (33%). The activities of glucose-6-phosphate dehydrogenase and hexokinase in the tumors were still significantly altered from those in tumors of intact animals, whereas the early increase in aspartate aminotransferase activity (Day 1) was no longer apparent. In the last column of Table 2 are the data obtained for tumors from animals at 14 days after ovariectomy. At this time, there was a suggestion that tumor diameter and volume had decreased. The activity of NADP-malate dehydrogenase in these neoplasms was significantly reduced (45%), as was the level of RNA (40%). The RNA/DNA ratio and the activities of glucose-6-phosphate dehydrogenase, phosphoglucomutase, pyruvate kinase, and hexokinase, which were significantly altered in the DMBA-induced mammary tumors examined soon after ovariectomy, were still altered in these neoplasms at 14 days by measurement of the Schneider method, with the use of the orcinol reaction for the determination of ribose (4) and the diphenylamine reaction for deoxyribose determination (6).

Aliquots of the original tissue homogenate were extracted for lipids with diethyl ether; separation of cholesterol, cholesterol esters, free fatty acids, and triglycerides was accomplished by thin-layer chromatography. The isolated lipids were localized, extracted from the gel, and determined quantitatively (20).
### Table 1

**Effect of ovariectomy on the growth of DMBA-induced mammary tumors**

In Experiment 1, animals were ovariectomized when the 1st tumor that appeared reached approximately 2.0 x 2.0 cm. Animals were sacrificed 1, 5, or 14 days after ovariectomy and only the 1st tumor was taken for biochemical analysis. In Experiment 2, animals were ovariectomized 35 days after the 1st tumor that appeared had reached approximately 0.5 x 0.5 cm. Animals were sacrificed 1, 5, or 14 days after ovariectomy, and the 1st, 2nd and 3rd tumors that appeared were taken for biochemical analysis.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Ovariectomy</th>
<th>Days after ovariectomy</th>
<th>Day 1</th>
<th>Day 5</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Palpation before operation</td>
<td>Mean diameter (cm)</td>
<td>Volume (cu cm)</td>
<td>Mean diameter (cm)</td>
<td>Volume (cu cm)</td>
</tr>
<tr>
<td>E1: early ovariectomy</td>
<td>Palpation</td>
<td>2.12 ± 0.10 (5)</td>
<td>5.11 ± 0.61</td>
<td>1.93 ± 0.21 (6)</td>
<td>4.40 ± 1.20</td>
</tr>
<tr>
<td></td>
<td>Measurement at sacrifice</td>
<td>1.86 ± 0.14</td>
<td>3.60 ± 0.71</td>
<td>1.53 ± 0.16</td>
<td>2.19 ± 0.73</td>
</tr>
<tr>
<td>E2: delayed ovariectomy</td>
<td>Palpation</td>
<td>1.59 ± 0.23 (9)</td>
<td>2.97 ± 0.90</td>
<td>1.74 ± 0.24 (12)</td>
<td>4.53 ± 1.76</td>
</tr>
<tr>
<td></td>
<td>Measurement at sacrifice</td>
<td>1.81 ± 0.25</td>
<td>4.51 ± 1.57</td>
<td>1.58 ± 0.21</td>
<td>3.18 ± 0.99</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 5</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA (µg/mg)</td>
<td>6.7 ± 0.4a</td>
<td>5.9 ± 0.8</td>
</tr>
<tr>
<td>DNA (µg/mg)</td>
<td>9.9 ± 0.6</td>
<td>9.1 ± 0.9</td>
</tr>
<tr>
<td>RNA/DNA ratio</td>
<td>0.71 ± 0.04</td>
<td>0.67 ± 0.11</td>
</tr>
<tr>
<td>G6PDCc</td>
<td>0.520 ± 0.038</td>
<td>0.279 ± 0.062b</td>
</tr>
<tr>
<td>ICD</td>
<td>0.737 ± 0.048</td>
<td>0.800 ± 0.047</td>
</tr>
<tr>
<td>ME</td>
<td>0.077 ± 0.008</td>
<td>0.076 ± 0.010</td>
</tr>
<tr>
<td>GPI</td>
<td>4.95 ± 0.27</td>
<td>6.99 ± 1.03</td>
</tr>
<tr>
<td>PGM</td>
<td>0.086 ± 0.006</td>
<td>0.085 ± 0.024</td>
</tr>
<tr>
<td>aGDP</td>
<td>0.082 ± 0.023</td>
<td>0.050 ± 0.010</td>
</tr>
<tr>
<td>GDH</td>
<td>0.028 ± 0.003</td>
<td>0.031 ± 0.004</td>
</tr>
<tr>
<td>AAT</td>
<td>1.09 ± 0.081</td>
<td>1.88 ± 0.296b</td>
</tr>
<tr>
<td>PYK</td>
<td>8.34 ± 0.771</td>
<td>9.96 ± 1.73</td>
</tr>
<tr>
<td>HK</td>
<td>0.037 ± 0.004</td>
<td>0.163 ± 0.010b</td>
</tr>
<tr>
<td>GK</td>
<td>0.002 ± 0.001</td>
<td>0.003 ± 0.002</td>
</tr>
<tr>
<td>CHOL</td>
<td>2.59 ± 0.08</td>
<td>1.71 ± 0.22b</td>
</tr>
<tr>
<td>FFA</td>
<td>2.85 ± 0.36</td>
<td>2.51 ± 0.29</td>
</tr>
<tr>
<td>TG</td>
<td>13.2 ± 6.0</td>
<td>2.0 ± 1.6</td>
</tr>
</tbody>
</table>

