The Estradiol-17β Enzyme Systems of Human Choriocarcinoma Derived from Women

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SUMMARY

Homogenates of seven specimens of human choriocarcinoma taken from women were assayed for three 17β-estradiol-sensitive enzyme systems: a DPN-linked dehydrogenase, a TPN-linked dehydrogenase, and an estradiol-sensitive transhydrogenase. Activities were found only in the soluble fraction of the cell precipitatable at half saturation with ammonium sulfate and ranged from 1 to 50 per cent of those found in term placenta.

Following the original observation by Villee and Hagerman (10) that oxygen uptake in human placental tissue is stimulated by catalytic quantities of estradiol-17β, at least three enzyme systems have been shown to be intimately concerned with this steroid (2, 5, 6, 9), a DPN-linked dehydrogenase, a TPN-linked dehydrogenase, and a transhydrogenase. Since intrauterine choriocarcinoma is considered to be the neoplastic analog of placenta (8), it becomes of importance to have information about these reactions in this tissue. In the present experiments, activities for the three systems in homogenates of choriocarcinoma derived from women are compared with those found in similar homogenates of term placenta.

MATERIALS AND METHODS

Tissues.—Term placentas were obtained within 30 minutes of delivery. Choriocarcinoma specimens were obtained directly from the patient at the time of surgery or from the hamster cheek pouch (3). With each strain of human choriocarcinoma maintained in the hamster, a cycle was established which resulted in maximum growth on a particular day following transplantation. Tissues were harvested on this day for assay. The strain reported in Table 1 was removed from a patient at the time of post mortem; she had received many courses of therapy with amethopterin, a folic acid antagonist which has produced several apparently total remissions in this disease (4).

Preparation of enzyme.—All procedures were carried out between 0° and 2° C. in double-distilled, deionized water. Because of the marked lability of these systems, despite efforts to exclude heavy metals and protect —SH groups as previously noted by other workers, a preparative procedure was devised to eliminate dialyzable substances by ammonium sulfate precipitation (which also stabilizes the enzymes) and thus shorten to 3 hours the time from the harvesting of the tissue to the final assay. A 10 per cent homogenate (w/v) was made with a glass homogenizer fitted with a Teflon pestle in a solution containing 0.25 M sucrose, 0.01 M EDTA, 0.001 M cysteine, and 0.02 M Tris-HCl buffer, pH 7.4, at 23° C. (Solution A). The homogenate was centrifuged at 109,000 × g for 45 minutes. Phase microscopy revealed that all particles were sedimented. To the clear, cherry-red supernatant was added an equal volume of saturated ammonium sulfate. The precipitate was centrifuged at 109,000 × g for an additional 45 minutes. The supernatant was used as the enzyme preparation. Experiments with other preparations, which were kept for 24 hours at 4° C. in the presence of 10 per cent ammonium sulfate, yielded similar results.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>(1) DPN-linked dehydrogenase</th>
<th>(2) TPN-linked dehydrogenase</th>
<th>(3) Transhydrogenase</th>
<th>Ratio of (3):(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human term pla-centa</td>
<td>2.09 ± 0.70 (6)</td>
<td>130 ± 33 (5)</td>
<td>23 ± 2.3 (5)</td>
<td>.10</td>
</tr>
<tr>
<td>Human choriocarcinoma</td>
<td>2.6 ± 0.5 (8)</td>
<td>1.5 ± 0.7 (5)</td>
<td>1.6 ± 0.1 (6)</td>
<td>.62</td>
</tr>
</tbody>
</table>

* Results expressed as units per mg protein on the final enzyme preparation. Number of specimens assayed in parentheses. Each single specimen was taken from the hamster 7 days after transplantation, the time of maximal growth. Data given as mean ± 1 S.D. See text for explanation.

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sulfate, reprecipitated from 0.2 per cent EDTA and buffered to pH 6.8 at 0°C. The mixture (50 per cent saturated ammonium sulfate) was immediately centrifuged at 1500 × g for 30 minutes. The resulting white precipitate was resuspended in a volume of solution A equal to the original and reprecipitated by the addition of an equal volume of saturated ammonium sulfate exactly as before. The final precipitate was resuspended in an appropriate solution and assayed immediately.

