Steroid Influences on Respiration, Glycolysis, and Levels of Pyridine Nucleotide-linked Dehydrogenases of Experimental Mammary Cancers

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SUMMARY
Measurement of respiration, glycolysis, and levels of soluble pyridine nucleotide-linked dehydrogenases provides the first biochemical characterization of hormone-dependent mammary cancer. The levels of lactic dehydrogenase in mammary cancer induced by 3-methylcholanthrene in rats were correlated, respectively, with progression or regression brought about by appropriate modifications of the hormonal status.

Arranged quantitatively, the dehydrogenases formed individually characteristic patterns in mammary cancer and in normal mammary glands. Malic dehydrogenase (DPN) had the greatest activity in the normal mammary glands; lactic dehydrogenase occupied the first rank in cancer. The level of malic enzyme (TPN) was low in mammary cancer.

Many mammary cancers of ovariectomized rats treated with large doses of estradiol-17β developed a vast accumulation of lipides in the epithelial cells—a newly recognized quality of hormonal responsiveness in carcinoma of the breast.

In the present experiments multiple mammary cancers were induced in rats, and the levels of six soluble pyridine nucleotide-linked enzymes were determined in the tumors before and after (a) the withdrawal of steroids by oophorectomy, (b) the administration of sex steroids, (c) pregnancy, and (d) lactation. It was found that these modifications of the steroid status exerted great effects on the levels of lactic dehydrogenase,1 glucose-6-phosphate dehydrogenase, and malic enzyme (TPN) in the cancers. Changes in the endocrine status exerted smaller effects on the levels in the tumors of 6-phosphogluconic dehydrogenase, isocitric dehydrogenase, and malic dehydrogenase (DPN). The presence or absence of ovaries did not influence the levels of 6-PGD, G-6-PD, lAD, and LAD in transplanted Walker carcinoma or fibrosarcoma in rats.

In the present work, it was also found that the 3-methylcholanthrene-induced mammary cancers had the high aerobic and anaerobic glycolysis which Warburg (22) discovered are metabolic characteristics common to all malignant tumors.

Many mammary cancers in the rat induced by the oral administration of 3-methylcholanthrene are hormone-dependent, since they regress after appropriate modification of the steroid status of the hosts; other mammary cancers do not shrink and, accordingly, are hormone-independent. The property of hormone dependence, recently recognized in these tumors (11, 12), is unique among experimental mammary cancers; hormone dependence is a function of the carcinoma and is not inherent in the host. The present study revealed biochemical differences between these classes of neoplasms.

One of the considerations leading to the determination of pyridine nucleotide-linked dehydrogenases was that hydrogen atoms and electrons

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at critical sites of the steroid molecule are of decisive importance in the promotion of cell growth (19) by compounds exerting androgenic or estrogenic effects. Evidence has been accumulated which has elucidated ways in which simple hydrogen atoms and electrons at critical sites in the steroid molecule can exert vast effects on biological systems. Talalay and Williams-Ashman (20) discovered the cyclical function of oxidation-reduction of steroid hormones which mediates hydrogen transfer between DPN and TPN. Such transhydrogenation of pyridine nucleotides by enzymes in the presence of catalytic quantities of steroid hormones made it desirable to investigate the level of pyridine nucleotide-linked enzymes in mammary cancer. Evidence (7-9) has been presented that estradiol-17β-linked transhydrogenation is operative in the human mammary glands and in certain cancers thereof.

Earlier, Meister (17) found that the levels of LAD in tumors of rodents are frequently higher than in the corresponding tissues of origin. Wenner et al. (23) observed that LAD, MDH, and ICD are present in tumors in amounts comparable to those in normal tissues. Delbrück et al. (9) found that tumors contain considerable quantities of LAD and MDH.

MATERIALS AND METHODS

Biological.—Observations were made on three malignant tumors, all derived from mammary glands. Walker carcinoma, and a fibrosarcoma originating after implantation of 3-methylcholanthrene in the mammary gland of a female rat, were transplanted intramuscularly in the hind legs of adult female rats, age 100 days. Some of these animals were intact normal females; other groups had been ovariectomized 21 days earlier.

Mammary carcinoma was induced in Sprague-Dawley female rats by the intragastric administration of 3-methylcholanthrene, 10 mg. daily, for 50 days commencing at age 50 days. At age 100 days, each rat had multiple mammary cancers. The experiments were begun 40-50 days after 3-methylcholanthrene had been discontinued. Tumors were removed under ether anesthesia from intact female rats which were in estrus as determined by the presence of cornified cells in the vaginal smear; ovariectomy was performed at the same sitting. Tumors from ovariectomized rats were harvested, under ether anesthesia, 21 days after ovariectomy, and the injection of estradiol-17β was started immediately. Histological sections of every tumor were examined. The size of the tumors was measured at frequent intervals during the experiments.