**a** Mean ± S.E. Numbers in parentheses, no. of tumors analyzed.

**b** Significantly different (p < 0.05) compared with intact (before ovariectomy) animals.

### Table 2

**Effect of early ovariectomy on biochemical characteristics of mammary tumors induced by DMBA**

Ovariectomy was performed when the 1st tumor to appear had reached 2.0 x 2.0 cm. Animals were sacrificed 1, 5, or 14 days after ovariectomy and only the 1st tumor to appear was taken for subsequent analysis.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control (intact)</th>
<th>Day 1</th>
<th>Day 5</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6PDCc</td>
<td>0.520 ± 0.038</td>
<td>0.279 ± 0.062b</td>
<td>0.203 ± 0.033b</td>
<td>0.216 ± 0.037b</td>
</tr>
<tr>
<td>ICD</td>
<td>0.737 ± 0.048</td>
<td>0.800 ± 0.047</td>
<td>0.561 ± 0.056</td>
<td>0.678 ± 0.143</td>
</tr>
<tr>
<td>ME</td>
<td>0.077 ± 0.008</td>
<td>0.076 ± 0.010</td>
<td>0.065 ± 0.010</td>
<td>0.043 ± 0.008b</td>
</tr>
<tr>
<td>GPI</td>
<td>4.95 ± 0.27</td>
<td>6.99 ± 1.03</td>
<td>5.17 ± 0.56</td>
<td>5.50 ± 0.89</td>
</tr>
<tr>
<td>PGM</td>
<td>0.086 ± 0.006</td>
<td>0.085 ± 0.024</td>
<td>0.044 ± 0.009b</td>
<td>0.047 ± 0.010b</td>
</tr>
<tr>
<td>aGDP</td>
<td>0.082 ± 0.023</td>
<td>0.050 ± 0.010</td>
<td>0.068 ± 0.014</td>
<td>0.042 ± 0.011</td>
</tr>
<tr>
<td>GDH</td>
<td>0.028 ± 0.003</td>
<td>0.031 ± 0.004</td>
<td>0.011 ± 0.002b</td>
<td>0.024 ± 0.005</td>
</tr>
<tr>
<td>AAT</td>
<td>1.09 ± 0.081</td>
<td>1.88 ± 0.296b</td>
<td>1.38 ± 0.107</td>
<td>1.36 ± 0.232</td>
</tr>
<tr>
<td>PYK</td>
<td>8.34 ± 0.771</td>
<td>9.96 ± 1.73</td>
<td>5.59 ± 0.72b</td>
<td>3.34 ± 0.692b</td>
</tr>
<tr>
<td>HK</td>
<td>0.037 ± 0.004</td>
<td>0.163 ± 0.010b</td>
<td>0.103 ± 0.010b</td>
<td>0.116 ± 0.26b</td>
</tr>
<tr>
<td>GK</td>
<td>0.002 ± 0.001</td>
<td>0.003 ± 0.002</td>
<td>0.002 ± 0.001</td>
<td>0.000</td>
</tr>
<tr>
<td>CHOL</td>
<td>2.59 ± 0.08</td>
<td>1.71 ± 0.22b</td>
<td>2.96 ± 0.68</td>
<td>3.05 ± 0.68</td>
</tr>
<tr>
<td>FFA</td>
<td>2.85 ± 0.36</td>
<td>2.51 ± 0.29</td>
<td>2.75 ± 0.25</td>
<td>2.11 ± 0.24</td>
</tr>
<tr>
<td>TG</td>
<td>13.2 ± 6.0</td>
<td>2.0 ± 1.6</td>
<td>10.4 ± 4.0</td>
<td>3.0 ± 1.4</td>
</tr>
</tbody>
</table>