Enzyme assay.—Dehydrogenase assays were carried out in a final volume of 0.6 ml in a solution containing 0.25 M sucrose, 0.01 M nicotinamide, 0.06 M Tris-HCl buffer, pH 7.4 at 23°C, 3.3 mg. crystalline bovine albumin, 10⁻⁴ M estradiol-17β (0.005 ml of 10⁻² M propylene glycol solution), 10⁻³ M TPN or DPN, and from 0.05 to 0.4 ml. of enzyme. The reaction was started by the addition of oxidized nucleotide, and the rate of change of optical density at 340 m is followed in a Beckman DU spectrophotometer equipped with thermospacers. Control cells contained all constituents except steroid substrate (including propylene glycol) and did not reduce oxidized nucleotide. The reaction curve was usually linear for at least 20 minutes and was linearly proportional to enzyme concentration for both placenta and choriocarcinoma (Chart 1).

Although the rate of nucleotide reduction in choriocarcinoma was low, the nature of the reaction was confirmed by the use of estradiol-C₁⁴. Sixty-one m moles of estradiol-C₁⁴ having a specific activity of 270 dpm/m mole (New England Nuclear) was added as substrate to the assay system described. A control cuvette was run without added nucleotide. Following a 3-hour incubation, the reaction mixtures were acidified with 2 N HCl and extracted 4 times with 10 ml. methylene chloride. The methylene chloride was pooled, washed once with 15 ml. water, taken to dryness under nitrogen, resuspended in methanol, and placed on paper. Descending chromatography was performed in a cyclohexane (50):benzene (100):methanol (100):water (20) system for 14 hours.

![Chart 1.—Rate of reaction as a function of enzyme concentration](chart.png)

Control spots containing nonradioactive estradiol and estrone were run on each side of the extraction mixtures, and the position of the appropriate radioactive steroid was determined by extrapolating from color developed in the nonradioactive spots by the use of equal parts of ferric chloride (1 per cent) and ferricyanide (1 per cent). Steroid was eluted from these areas with methanol, taken to dryness under nitrogen, and counted in a liquid scintillation counter. Recovery of radioactivity was approximately 80 per cent. No significant radioactivity counts above background appeared in the estrone spot derived from the system without added nucleotide. The m moles estrone produced (extrapolating to 100 per cent recovery of radioactivity) was 16.4 for the TPN-linked dehydrogenase and 23.4 for the DPN-linked dehydro-
genase. If one assumes that the quantity of estrone produced was proportional to the optical density increase at 340 m\(\mu\) (molar extinction coefficient for the reduced nucleotides was taken as \(6.22 \times 10^3\)), the m\(\mu\)moles estradiol converted to estrone during the 3-hour incubation was 15.4 for the TPN-linked dehydrogenase and 19.3 for the DPN-linked dehydrogenase.

Transhydrogenase assays were carried out in a final volume of 0.5 ml in a solution containing 0.25 M sucrose, 0.01 M nicotinamide, 0.06 M Tris-HCl buffer, pH 7.4 at 25\(^\circ\) C. A catalytic quantity of TPN, \(10^{-5}\) M, was first reduced by excess glucose-6-phosphate and glucose-6-phosphate dehydrogenase. Upon the completion of this reaction, \(10^{-3}\) M DPN was added and the rate of optical density change at 340 m\(\mu\) determined. The difference in rate between cells with and without \(10^{-7}\) M 17\(\beta\)-estradiol was taken as estradiol-sensitive transhydrogenase. Generation of TPNH with isocitrate and isocitric dehydrogenase resulted in similar findings. Again the reaction was linear for at least 20 minutes and was linearly proportional to enzyme concentration.

In all cases, one unit of enzyme activity was defined as a change in optical density of 0.001 per minute under the conditions stated.

Protein determination.—Protein was determined by the method of Lowry et al. (7) on the final enzyme preparation.

RESULTS

No activity for the three reactions could be detected in the particles, nuclei, or supernatant from the ammonium sulfate precipitation in either term placenta or choriocarcinoma. Human lung and hamster uterus did not possess activity. Hamster liver possessed a DPN-linked estradiol dehydrogenase and a soluble transhydrogenase not stimulable by estradiol under these conditions. Testosterone and estradiol-17\(\alpha\) were inactive for the three reactions under the conditions stated. For both placenta and choriocarcinoma, in each separate assay, the enzyme activity expressed per mg protein on the final preparation was essentially the same as per 100 mg wet weight on the original tissue.

