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Distribution of Lysozyme, a_1-Antichymotrypsin and a_1-Antitrypsin in Adenocarcinomas of the Stomach and Large Intestine

An Immunohistochemical Study

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Summary. Lysozyme, a_1-Antichymotrypsin and a_1-Antitrypsin were demonstrated by an immunoperoxidase technique (PAP) in malignant cells of adenocarcinomas of the stomach but not of the large intestine. Lymph-node metastases showed identical immunoreactivity to that of the primary tumour. Neoplasms arising from the cardia, the body and the pyloric antrum of the stomach showed different immunostaining reactions. It seems that these differences partly reflect the distribution of lysozyme, a_1-Antichymotrypsin and a_1-Antitrypsin in the normal gastric mucosa. The usefulness of our findings in the identification of the primary tumour in cases of lymph node metastases of unknown origin, is also discussed.

Key words: Gastrointestinal tumours – Lysozyme – a_1-Antichymotrypsin – a_1-Antitrypsin

a_1-Antitrypsin (a_1AT) and a_1-Antichymotrypsin (a_1AChy) have been recently demonstrated immunohistochemically in various epithelial cells of the gastrointestinal tract (G.I.T.) (Geboes et al. 1982; Kittas et al. 1982). The distribution of lysozyme in the G.I.T. has been also previously studied (Mason and Taylor 1975; Klockars and Reitamo 1975; Montero and Erlandsen 1978). Moreover, lysozyme has been found immunohistochemically in an adenocarcinoma of the stomach and has been considered to be a marker for malignant Paneth cells (Heitz and Wegmann 1980). To our knowledge there is no report in the literature concerning the presence of a_1AT and a_1AChy in adenocarcinomas of the G.I.T.

The objective of this study was to demonstrate with an immunoperoxidase technique the presence of a_1AT, a_1AChy and lysozyme within tumour cells in patients with adenocarcinomas of the stomach and large intestine,

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and to evaluate the usefulness of our findings in the field of gastrointestinal tumour markers.

Material and Methods

We studied formalin fixed, paraffin embedded tissue from 20 patients with adenocarcinomas of the stomach and 15 patients with adenocarcinomas of various parts of the large intestine. Four of the tested gastric tumours had arisen in the cardia, 6 in the body and 10 in the pyloric antrum of the stomach. On the basis of histological characteristics these tumours were classified as intestinal or diffuse type according to Lauren (1965). The predominant pattern was essential in the classification of tumours showing features of both histological types. Invasiveness of the malignant neoplasms was assessed in Haematoxylin and Eosin stained sections. Infiltrated lymph-nodes were found in 12 cases of gastric and in 9 cases of intestinal carcinomas and they were included in the immunohistochemical study.

The presence of lysozyme, a_1AChy and a_1AT was demonstrated in 5 μm paraffin sections with the peroxidase-antiperoxidase (PAP) technique of Sternberger et al. (1970) modified by Taylor (1974).

To block endogenous peroxidase activity prior to immunostaining, the sections were treated with a solution of 0.3% H_2O_2 in methanol. Background staining was reduced by flooding the sections with normal swine serum. Lysozyme, a_1AChy and a_1AT rabbit antisera were obtained from Dakopatts (Copenhagen, Denmark). They were applied on the sections at a dilution of 1:200 for lysozyme and 1:100 for a_1AChy and a_1AT. Swine anti-rabbit serum and the PAP complex were also obtained from Dakopatts and they were subsequently used at dilutions of 1:40 and 1:60 respectively. The histochemical reaction for peroxidase was carried out using a freshly prepared 3,3-diaminobenzidine tetrahydrochloride solution (6 mg of DAB with 0,01% H_2O_2 in 10 ml of tris buffer). All sections were counterstained with haematoxylin. Substitution of nonimmune rabbit serum for the lysozyme, a_1AChy and a_1AT antisera, and a slide treated with DAB reagent alone, served as controls.

Results

The immunohistological distribution of lysozyme, a_1AChy and a_1AT in gastric adenocarcinomas is depicted in Table 1.

Lysozyme was demonstrated as brown deposits in the cytoplasm of the malignant cells of 1 intestinal and 2 diffuse types of carcinoma of the cardia (Fig. 1), while the remaining case was lysozyme-negative. There was no positive staining for both a_1AChy and a_1AT in any of the four tested cases of cardiac adenocarcinomas.