The steroids were dissolved in ethyl alcohol and diluted with sesame oil to make the final concentration 10 per cent. The solution (0.2 ml.) was injected intramuscularly daily; dosage refers to the amount administered each day. Throughout this paper, unless otherwise designated, percentage refers to differences from mean values obtained in tumors of normal intact female rats which were rated 100 per cent.

**Enzyme assays and units.**—The tumors were excised rapidly, weighed, and homogenized in an ice-cold solution of 0.15 N NaCl containing 0.003 M NaHCO₃; the enzyme solutions were kept thereafter in an ice bath. The homogenates were centrifuged at 11,000 × g for 15 minutes in a refrigerated centrifuge. Assays were carried out promptly. The nitrogen content of the organs was determined by a micro-Kjeldahl technic. Pieces of each tumor were dried to constant weight at 100° C., and the fat content was determined by difference in weight after continuous extraction with ethyl ether, followed by petroleum ether, for 48 hours.

Spectrophotometric determinations were made with a Beckman model DU spectrophotometer at 25° C. in an air-conditioned room with either pyrex or silica cells of a 1-cm. light path. The reduction (or oxidation) of pyridine nucleotides was followed by measurement of change of absorbance at 340 μm. The molar extinction coefficient of reduced pyridine nucleotides at 340 μm was assumed to be 6,220. Only the initial velocity of oxidation or reduction of pyridine nucleotides was measured; these conditions yielded zero-order kinetics for all the dehydrogenases. All the enzyme determinations were carried out in the presence of Tris buffer (250 μmoles/3 ml) at pH 7.4.

6-PGD and G-6-PD were determined by a modification (14) of the double substrate method of Glock and McLean (5). LAD was measured by the method of Kubowitz and Ott (15). ICD (18), MDH (16), and malic enzyme (19) were measured by methods devised by Ochoa and his school.

One unit of 6-PGD, or of G-6-PD, or of ICD, or of malic enzyme is defined as the enzyme activity which oxidized 1 μmole of DPNH/minute under stated conditions. One unit of LAD or MDH is defined as the enzyme activity which reduced 1 μmole of TPN/minute under the stated conditions. The units are expressed in terms of 1 gm. of fresh tissue (wet weight) or per mg. of nitrogen.

**Manometric methods.**—Slices were cut by the method of Deutsch (3). Respiration was determined manometrically at 37° C. in Warburg flasks.
containing 0.2 ml. of 20 per cent KOH on a filter paper roll in the center well. The slices were immersed in 2 ml. of Krebs-Ringer phosphate solution (21) at pH 7.4, with 0.2 per cent glucose, but without calcium. The gas phase was 100 per cent O₂. When it was desired, the medium contained 5 × 10⁻⁴ moles recrystallized 2,4-dinitrophenol. For the glycolysis measurements the flasks contained 2 ml. of Krebs-Ringer bicarbonate solution (21) at pH 7.4, with 0.2 per cent glucose, but without calcium. Anaerobic glycolysis was determined manometrically with 95 per cent N₂—5 per cent CO₂ as the gas phase. Aerobic glycolysis was estimated by the direct method of Warburg (4) with 95 per cent O₂ — 5 per cent CO₂ as the gas phase. In calculating the glycolytic value, the conventional assumption was made that the respiratory quotient was 1.0. The lactic acid content of the media from 80 flasks were determined by an enzymatic analysis (10). The data obtained from these analyses indicated that the CO₂ displaced from the bicarbonate medium by lactic acid accounted for the CO₂ formed in excess of the respiratory CO₂, thus validating the procedure as a measurement of glycolysis by the mammary cancers.

At the end of each manometric experiment, the slices were rinsed in distilled water, blotted, and then dried overnight in an oven at 105°C. Fat was extracted from the dry tissue of the individual slices by refluxing with carbon tetrachloride in a fat extractor. Q values were calculated as μl/mg of dry nonfat tissue/hour at 37°C. The respiration values, Qₜₒ and QᵦNP cited were those obtained during the 1st hour of measured respiration. The rate of glycolysis diminished with time, and it should be noted that the values cited for aerobic glycolysis, Qₜₒ, and anaerobic glycolysis, Qᵦ, were those obtained during the 1st ½ hour of measurement.

