Histochemical Observations of α-Glycerophosphate Dehydrogenase Activity in Human Tumors

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SUMMARY

A histochemical study was made of α-glycerophosphate dehydrogenase in human tumors of 230 cases. Enzymatic activity was generally confined to the cell plasma in neoplastic cells, and stromal reaction was usually low or absent. The stainability for α-glycerophosphate dehydrogenase was constant in normal tissue, whereas that of neoplastic cells was variable. Squamous-cell carcinomas showed varying degrees of enzymatic activity. Adenocarcinoma exhibited a moderate to strong activity, and 47 percent of the stomach cancers seemed to be moderately reactive. Adenomas and fibroadenomas in the breast showed a moderate activity.

Activity and localization for α-glycerophosphate dehydrogenase showed no characteristics, and no correlation was found with the degree of differentiation and malignancy.

Although it has already been reported that α-glycerophosphate dehydrogenase is important for neutral fat and phospholipide metabolism and that it catalyzes the reversible oxidation of L-α-glycerophosphate (5), there are few reports concerning a histochemical detection of α-glycerophosphate dehydrogenase in human neoplasms (1, 7). The biological significance of α-glycerophosphate dehydrogenase in tumor tissue from the biochemical and histochemical viewpoints is still not clarified at the present time. The present experiments were attempted to determine the localization of α-glycerophosphate dehydrogenase activity in human neoplastic tissue and to investigate a possible relationship between neoplastic cells and homologous non-neoplastic cells.

MATERIALS AND METHODS

The human tumors consisted of 140 malignant and 90 benign tumors. The blocks were obtained from surgical specimens. All specimens were immediately frozen in dry ice, and fresh frozen sections, 12–18 μ thick, were made in a cryostat at −20° C. with the use of a slide microtome. After being dried at room temperature, these sections were incubated in the following solution at 37° C. for 1 hour for the histochemical study of α-glycerophosphate dehydrogenase. The incubation solution consisted of 4 ml. of 1 M sodium α-glycerophosphate, 8 ml. of Nitro-BT, 2.5 mg. of 100 per cent DPN, 11 ml. of 0.1 M phosphate buffer, pH 7.6, to which 2 ml. of 0.1 M potassium cyanide was added, and the solution was adjusted with 0.5 M hydrochloride to pH 7.6 (2). After incubation, the sections were fixed in 10 per cent neutral formalin for over 10 minutes, rinsed in distilled water, and mounted in balsam. Positive enzymatic activity was revealed by a blue color. All specimens were histopathologically diagnosed on sections stained with hematoxylin and eosin.

RESULTS

The stainability for α-glycerophosphate dehydrogenase activity in tissue sections with Nitro-BT used as an electron acceptor showed a fine bluish pigment. The grades of enzymatic intensity were arbitrarily divided into the following groups: 0 (negative), ± (trace), +1 (slight), +2 (moderate), +3 (high or strong), and +4 (the highest or most intense). The degrees of color reaction in both malignant and normal tissues were determined by different stainability in the same section.

Oral carcinoma.—Epidermoid cancer with high keratinization (sixteen cases) exhibited a low to moderate activity. The staining intensity was usually weak and the reaction was generally confined to the cell plasma of the neoplastic cells. Squamous-cell carcinomas showed varying degrees of enzymatic activity, ranging from low to strong. Adenocarcinoma exhibited a moderate to strong activity, and 47 percent of the stomach cancers seemed to be moderately reactive. Adenomas and fibroadenomas in the breast showed a moderate activity.

Adenocarcinoma.—Adenocarcinomas of the stomach exhibited varying degrees of enzymatic activity. The staining intensity was usually weak and the reaction was generally confined to the cell plasma of the neoplastic cells. Adenocarcinomas of the breast showed a moderate activity.

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Oral carcinoma.—Epidermoid cancer with high keratinization (sixteen cases) exhibited a low to moderate activity.
marked enzymatic activity in the malignant epithelium and no activity in the keratinized portion. The stromal elements were usually devoid of this enzymatic reaction. The normal oral squamous epithelium showed a moderate $\alpha$-glycerophosphate dehydrogenase activity in all the epithelial layers except for the superficial keratinized layer (Table 1).