Table 1. Staining of adenocarcinomas of the stomach for Lysozyme, a_1AChy, and a_1AT, by an immunoperoxidase technique

Site of tumour	Histological type	No. of tested cases	No. of Ly(+) cases	No. of a_1AChy(+) cases	No. of a_1AT(+) cases
Cardia	Intestinal	2	1	–	–
	Diffuse	2	2	–	–
Body	Intestinal	3	2	1	–
	Diffuse	3	2	1	–
Antrum	Intestinal	5	2	4	3
	Diffuse	5	1	3	2
Total		20	10	9	5

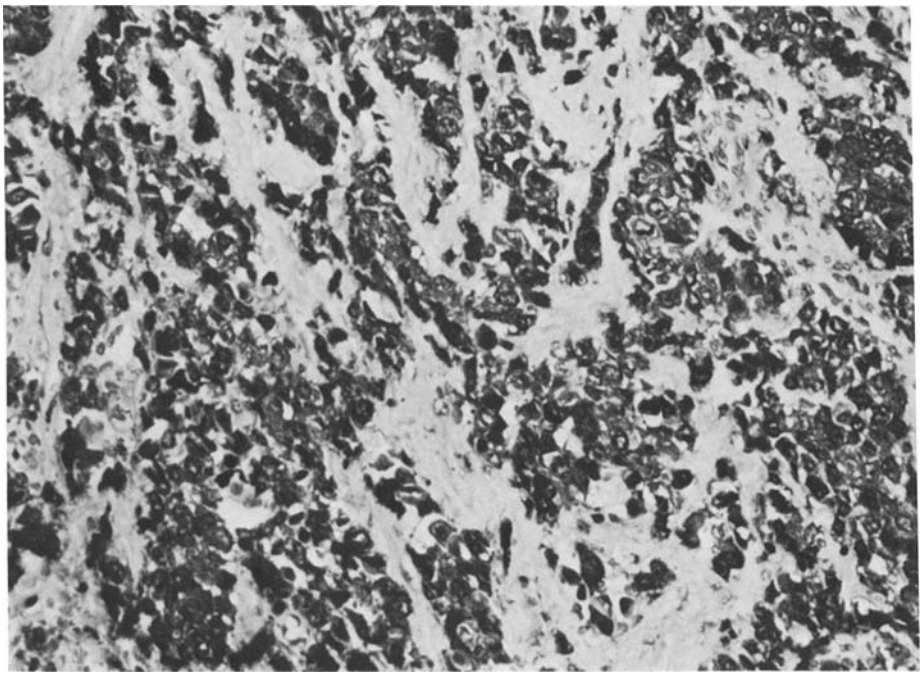

Fig. 1. Diffuse type of adenocarcinoma arising in the cardiac area of the stomach. Malignant cells positive for lysozyme. Peroxidase-antiperoxidase technique (PAP) Magn. ×272

In the tumours from the body of the stomach, 2 intestinal and 2 diffuse types of adenocarcinomas showed malignant cells positive for lysozyme while the other two cases, one from each histological type, contained lysozyme-negative and a_1AChy-positive malignant cells (Fig. 2). All these tumours were a_1AT-negative.

The immunostaining of the malignant cells of the antral tumours was more complicated. One intestinal and two diffuse adenocarcinomas were completely negative for all the tested enzymes. Two intestinal and one diffuse adenocarcinomas showed areas positive for lysozyme and areas positive for a_1AChy and a_1AT (Fig. 3). The remaining cases were lysozyme-negative and either a_1AChy, a_1AT-positive or only a_1AChy-positive.

As far as the histological pattern of the tumours was concerned, the intestinal types appeared to show more intense staining for all enzymes than the diffuse ones. However, there was no definite correlation between the immunohistochemical reaction and invasiveness of the tumour.

The immunostaining of the gastric mucosa adjacent to carcinoma depended on the existence or absence of dysplastic areas in the material studied. In those fields where the gastric mucosa appeared morphologically normal, a_1AChy and a_1AT were only found in cells of deeper pyloric glands which were occasionaly seen in the sections (Fig. 4). Additionally, lysozyme was immunohistochemically demonstrated not only in normal pyloric but also in cardiac glands. In some cases the presence of these enzymes in normal appearing mucosa was independent of their existence in the adjacent

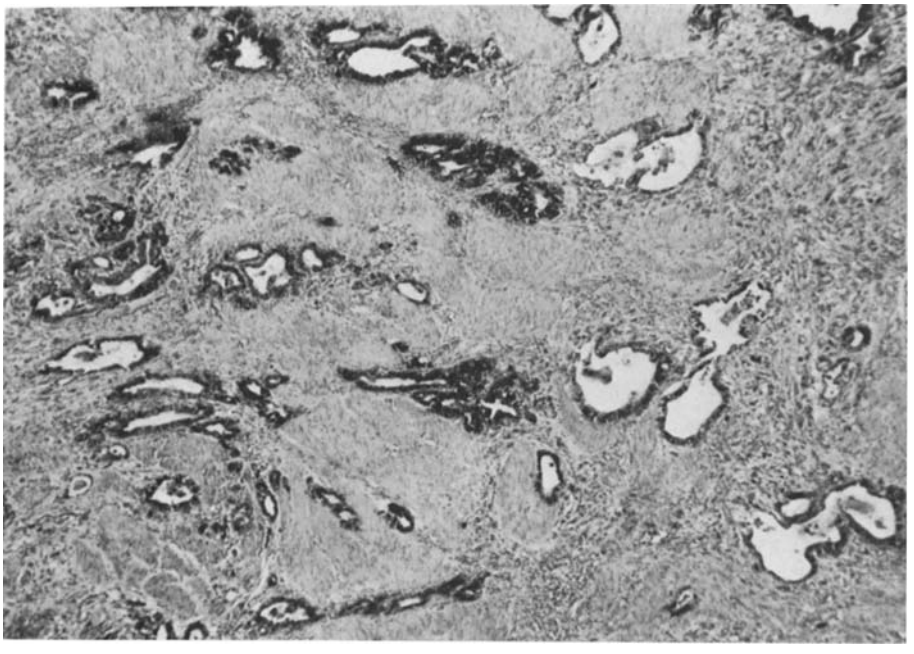

Fig. 2. Intestinal type of adenocarcinoma of the body of the stomach. Numerous malignant cells positive for a_1AChy. PAP ×42