It was of interest to compare the rates of enzyme activities (μmoles/gm wet weight/min at 25°C) with comparable manometric data. In order to do this, a conversion factor was derived to convert conventional Q values to μmoles/gm wet weight/min at 25°C. The derivation makes the conventional assumption that the temperature coefficient, Q₁₀, for these metabolic processes is 2, and thus the rates decreased 2.4 times between 37°C and 25°C. The wet weight/dry weight ratio of mammary cancers was uniformly 5. By the equivalents —22.4 μl/μmole, 1000 mg/gm, 60 min/hr—the conversion factor was derived

\[
\frac{1}{22.4} \times \frac{1000}{5} \times \frac{1}{60} \times \frac{1}{2.4} = 0.062.
\]

Therefore, the values of \( Q \times 0.062 \) yield μmoles/gm wet weight/min at 25°C.

RESULTS

Dehydrogenases in mammary cancer and in normal mammary glands.—The levels of LAD and MDH, both in mammary cancer and in normal hyperplastic mammary gland as well, exceeded by several orders of magnitude the activities of the other dehydrogenases which were investigated (Tables 1, 2). In the mammary glands, normal or malignant, which were studied the level of G-6-PD was higher than that of 6-PGD (Tables 1–3).

Arranged quantitatively, the levels of soluble pyridine nucleotide-linked dehydrogenases form patterns which are characteristic for each class of tissue (Table 4). The levels of LAD in each malignant tumor were impressively high, exceeding the values obtained for this enzyme in normal mammary glands. In each cancer the level of LAD exceeded that of other dehydrogenases.

The mammary glands in rats on day 15 of pregnancy are hyperplastic and embedded in fat, the acini have no lumina, and they do not contain milk; ether-extractable lipides were 43.4 ± 8 per cent of wet weight of the ensemble. In the mammary glands of pregnancy, the level of MDH exceeded that of other dehydrogenases.

The mammary glands in rats on day 15 of lactation are hyperplastic and secrete milk copiously; ether-extractable lipides were 24.6 ± 12 per cent of wet weight of the glands. As in pregnancy (Table 2), the level of MDH in lactating mammary glands exceeded that of other dehydrogenases. The levels of all the dehydrogenases (except ICD) which were investigated were higher in the hyperplastic mammary glands during lactation than in those of the pregnant rat (day 15); the levels of ICD were similar in both classes of mammary glands. With reference to mammary glands of pregnant rats, the most impressive increases of dehydrogenases in the lactating mammary glands concerned malic enzyme and G-6-PD (Table 2).

The mammary cancers grew rapidly during pregnancy, yet the only impressive difference from the breast cancers of virgin intact females in dehydrogenases concerned LAD (Table 1); during pregnancy the levels of lactic dehydrogenase of mammary neoplasms were significantly higher than they were in the cancer of intact nonpregnant females. In pregnancy at day 15, the following enzymes were at higher levels in mammary cancer than in adjacent mammary glands: LAD, ICD, G-6-PD, 6-PGD; the levels of MDH were rather
### TABLE 1