Pleomorphic adenoma of salivary glands.—Nine cases of mixed tumor of salivary glands and pleomorphic adenoma in oral mucosae were examined. In general, the majority of adenomatous cells of pleomorphic adenoma showed a low enzymatic reaction—e.g., a low reaction in five of the nine cases and a moderate reaction in three cases. The normal gland in subepithelial tissue of the palate and the buccal region revealed a marked enzymatic activity in duct cells and a moderate to low activity in acini.

Ameloblastoma.—Seven cases of ameloblastoma were studied. A slight reaction was observed in three cases, and a moderate and high activity in two cases. The neoplastic epithelium of this tumor was positive for $\alpha$-glycerophosphate dehydrogenase and generally slightly positive in the stroma.

Stomach tumor.—Sixty-nine cases of gastric cancer consisted of 54 cases of adenocarcinoma and fifteen cases of undifferentiated cancer; six cases of gastric polyp and four cases of gastritis were also examined (Table 1). Most cases of differentiated adenocarcinoma of the stomach showed a moderate activity for $\alpha$-glycerophosphate dehydrogenase. In well differentiated adenocarcinoma the enzymatic activity was confined to the neoplastic epithelium (Figs. 7, 8, 14), and in its undifferentiated type the activity was variable in the neoplastic cells (Figs. 11, 12). Forty-seven per cent of the cases of stomach cancer (32 of 69 cases) showed a moderate activity for $\alpha$-glycerophosphate dehydrogenase. The surface epithelium of the stomach polyp was stained slightly for $\alpha$-glycerophosphate dehydrogenase reaction (Fig. 10).

Rectal cancer.—Ten cases of rectal adenocarcinoma and one case each of sigmoid cancer and malignant polyp were examined (Table 1). Malignant epithelia of rectal cancer were usually moderately reactive (Fig. 18).

Pancreatic cancer.—Two cases of adenocarcinoma in the pancreas showed a low and moderate enzymatic activity in the neoplastic epithelium.

Tumors of female genital organs.—Twenty-two cases of squamous-cell cancer in cervix uteri, one case of chorioepithelioma, three cases of ovarian adenocarcinoma, and one case of Kruckenberg's tumor were studied (Table 1). All the squamous-cell carcinomas contained a low to marked enzymatic reaction in the neoplastic epithelium; however, the surrounding necrotic areas in cancer foci had a somewhat higher activity than did peripheral cells. The activity of chorioepithelioma was low, that of ovarian cystoadenocarcinoma low, that of two cases of papillary adenocarcinoma high, and that of Kruckenberg's tumor moderate (Figs. 3, 4). The stromal elements of squamous-cell cancer in cervix uteri contained a trace and low enzymatic activity. The homologous squamous epithelium of the cervix exhibited a moderate enzymatic activity in the basal-cell layer to the parakeratotic layer, as

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**TABLE 1**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Total Cases</th>
<th>Degrees of activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermoid ca., mouth</td>
<td>18</td>
<td>± 1 2 3 4 0</td>
</tr>
<tr>
<td>Squamous ca., uterus</td>
<td>22</td>
<td>± 1 2 3 4 0</td>
</tr>
<tr>
<td>Adenoc., stomach</td>
<td>54</td>
<td>± 1 2 3 4 0</td>
</tr>
<tr>
<td>Undiff. ca., stomach</td>
<td>15</td>
<td>± 1 2 3 4 0</td>
</tr>
<tr>
<td>Polyp, stomach</td>
<td>6</td>
<td>± 1 2 3 4 0</td>
</tr>
<tr>
<td>Adenoc., rectum</td>
<td>12</td>
<td>± 1 2 3 4 0</td>
</tr>
<tr>
<td>Adenoc., breast</td>
<td>7</td>
<td>± 1 2 3 4 0</td>
</tr>
<tr>
<td>Adenoma and fibroadenoma,</td>
<td>28</td>
<td>± 1 2 3 4 0</td>
</tr>
<tr>
<td>breast</td>
<td></td>
<td>± 1 2 3 4 0</td>
</tr>
<tr>
<td>Adenoc., thyroid gl.</td>
<td>2</td>
<td>± 1 2 3 4 0</td>
</tr>
<tr>
<td>Struma nodosa</td>
<td>21</td>
<td>± 1 2 3 4 0</td>
</tr>
<tr>
<td>Pleomorphic adenoma, salivary gl.</td>
<td>9</td>
<td>± 1 2 3 4 0</td>
</tr>
<tr>
<td>Adenoc., pancreas</td>
<td>2</td>
<td>± 1 2 3 4 0</td>
</tr>
<tr>
<td>Adenoc., ovary</td>
<td>3</td>
<td>± 1 2 3 4 0</td>
</tr>
</tbody>
</table>