**a** Mean ± S.E.

**b** Significantly different (p < 0.05) from intact (control) animals.

**c** The abbreviations used are: G6PDC, glucose-6-phosphate dehydrogenase; ICD, NADP-isocitrate dehydrogenase; ME, NADP-malate dehydrogenase; GPI, glucosephosphate isomerase; PGM, phosphoglucomutase; aGDP, α-glycerolphosphate dehydrogenase; GDH, glutamate dehydrogenase; AAT, aspartate aminotransferase; PYK, pyruvate kinase; HK, hexokinase; GK, glucokinase; CHOL, cholesterol; FFA, free fatty acids; and TG, triglycerides.
Effect of Delayed Ovariectomy on the Biochemistry of Mammary Tumors. In this experiment, ovariectomy was delayed until 35 days after the 1st neoplasm that appeared had reached 0.5 x 0.5 cm. In effect, this delay allowed the neoplasm to become established, and a significant decrease in tumor diameter and volume was obtained at 14 days after ovariectomy. Neoplasms examined 1 day after ovariectomy demonstrated increased activity of aspartate aminotransferase (78%) and hexokinase (286%) and a decrease in the activity of glutamate dehydrogenase (40%) and the level of cholesterol (37%). By 5 days after ovariectomy, before significant changes in tumor size had occurred, several biochemical parameters were significantly altered in the neoplasms, compared with those in tumors of intact animals. Both the amount of RNA and the RNA/DNA ratio were decreased, at 34% and 44%, respectively. In addition, significant decreases in the activities of the following enzymes were first noted at this time: glucose-6-phosphate dehydrogenase, 64%; NADP-malate dehydrogenase, 48%; phosphoglucomutase, 51%; and pyruvate kinase, 66%. Glutamate dehydrogenase and hexokinase activity, which had shown changes as early as 1 day after ovariectomy, were still altered in the tumors at 5 days after ovariectomy, whereas aspartate aminotransferase activity was no longer significantly elevated in the neoplasms at this time. Carcinogen-induced carcinomas analyzed at 14 days after ovariectomy showed many significant changes from neoplasms of intact rats. RNA levels and RNA/DNA ratios were decreased, at 36 and 51%, respectively, as were the activities of glucose-6-phosphate dehydrogenase, 70%, NADP-isocitrate dehydrogenase (39%), phosphoglucomutase (51%), glutamate dehydrogenase (43%), and pyruvate kinase (64%). Hexokinase activity was still elevated (284%) in these neoplasms at 14 days after ovariectomy. Thus, the DMBA-induced neoplasms demonstrated numerous biochemical changes after ovariectomy of the tumor-bearing host.

DISCUSSION

An important feature of the DMBA-induced mammary carcinoma of the rat is that most of these neoplasms will regress after removal of the ovaries of the host. These tumors are hormone dependent and require the secretions of the ovary to maintain continued growth of the neoplasm. The purpose of the study reported here was to identify some biochemical parameters that would reflect alterations in the growth of these tumors. Further, by examination of a variety of biochemical and points at several different time intervals after ovariectomy of the tumor-bearing host, it was hoped that some significant biochemical changes would be demonstrated prior to measurable decreases in tumor size.

Only a few studies have examined biochemical changes in hormone-dependent, carcinogen-induced tumors after ablation of endocrine organs of the host. Rees and Huggins (27) reported that there was a 66% decrease in glucose-6-phosphate dehydrogenase and a 21% decrease in NADP-isocitrate dehydrogenase activities in MCA-induced tumors at 21 days after ovariectomy. Hershey et al. (14) found that glucose-6-phosphate dehydrogenase activity was decreased by 50% in regressing MCA-induced carcinomas examined 14 days after ovariectomy. In an earlier study, we demonstrated that the activities of glucose-6-phosphate dehydrogenase, NADP-isocitrate dehydrogenase, NADP-malate dehydrogenase, and phosphoglucomutase were significantly reduced in MCA-induced tumors at 2 to 3 weeks after ovariectomy (20).