**EFFECTS OF HORMONAL MODIFICATIONS ON LEVELS OF PYRIDINE NUCLEOTIDE-LINKED ENZYMES IN MAMMARY CANCER**

The results are expressed in units per gram of tumor, wet weight. The hormonal modifications had been in effect 21 days before the tumors were harvested. Means with standard deviation (±) are given; in addition, the values for each enzyme are expressed in percentage of the values for that enzyme in intact adult female rats.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NO. TUMORS</th>
<th>6-Phosphogluconic</th>
<th>Glucose-6-phosphate</th>
<th>Isocitric</th>
<th>Lactic</th>
<th>Malic</th>
<th>ENZYME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact adult females</td>
<td>25</td>
<td>2.84±0.62 (100%)</td>
<td>5.05±1.48 (100%)</td>
<td>7.57±1.81 (100%)</td>
<td>90.1±28 (100%)</td>
<td>55.8±14.9 (100%)</td>
<td>0.28±0.09 (100%)</td>
</tr>
<tr>
<td>Intact; dihydrotestosterone, 1 mg.</td>
<td>11</td>
<td>2.18±0.78 (77%)</td>
<td>3.21±0.82 (54%)</td>
<td>7.14±1.89 (97%)</td>
<td>79.8±22 (89%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovariectomized; hormone-dependent tumors</td>
<td>11</td>
<td>1.13±0.41 (40%)</td>
<td>2.04±2.02 (34%)</td>
<td>6.30±1.05 (84%)</td>
<td>47.7±15.6 (53%)</td>
<td>43.7±15.4 (75%)</td>
<td>0.62±0.32 (221%)</td>
</tr>
<tr>
<td>Ovariectomized; hormone-independent tumors</td>
<td>8</td>
<td>2.36±1.38 (85%)</td>
<td>3.09±1.26 (52%)</td>
<td>8.46±1.26 (115%)</td>
<td>127.4±37.7 (141%)</td>
<td>38.8±7.2 (97%)</td>
<td>0.76±0.50 (271%)</td>
</tr>
<tr>
<td>Ovariectomized; estradiol-17β, 10μg.</td>
<td>8</td>
<td>2.37±0.67 (85%)</td>
<td>5.82±1.24 (98%)</td>
<td>7.20±1.15 (98%)</td>
<td>111.5±17 (124%)</td>
<td>50.2±12.3 (96%)</td>
<td>0.34±0.07 (121%)</td>
</tr>
<tr>
<td>Ovariectomized; estradiol-17β, 50μg.</td>
<td>7</td>
<td>4.95±1.55 (150%)</td>
<td>7.83±3.58 (139%)</td>
<td>5.77±1.57 (78%)</td>
<td>118±26 (131%)</td>
<td>46.4±5.4 (90%)</td>
<td>3.11±1.37 (1111%)</td>
</tr>
<tr>
<td>Pregnancy; day 15</td>
<td>5</td>
<td>2.37±0.75 (90%)</td>
<td>6.55±2.70 (110%)</td>
<td>8.46±1.34 (115%)</td>
<td>108±16 (120%)</td>
<td>49.2±15.0 (120%)</td>
<td>0.35±0.08 (122%)</td>
</tr>
<tr>
<td>Lactation; day 15</td>
<td>5</td>
<td>5.31±0.87 (116%)</td>
<td>5.61±0.84 (94%)</td>
<td>5.92±1.86 (80%)</td>
<td>69.9±6 (78%)</td>
<td>60.5±11.1 (104%)</td>
<td>0.39±0.16 (156%)</td>
</tr>
</tbody>
</table>

### TABLE 2

**RESPIRATION, GLYCOLYSIS, AND LEVELS OF DEHYDRGENASES IN MAMMARY GLANDS AND IN MAMMARY CANCERS IN PREGNANCY AND LACTATION**

Dehydrogenase results are expressed in μmoles of pyridine nucleotide (oxidized or reduced)/min/gm wet weight or per mg. of nitrogen. Manometric results are expressed in gmoles/min/gm wet weight, at 23°C.

<table>
<thead>
<tr>
<th>NITROGEN</th>
<th>6-PGD</th>
<th>G-6-PD</th>
<th>ICD</th>
<th>LAD</th>
<th>MDH</th>
<th>MALIC ENZYME</th>
</tr>
</thead>
<tbody>
<tr>
<td>(gm/100 gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mammary cancer</td>
<td>1.77</td>
<td>2.58</td>
<td>6.28</td>
<td>9.22</td>
<td>11.53</td>
<td>32.6</td>
</tr>
<tr>
<td>Mammary gland</td>
<td>1.94</td>
<td>1.71</td>
<td>2.09</td>
<td>18.1</td>
<td>36.2</td>
<td>1.19</td>
</tr>
<tr>
<td>Ratio*</td>
<td></td>
<td>205%</td>
<td>367%</td>
<td>441%</td>
<td>504%</td>
<td>123%</td>
</tr>
<tr>
<td>Mammary cancer</td>
<td>0.15</td>
<td>0.55</td>
<td>0.52</td>
<td>0.73</td>
<td>1.83</td>
<td>0.02</td>
</tr>
<tr>
<td>Mammary gland</td>
<td>0.09</td>
<td>0.13</td>
<td>0.16</td>
<td>1.55</td>
<td>1.97</td>
<td>0.09</td>
</tr>
<tr>
<td>Ratio*</td>
<td></td>
<td>167%</td>
<td>269%</td>
<td>254%</td>
<td>55%</td>
<td>93%</td>
</tr>
<tr>
<td>Mammary cancer</td>
<td>2.17</td>
<td>3.61</td>
<td>4.30</td>
<td>5.29</td>
<td>87.3</td>
<td>47.3</td>
</tr>
<tr>
<td>Mammary gland†</td>
<td>1.85</td>
<td>6.83</td>
<td>26.72</td>
<td>2.03</td>
<td>52.5</td>
<td>85.7</td>
</tr>
<tr>
<td>Ratio*</td>
<td></td>
<td>152%</td>
<td>238%</td>
<td>296%</td>
<td>318%</td>
<td>152%</td>
</tr>
</tbody>
</table>

* Cancer/mammary gland X 100.
† The respiratory quotient of lactating mammary gland was 1.4.
similar in both states; the activity of malic enzyme in mammary cancer was much reduced (Table 2) below the level found in the mammary glands.