* Degrees of activity: —, negative; ±, trace; +1, slight; +2, moderate; +3, strong; +4, most intense.
observed in the normal oral epithelium. The distribution of α-glycerophosphate dehydrogenase in squamous-cell cancer was similar to the homologous non-neoplastic epithelium.

**Breast tumors.**—Seven cases of adenocarcinoma and 23 cases of the so-called mastopathy were studied (Table 1). α-Glycerophosphate dehydrogenase was significantly reduced in the neoplastic epithelium of both adenocarcinoma and adenoma of the breast (Fig. 1). It was moderate in amount in eleven out of 23 cases of adenosis and fibroadenosis. The stroma of breast tumors generally showed a low enzymatic activity in fibrous components in periductal and periacinous regions (Fig. 2).

**Thyroid tumors.**—Two cases of thyroid cancer and 21 cases of struma nodosa were studied (Table 1). Thyroid cancer was markedly reactive for α-glycerophosphate dehydrogenase in the malignant epithelium, 46 per cent of 21 cases of struma nodosa showed a low enzymatic activity, and many cases of goiter showed a slight to moderate activity in their epithelia.

**Tumors of the nervous system.**—Seven cases of meningioma, four cases of glioma, one case each of glioblastoma, neurinoma of the skin, astrocytoma, and ganglioma, and two cases of pinealoma were examined. In cases of meningioma, α-glycerophosphate dehydrogenase was slight in three cases, moderate in three cases, and strong in one case. Meningiofibroma showed a somewhat lower activity than multiform type. Of the four cases of glioma studied the enzymatic intensity was low and moderate in two cases. The enzymatic activity of astrocytoma was the highest; two cases of pinealoma and a case of ganglioma had moderate, a case of neurinoma had low, and a case of glioblastoma had a trace of activity (Fig. 6).

**Others.**—One case each of epithelial tumor, basalioma, and melanoma, and one case each of fibrosarcoma, liposarcoma, lymphosarcoma, and osteosarcoma were studied, as well as one case of adenocortical adenoma. The grade of enzymatic intensity of basalioma was moderate and that of melanoma low. The enzymatic intensity in mesenchymal malignant tumors was moderate in liposarcoma (Fig. 5), and in other sarcomas it was low.

**DISCUSSION**

α-Glycerophosphate dehydrogenase catalyzes the oxidation of 1-α-glycerophosphate to dihydroxyacetone phosphate, which constitutes an important intermediate for neutral fats and phospholipide synthesis (4). However, the biological significance of α-glycerophosphate dehydrogenase in tumor tissue has not included reference to histochemical studies of the enzymatic activity.

The data reported in the present study indicated that the malignant proliferating epithelium usually stained moderately for α-glycerophosphate dehydrogenase, with a suggestion that it would show a noncharacteristic distribution in tumor tissue. In squamous-cell carcinoma the enzymatic intensity showed little difference between high-keratinized epidermoid cancer developed in the oral cavity and non- or low-keratinized squamous-cell cancer in cervix uteri. Besides, the distribution pattern of the neoplastic epithelium of squamous-cell cancer was similar to that of the homologous non-neoplastic epithelium. It was assumed on the basis of the present report, as well as previous data (3), that the intensity and localization of α-glycerophosphate dehydrogenase in human squamous-cell cancer were nonspecific.