Table 1 shows the specific activity in units per mg. protein for these three reactions in term placenta compared with one strain of human choriocarcinoma carried in heterologous transplant. Two observations stand out: First, the amount of activity in this choriocarcinoma, when compared with term placenta, was extremely low—roughly 1 per cent for the dehydrogenases and 7 per cent for the transhydrogenase; and, second, although the ratio of the two dehydrogenases was almost identical in the two tissues, TPN reduction being about one-half that of DPN reduction, the transhydrogenase was 6 times greater relative to the DPN-linked dehydrogenase in choriocarcinoma as compared with placenta (last column, Table 1). In view of the marked differences in specific activities of both of these reactions in choriocarcinoma compared with placenta, the significance of this difference in ratio is statistically uncertain. It was not due to a relative lessening of the nonestradiol-sensitive transhydrogenase in choriocarcinoma. Mixing placental enzyme with choriocarcinoma enzyme under the conditions stated resulted in no lowering of the activity of the placental reactions for any of the systems.

Table 2 shows a series of single assays performed on several specimens of human choriocarcinoma taken from the patient and from the hamster cheek pouch. There have been no specimens of definitely viable human choriocarcinoma derived from women completely devoid of activity for any of the three reactions. The last two specimens were from the same patient, No. 7—the bottom one was tissue obtained at post mortem, and the one immediately above it represents this same tissue after several generations in the hamster. The values are comparable. The activities of most of these specimens were very low except for case No. 2 which was taken directly from a patient who had not been treated.

DISCUSSION

These data taken together imply that a wide range of activities for the estradiol-17\(\beta\)-sensitive enzyme systems are to be found in choriocarcinoma. The fact that the values are low compared

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**TABLE 2**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Source</th>
<th>Therapy</th>
<th>TPN-linked dehydrogenase</th>
<th>Transhydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Biop.</td>
<td>+</td>
<td>2.5</td>
<td>9.8</td>
</tr>
<tr>
<td>2</td>
<td>Biop.</td>
<td>0</td>
<td>1.0</td>
<td>10.0</td>
</tr>
<tr>
<td>3</td>
<td>Ham.</td>
<td>0</td>
<td>3.1</td>
<td>14.1</td>
</tr>
<tr>
<td>4</td>
<td>Ham.</td>
<td>+</td>
<td>2.6</td>
<td>1.4</td>
</tr>
<tr>
<td>5</td>
<td>Ham.</td>
<td>+</td>
<td>2.3</td>
<td>2.8</td>
</tr>
<tr>
<td>6</td>
<td>Ham.</td>
<td>+</td>
<td>3.7</td>
<td>2.1</td>
</tr>
<tr>
<td>7</td>
<td>Ham.</td>
<td>+</td>
<td>3.0</td>
<td>8.1</td>
</tr>
<tr>
<td>7</td>
<td>P.M.</td>
<td>+</td>
<td>3.4</td>
<td>7.2</td>
</tr>
</tbody>
</table>

* Expressed as units per mg. protein on the final enzyme preparation. See text for explanation.
with term placenta shows that in choriocarcinoma either the activity per cell is uniformly low or only a small number of cells actually possess activity. In those cases in which the values are very low, there is a tenfold variation in the TPN-linked dehydrogenase (14–140) and a fourfold variation in the transhydrogenase (7–30), but no appreciable variation in the DPN-linked dehydrogenase (25–37). This suggests that these reactions vary independently of each other, but the series is too small to offer substantial evidence bearing on the controversy over the mechanism of the transhydrogenation reaction. In this small series, there is no correlation between enzyme activity and the origin of the tissue, the institution of therapy, the response to therapy, or the hormonal status of the patient. These studies are consistent with previous enzymatic studies of tumor (1) and reconfirm the idea that tumor is dedifferentiated with respect to its normal analog.

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REFERENCES

The Estradiol-17β Enzyme Systems of Human Choriocarcinoma Derived from Women

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