The mammary cancers grew sluggishly during lactation; indeed, many tumors regressed. On day 15 of lactation, the levels of LAD and of ICD of the mammary cancers that persisted despite the post partum state were significantly lower, and the activity of malic enzyme slightly higher, than values procured from the breast cancers of normal intact females (Table 1). Other enzymes did not differ significantly in the two states. It will be seen (Table 2), that in mammary cancers during lactation, only LAD and ICD levels exceeded the values of dehydrogenases in adjacent normal lactating mammary gland.

Endocrine effects on levels of dehydrogenases of mammary cancers.—All the mammary cancers of intact female rats were classified as papillary adenocarcinoma (Fig. 1). Many of the tumors 0.78 gm/100 gm wet weight. In hormone-dependent mammary cancer after ovariectomy (Table 1) the activity of malic enzyme increased (291 per cent), while the levels of other dehydrogenases decreased. The greatest decline was in the concentrations of G-6-PD (34 per cent), 6-PGD (40 per cent), and LAD (58 per cent) (Table 1).

In hormone-independent mammary cancers, ovariectomy resulted in an increase in the activity of LAD (141 per cent) (Table 1). The level of malic enzyme of these neoplasms was increased (271 per cent) and that of G-6-PD was decreased (52 per cent) in reference to the mammary cancer of intact rats; the level of 6-PGD was not altered significantly.

In ovariectomized rats given injections of estradiol-17β, 10 μg., the atrophic effects in hormone-dependent tumors were not observed, and papillary epithelial cancer cells reappeared (Fig. 3). The activities of the dehydrogenases and malic

Table 3
Lack of Effect of Ovariectomy on Pyridine Nucleotide-Linked Dehydrogenases in Transplanted Walker Carcinoma and Fibrosarcoma

<table>
<thead>
<tr>
<th>Tumors</th>
<th>6-Phosphogluconic</th>
<th>Glucose-6-phosphate</th>
<th>Isocitric</th>
<th>Lactic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walker carcinoma; intact females</td>
<td>2.00</td>
<td>6.38</td>
<td>1.63</td>
<td>126.7</td>
</tr>
<tr>
<td></td>
<td>1.21–3.01</td>
<td>5.31–7.75</td>
<td>1.21–9.02</td>
<td>118.7–135.9</td>
</tr>
<tr>
<td>Walker carcinoma; ovariectomized rats</td>
<td>2.50</td>
<td>5.41</td>
<td>1.44</td>
<td>99.1</td>
</tr>
<tr>
<td></td>
<td>1.99–2.99</td>
<td>4.44–7.58</td>
<td>1.15–1.92</td>
<td>85.9–119.9</td>
</tr>
<tr>
<td>Fibrosarcoma; intact females</td>
<td>1.79</td>
<td>2.48</td>
<td>2.68</td>
<td>42.1</td>
</tr>
<tr>
<td></td>
<td>0.80–3.01</td>
<td>1.80–3.17</td>
<td>2.86–3.11</td>
<td>25.1–33.8</td>
</tr>
<tr>
<td>Fibrosarcoma; ovariectomized rats</td>
<td>1.60</td>
<td>2.79</td>
<td>2.98</td>
<td>45.8</td>
</tr>
<tr>
<td></td>
<td>1.07–2.16</td>
<td>1.99–3.82</td>
<td>2.19–3.77</td>
<td>27.9–80.9</td>
</tr>
</tbody>
</table>

Table 4
Relative Levels of Dehydrogenases in Mammary Cancer and in Hyperplastic Mammary Glands

| Mammary gland: pregnancy, day 15 | MDH > LAD > ICD > G-6-PD > 6-PGD | Malic enzyme |
| Mammary gland: lactation, day 15 | MDH > LAD > G-6-PD > Malic enzyme > 6-PGD > ICD |

| Mammary cancer | LAD > MDH > ICD > G-6-PD > 6-PGD | Malic enzyme |
| Walker carcinoma* | LAD > G-6-PD > 6-PGD > ICD |
| Fibrosarcoma* | LAD > ICD > G-6-PD > 6-PGD |

* Levels of MDH and malic enzyme were not measured.
enzyme in the neoplasms were restored to the levels found in mammary cancers of normal intact female rats. Hormone responsiveness could no longer be recognized by differences in dehydrogenase levels.