In contrast, less data are available on the influence of ovariectomy on the biochemistry of DMBA-induced tumors. Stevens (34) found no significant alterations in the concentrations of DNA, RNA, and protein in neoplasms at various intervals up to 48 hr after ovariectomy. Smith et al. (32) reported that acidic nuclear protein content was higher in growing DMBA-induced neoplasms than in static carcinogen-induced tumors. Gorlich and Heise (10) found a decrease in aspartate transcarbamylase activity in DMBA-induced tumors that were regressing as a result of administration of testosterone propionate, whereas the activity of aspartate aminotransferase was unchanged in these neoplasms.

The results reported here are an extension of our earlier findings in which neoplasms were examined at 14 to 21 days after ovariectomy (20). In that earlier study, we reported that the activities of glucose-6-phosphate dehydrogenase, NADP-isocitrate dehydrogenase, glucosephosphate isomerase, and phosphoglucomutase were significantly reduced in the levels of RNA were significantly reduced in the regressing neoplasms at 2 to 3 weeks after ovariectomy of the host. From the data seen in Tables 2 and 3, significant decreases in the activities of glucose-6-phosphate dehydrogenase, NADP-malate dehydrogenase, phosphoglucomutase, glutamate dehydrogenase, and pyruvate kinase were observed in neoplasms at 5 days after ovariectomy. At 5 days after ovariectomy, no significant decrease in tumor size or volume could be detected, probably because of the accuracy inherent to palpation measurements. Thus, since most of the DMBA-induced tumors regress after ovariectomy, it appears that certain decreases in enzyme activity may precede an actual decrease in tumor size. Several of the above-mentioned enzymes have been examined in earlier studies for their relationship to tumor growth as influenced by hormonal administration. Recently, we reported that the administration of testosterone propionate to DMBA-induced tumor-bearing rats caused a decrease in glucose-6-phosphate dehydrogenase activity in the neoplasms, a result which resembled the effects of ovariectomy (21). Similar findings were reported with the R3320AC mammary tumor, a transplantable autonomous neoplasm that responds to treatment with hormones (21).

The effect of administration of estrogens on the activities of many of the enzymes discussed above is somewhat more complicated. The amount of estrogen administered is critical, since small doses maintain or even stimulate mammary cancer growth (11, 27, 28), whereas larger doses produce regression or a decrease in neoplastic growth (11, 17, 20, 21, 36). Glucose-6-phosphate dehydrogenase appears to play a most important role in the metabolism of the neoplasm, since the reduced pyridine nucleotide produced by the
Table 3

Effect of delayed ovariectomy on biochemical characteristics of mammary tumors induced by DMBA

Ovariectomy was performed 35 days after the 1st tumor that appeared had reached approximately 0.5 x 0.5 cm. Animals were sacrificed 1, 5, or 14 days after ovariectomy and all tumors were excised, weighed separately, and frozen. Each tumor was subsequently analyzed separately. Abbreviations are as in Table 2.

<table>
<thead>
<tr>
<th>End point</th>
<th>Control (intact)</th>
<th>Day 1</th>
<th>Day 5</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA (μg/mg tumor)</td>
<td>6.7 ± 0.4a</td>
<td>6.2 ± 0.6</td>
<td>4.4 ± 0.4b,c</td>
<td>4.3 ± 0.4b,c</td>
</tr>
<tr>
<td>RNA (μg/mg tumor)</td>
<td>9.9 ± 0.6</td>
<td>10.2 ± 0.8</td>
<td>11.9 ± 1.7</td>
<td>13.9 ± 2.1</td>
</tr>
<tr>
<td>RNA/DNA ratio</td>
<td>0.71 ± 0.04</td>
<td>0.61 ± 0.05</td>
<td>0.40 ± 0.4b,c</td>
<td>0.35 ± 0.03b,c</td>
</tr>
</tbody>
</table>

Enzyme activity (μmoles pyridine nucleotide reduced or oxidized/min/mg DNA)