A histochemical demonstration of α-glycerophosphate dehydrogenase activity in the gastrointestinal tract was carried out by previous investigator (2) and that in carcinoma of the large intestine in man was reported by Wattenberg (6). Wattenberg reported that the enzymatic reactivity in normal colon mucosae exhibited a moderate intensity, that of the cells at the base of crypts being the highest and that of the surface epithelium slight. Both poorly and well differentiated carcinoma cells in the colon showed a weak activity for this enzyme, and a considerably higher proportion of poorly differentiated neoplastic cells exhibited an intense staining. In the present study the enzymatic activity of both well differentiated adenocarcinoma and undifferentiated cancer in the human stomach was moderate in 81 cases of 67 cases; ten cases of twelve rectum cancers showed a moderate to high enzymatic activity. The enzymatic intensity for all gastrointestinal cancer cases was moderate in the malignant and polyp epithelia. However, the highest staining in the neoplastic epithelium was observed in a few cases in stomach and rectum specimens. The significance of the highest staining in gastrointestinal adenocarcinoma has not been clarified. Furthermore, it must be admitted that those cases with the most intense activity were not always so advanced in malignancy. Possibly, those differences indicated in the enzymatic intensity may be related to quite different primary cells in the gastrointestinal tract.

Studies on normal salivary glands indicated that most of the oxidative and reducing enzymes were present in the duct system, with a high to the highest staining reaction (9). In the present study, pleomorphic adenoma which developed from minor salivary glands in oral mucosae showed a varying
intensity for α-glycerophosphate dehydrogenase. In general, the minor salivary gland present in the connective tissue under the oral epithelium was the so-called mucous gland, and a dehydrogenase was usually localized in the basal layer in acini with a relatively low grade of activity. Further studies for α-glycerophosphate dehydrogenase and other oxidative enzymes in mixed tumors of the salivary glands seem to be necessary.

An enzymatic histochemical study of dehydrogenase in the normal nervous system was carried out in detail by Thomas and Pearse (5). They reported that nerve cells exhibited slight enzymatic activity but that the surrounding gray matter showed a high activity, especially in glial cells. An astrocytoma group showed a slightly higher activity for α-glycerophosphate dehydrogenase than did a fibrous type in brain tumor. The finding that astrocytic cells showed more activity than other cells such as fibrous elements is assumed to be related to structural elements in nerve cells. At any rate, the significance of oxidative enzymes has not been fully clarified in normal nerve cells or in brain tumor cells. It should be approached by biological patterns in the normal nervous system in reference to tumors of this system.

REFERENCES
FIG. 7.—Adenocarcinoma of the stomach. In the lower part tumor cells showed the highest activity, whereas in the upper part the normal glands were slightly stained. X40.

FIG. 8.—Higher magnification of Figure 7. Stromal elements reacted weakly for a-glycerophosphate dehydrogenase. X400.

FIG. 9.—Adenocarcinoma of the stomach. Neoplastic epithelium exhibited low enzymatic activity in contrast with Figure 7. X40.

FIG. 10.—Stomach polyp. Weak activity was found in the epithelium of polyp. X200.
FIG. 11.—Undifferentiated carcinoma of the stomach. Low enzymatic activity was found in neoplastic cells, while large cells scattered among them revealed a comparatively strong activity. \( \times400 \).

FIG. 12.—Undifferentiated adenocarcinoma of the stomach. Malignant tumor cells showed intense activity. \( \times400 \).

FIG. 13.—Adenocarcinoma of the rectum. \( \alpha \)-Glycerophosphate dehydrogenase activity in the neoplastic epithelium was weaker than that of the homologous normal tissue. \( \times40 \).

FIG. 14.—\( \alpha \)-Glycerophosphate dehydrogenase activity in cystoadenocarcinoma of the stomach. \( \times200 \).
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