In ovariectomized rats given injections of estradiol-17β, 50 µg., for 9–21 days, many of the tumors underwent a remarkable transformation with a great accumulation of lipides in many of the mammary cancer cells (Fig. 4), while some epithelial cells in these tumors contained little fat. Other tumors had no great accumulation of lipides. The tumors in which this fatty change was pronounced had an appearance like lard, and they

level of G-6-PD (54 per cent) and of 6-PGD and LAD; the results were somewhat similar to the effects induced by ovariectomy, although less pronounced in magnitude.

Lack of effect of ovariectomy on Walker carcinoma and fibrosarcoma.—Neither the growth of these transplanted tumors nor the enzyme muster was remarkably different in intact female rats and in ovariectomized sisters (Table 3). The Walker carcinoma was characterized by a relatively high level of LAD and a low level of ICD. The activity of G-6-PD and 6-PGD in Walker carcinoma was of the same order of magnitude as in the mammary cancers induced with 3-methylcholanthrene.

**TABLE 5**

**EFFECTS OF HORMONAL MODIFICATIONS ON RESPIRATION AND GLYCOLYSIS OF MAMMARY CANCER SLICES**

The results are expressed in µl/mg of dry nonfat tissue. Q_{ONP} designates the respiration in the presence of 5 × 10^{-3} M 1,4-dinitrophenol (DNP). Hormonal modifications were in effect 21 days before tumors were harvested. Means with standard deviations (±) are given; in addition, the value for each metabolic parameter is expressed in percentage of the values for that parameter in intact adult female rats.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NO. TUMORS</th>
<th>RESPIRATION</th>
<th>GLYCOLYSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Qo</td>
<td>Q_{ONP}</td>
</tr>
<tr>
<td>Intact adult females</td>
<td>20</td>
<td>12.2 ± 2.1</td>
<td>21.8 ± 4.1</td>
</tr>
<tr>
<td>Ovariectomized</td>
<td>12</td>
<td>10.5 ± 4.5</td>
<td>15.8 ± 6.7</td>
</tr>
<tr>
<td>Ovariectomized; estradiol-17β, 10µg.</td>
<td>9</td>
<td>12.6 ± 2.3</td>
<td>25.0 ± 3.6</td>
</tr>
<tr>
<td>Ovariectomized; estradiol-17β, 50µg.</td>
<td>9</td>
<td>18.4 ± 4.9</td>
<td>26.6 ± 6.2</td>
</tr>
<tr>
<td>Pregnancy; day 15</td>
<td>7</td>
<td>14.7 ± 5.4</td>
<td>23.7 ± 7.5</td>
</tr>
</tbody>
</table>

The transplanted fibrosarcoma was characterized by a relatively high level of LAD (Table 3) and low levels of G-6-PD, ICD, and 6-PGD.

Respiration and glycolysis of mammary glands and cancers.—The 3-methylcholanthrene-induced mammary carcinomas of intact adult females provided respiration values, Qo, 12.2 ± 2.1 (Table 5). Variations of Qo, of the tumors were not observed during different stages of the estrus cycle; mean respiration values of the mammary cancers were obtained as follows: in estrus Qo, 12.3; in proestrus, 12.2; in diestrus 12.2. In the presence of DNP, respiration of the tumor slices was nearly doubled (Table 5). High rates of aerobic glycolysis (Q_{Glc} 12.0) and anaerobic glycolysis (Q_{Glc} 27.3) were observed.

The respiration values of the mammary glands

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NO. TUMORS</th>
<th>RESPIRATION</th>
<th>GLYCOLYSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Qo</td>
<td>Q_{ONP}</td>
</tr>
<tr>
<td>Intact adult females</td>
<td>20</td>
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<td>21.8 ± 4.1</td>
</tr>
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<td>15.8 ± 6.7</td>
</tr>
<tr>
<td>Ovariectomized; estradiol-17β, 10µg.</td>
<td>9</td>
<td>12.6 ± 2.3</td>
<td>25.0 ± 3.6</td>
</tr>
<tr>
<td>Ovariectomized; estradiol-17β, 50µg.</td>
<td>9</td>
<td>18.4 ± 4.9</td>
<td>26.6 ± 6.2</td>
</tr>
<tr>
<td>Pregnancy; day 15</td>
<td>7</td>
<td>14.7 ± 5.4</td>
<td>23.7 ± 7.5</td>
</tr>
</tbody>
</table>

The transplanted fibrosarcoma was characterized by a relatively high level of LAD (Table 3) and low levels of G-6-PD, ICD, and 6-PGD.