- **G6Pd**: 0.520 ± 0.038, 0.409 ± 0.067, 0.186 ± 0.035b,c, 0.157 ± 0.046b,c
- **ICD**: 0.737 ± 0.048, 0.769 ± 0.073, 0.543 ± 0.088, 0.450 ± 0.084b,c
- **ME**: 0.077 ± 0.008, 0.085 ± 0.008, 0.040 ± 0.006b,c, 0.049 ± 0.013c
- **GPI**: 4.95 ± 0.27, 5.73 ± 0.63, 4.66 ± 0.33, 3.83 ± 0.60c
- **PGM**: 0.086 ± 0.006, 0.072 ± 0.010, 0.042 ± 0.007b,c, 0.042 ± 0.010b,c
- **αGPD**: 0.082 ± 0.023, 0.046 ± 0.004, 0.032 ± 0.007, 0.055 ± 0.014
- **GDH**: 0.028 ± 0.003, 0.017 ± 0.003b, 0.019 ± 0.003b, 0.016 ± 0.003b
- **AAT**: 1.091 ± 0.081, 1.942 ± 0.15b, 1.272 ± 0.097b,c, 0.953 ± 0.149c
- **PYK**: 8.341 ± 0.771, 9.816 ± 0.493, 2.852 ± 0.431b,c, 3.035 ± 0.770b,c
- **HK**: 0.037 ± 0.004, 0.143 ± 0.014b, 0.102 ± 0.007b,c, 0.105 ± 0.014b
- **GK**: 0.002 ± 0.001, 0.001 ± 0.001, 0.001 ± 0.001, 0.002 ± 0.001

- **CHOL**: 2.59 ± 0.08, 1.64 ± 0.21b, 2.43 ± 0.37, 3.63 ± 0.50c
- **FFA**: 2.85 ± 0.36, 2.37 ± 0.34, 2.35 ± 0.33, 2.73 ± 0.28
- **TG**: 13.2 ± 6.0, 7.0 ± 4.2, 3.1 ± 1.3, 18.2 ± 9.3

- a Mean ± S.E.
- b Significantly different (p < 0.05) from the intact control.
- c Significantly different (p < 0.05) from the animals sacrificed 1 day after ovariectomy.

Enzyme-catalyzed reaction could be used as a cofactor in fatty acid synthesis or protein synthesis, as well as the overall pentose pathway, could be used as a source of ribose. Data have been presented which show that the administration of estrogens caused an increase in the activity of this enzyme in the face of a decrease in tumor growth (15, 17, 20, 21). In such cases, morphological evidence had demonstrated that the hormone caused a stimulation of a secretory state of the neoplasms and, in some cases, caused the accumulation of a milk-like fluid (15, 20, 21). Similar correlations have also been found for NADP-malate dehydrogenase and phosphoglucomutase. Thus, in hormone-dependent, carcinogen-induced mammary tumors, removal of the ovaries resulted in a decrease in enzyme activities, whereas administration of pharmacological doses of estrogens produced an increase in the activities of the same enzymes. Mammary tumors unaffected by administration of estrogens demonstrated no changes in the activities of the above enzymes (16).

The mammary gland, stimulated by pregnancy or large doses of estrogens, responds with an increase in the activities of the above enzymes and, during involution, decreases in the activities of the same enzymes have been reported (1, 2). However, the mammary gland of the tumor-bearing rat is relatively quiescent and is comprised primarily of adipose tissue. Although very few biochemical changes can be demonstrated in the mammary gland shortly after ovariectomy, morphological atrophy does occur with time after removal of the ovaries. In contrast, the uterus decreases more strikingly after ovariectomy and the activities of glucose-6-phosphate dehydrogenase and NADP-malate dehydrogenase decrease in the uterus (7). Endogenous levels of estrogen maintain the morphological and biochemical integrity of estrogen target organs. Although we have no data at present, it seems logical that administration of small doses of estrogen following ovariectomy would prevent ovariectomy-induced regression of DMBA-induced tumors as well as the decreases in the activities of these enzymes (11).

Correlations of enzyme activity with tumor growth rate have been reported by several investigators studying hepatomas. For example, pyruvate kinase and hexokinase activities were elevated in poorly differentiated or fast-growing hepatomas (8, 38). Although we recently reported that neither of these enzymes showed correlations between activity and growth rate of DMBA-induced tumors (19), the data presented here demonstrate that pyruvate kinase activity was significantly decreased at 5 days after ovariectomy. It would appear that pyruvate kinase, an important enzyme for...
Ovariectomy and DMBA-induced Mammary Tumors


21. Hilf, R., Michel, I., and Bell, C. Biochemical and Morphological
Hilf et al.


Biochemical Events Associated with Regression of 7,12-Dimethylbenz(a)anthracene-induced Mammary Carcinomas after Ovariectomy

Russell Hilf, Harold Goldenberg, Inge Michel, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/31/1/52

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/31/1/52.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.