Respiration and glycolysis of mammary glands and cancers.—The 3-methylcholanthrene-induced mammary carcinomas of intact adult females provided respiration values, Qo, 12.2 ± 2.1 (Table 5). Variations of Qo, of the tumors were not observed during different stages of the estrus cycle; mean respiration values of the mammary cancers were obtained as follows: in estrus Qo, 12.3; in proestrus, 12.2; in diestrus 12.2. In the presence of DNP, respiration of the tumor slices was nearly doubled (Table 5). High rates of aerobic glycolysis (Q_{Glc} 12.0) and anaerobic glycolysis (Q_{Glc} 27.3) were observed.

The respiration values of the mammary glands
during lactation were similar to those provided by mammary cancers. Slices of mammary gland from lactating rats yielded $Q_0$, 18.2 ± 4.1; this value was not increased by the addition of DNP.

The respiration of tumor slices from ovariectomized rats fell into two groups: (a) those with $Q_0$, and $Q_0^{DNP}$ in the normal range for tumors of intact rats and (b) those with lower levels. Values of $Q_0$, smaller than 10.9 were considered to be significantly lower than the mean $Q_0$, of tumors from intact rats. On this basis in ovariectomized rats, eight mammary cancers were assigned to the subnormal group $Q_0$, 7.9 ± 1.9 and $Q_0^{DNP}$ 12.8 ± 1.1. These tumors were found to have the atrophic histologic appearance of hormone-dependent neoplasms (Fig. 2). Moreover, four cancers of the breast in ovariectomized rats did not undergo steroid-withdrawal atrophy as determined by histologic methods and by continued growth of the tumors. In these hormone-independent tumors, the respiration values were $Q_0$, 15.8 ± 3.7 and $Q_0^{DNP}$ 24.7 ± 2.8; such differences between the two groups were statistically significant ($p < .005$).

When estradiol-17$\beta$, 10 $\mu$g., was administered to ovariectomized animals, the tumors could no longer be separated into groups according to their respiration; the value $Q_0$, 12.6, obtained was the level found in the tumors studied prior to ovariectomy. DNP increased the respiration of the tumors in every case (Table 5). When the daily dosage of estradiol-17$\beta$, 50 $\mu$g., was administered, the mean respiration increased slightly (Table 5). Again in every case, DNP increased the $Q_0$. Tumors from rats on day 15 of pregnancy had $Q_0$, values and the response to DNP which were characteristic of mammary cancers from intact rats. No treatment employed in this study significantly influenced the rates of the glycolytic components of the tumor metabolism.

DISCUSSION

The mammary glands of virgin female rats consist of small tubules embedded in much fat. This preponderance of adipose tissue makes the study of metabolic characteristics of the mammary tubules of virgin rats difficult; hence, in this work, biochemical characteristics of mammary cancers were compared with those of the hyperplastic mammary glands of pregnant or lactating rats.

The respiration values of mammary cancer growing in normal, pregnant, or lactating rats were similar to that of the mammary gland in lactation. The respiration of slices of mammary cancer increased nearly twofold in the presence of 2,4-dinitrophenol, presumably owing to the uncoupling of oxidation and oxidative phosphorylation; respiration of lactating mammary gland was not increased by 2,4-dinitrophenol in the concentration employed.

3-Methylcholanthrene-induced mammary cancers had the high aerobic glycolysis which is distinctive of the metabolism of neoplasms. The rate of glycolysis decreased in the presence of oxygen (Pasteur effect).

While the levels of other pyridine nucleotide-linked dehydrogenases were considerably influenced by the administration or withdrawal of steroids, the level of isocitric dehydrogenase was little affected by these hormonal modifications.

The level of malic enzyme was increased after ovariectomy in both hormone-dependent and hormone-independent mammary cancers. It would appear that this enzyme is present in the stroma of the tumors in addition to mammary epithelial cells, since mammary cancers with atrophic epithelium had increased levels. This increased level in ovariectomized rats was lowered to that found in intact normal female rats by the administration of moderate amounts (10 $\mu$g.) of estradiol-17$\beta$. However, the level of malic enzyme was increased impressively in the mammary cancers of ovariectomized rats receiving large doses (50 $\mu$g.) of estradiol-17$\beta$. The level of malic enzyme was considerably increased in the mammary glands during lactation.

In the mammary cancers, ovariectomy caused a considerable decrease in the level of glucose-6-phosphate dehydrogenase and a smaller decrement in 6-phosphogluconic dehydrogenase. It was of interest that the level of glucose-6-phosphate dehydrogenase declined in both hormone-dependent and in hormone-independent mammary cancers after ovariectomy. The reduced levels of Zwischenferment were restored to normal levels by the administration of estradiol-17$\beta$, 10 $\mu$g.

Glock and McLean (8) found that the concentrations of 6-phosphogluconic dehydrogenase and, especially, of glucose-6-phosphate dehydrogenase were increased in the mammary gland of the rat during lactation. These observations were confirmed in the present study; but no increase in these enzymes was observed in mammary cancers during lactation.
With the exception of malic enzyme, the activity levels of all the enzymes studied exceeded the levels of oxygen consumption and lactic acid production. In mammary cancers (Table 2), the activity of malic and lactic dehydrogenases are about two orders of magnitude greater than the rates of respiration and glycolysis. The levels of other enzymes measured did not differ from the rates of glycolysis and respiration by a factor greater than 10. The low levels of malic enzyme suggest that the activity of this enzyme may be a pacemaking step in carbohydrate synthesis in the mammary cancers.

When arranged in order of magnitude, the soluble dehydrogenases formed patterns which were characteristic for each tissue. The pattern of the dehydrogenases of the normal hyperplastic mammary glands of pregnant rats differed from that which distinguished the mammary cancers.

Malic dehydrogenase was the most active dehydrogenase in the normal mammary glands of pregnancy and lactation. Lactic dehydrogenase occupied the highest rank in mammary cancer. In two other neoplasms, the transplanted Walker carcinoma and fibrosarcoma, high levels of lactic dehydrogenase were found.

During lactation, only lactic and isocitric dehydrogenases were more active in the mammary cancers than in the adjacent mammary glands. During pregnancy, the levels of all the dehydrogenases (except malic enzyme) were higher in the cancers.

The 3-methylcholanthrene-induced hormone-dependent mammary cancers regress (a) in ovariectomized animals, (b) in rats treated with dihydrotestosterone, and (c) during lactation. In the present experiments, the levels of lactic dehydrogenase in the mammary cancers were reduced in each of these endocrine states.

The cancers grew at a rapid rate during pregnancy and in ovariectomized rats treated with estradiol-17β, 10 μg; in both of these hormonal states, the level of lactic dehydrogenase in the mammary cancers exceeded that found in the breast carcinomas of intact female rats. While the levels of lactic dehydrogenase activity could be well correlated with the growth rate of the mammary cancers, no such correlation existed between the level of LAD and the rate of glycolysis.

The data revealed many differences between hormone-dependent and hormone-independent mammary cancer. No biochemical differentiation between these classes has been described hitherto.

Hormone-dependent mammary cancers regress after ovariectomy. It was found in the present experiments that ovariectomy induced in tumors of this class decline in the levels of: (a) respiration, (b) glucose-6-phosphate dehydrogenase, (c) 6-phosphogluconic dehydrogenase, (d) lactic dehydrogenase. There was no great change in the level of isocitric dehydrogenase or in the rates of aerobic or anaerobic glycolysis. The level of malic enzyme was doubled.

Hormone-independent mammary cancers do not regress after ovariectomy, and their epithelial cells do not atrophy. In the present work, it was observed that, in tumors of this class, ovariectomy resulted in decline only in the levels of (a) glucose-6-phosphate dehydrogenase and (b) malic dehydrogenase; the levels of (c) malic enzyme and of (d) lactic dehydrogenase were increased.

Another novel observation was the finding of lipide-rich cancer cells in some of the mammary carcinomas of ovariectomized rats treated with estradiol-17β, 50 μg. The fat-crammed cancer cells are reminiscent of the fat-laden epithelial cells of the normal mammary gland in lactation, but the mammary cancers of rats lactating after parturition never had the vast accumulation of lipides found in ovariectomized rats treated with large amounts of estradiol-17β. The accumulation of lipides in the mammary cancer cells is a quality of hormone responsivity, and not all the treated rats displayed this effect.
REES AND HUGGINS—Steroid Influences on Mammary Cancer Metabolism

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