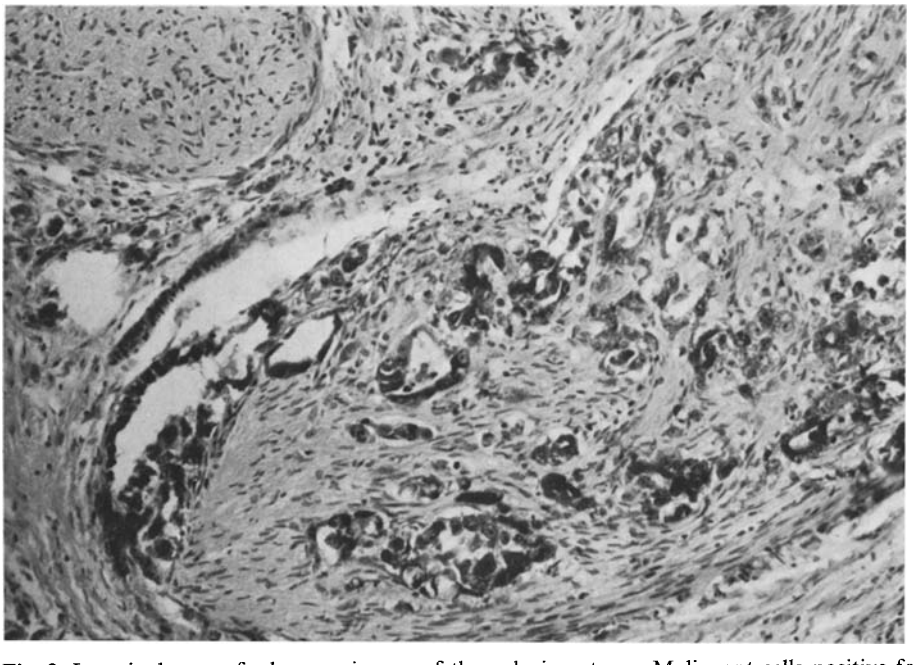

Fig. 3. Intestinal type of adenocarcinoma of the pyloric antrum. Malignant cells positive for a_1AT invading a nerve. PAP ×136

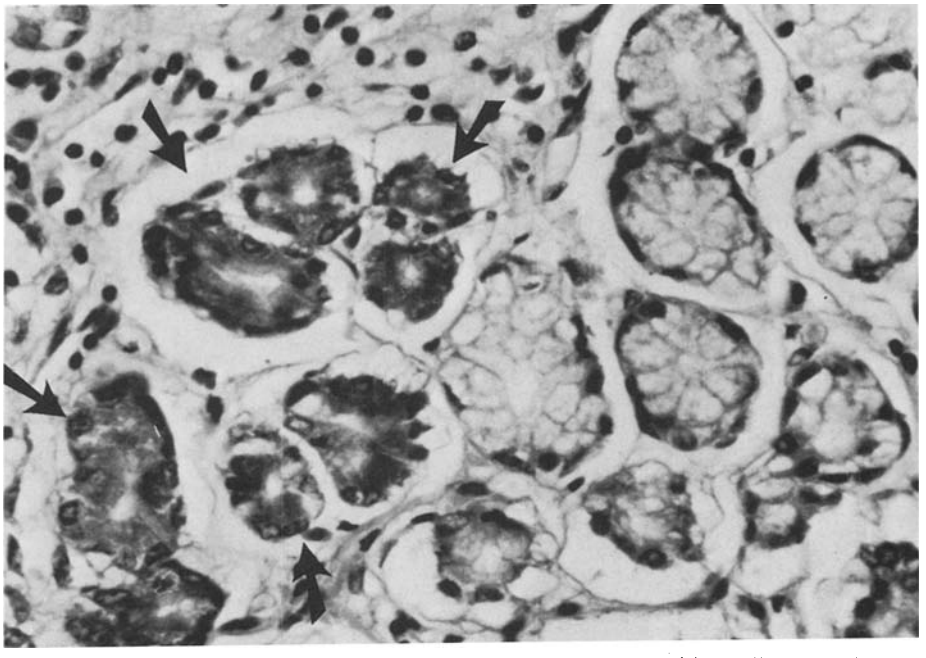

Fig. 4. Normal-appearing deeper pyloric glands. Several a_1AT-positive cells (*arrows*). PAP ×360

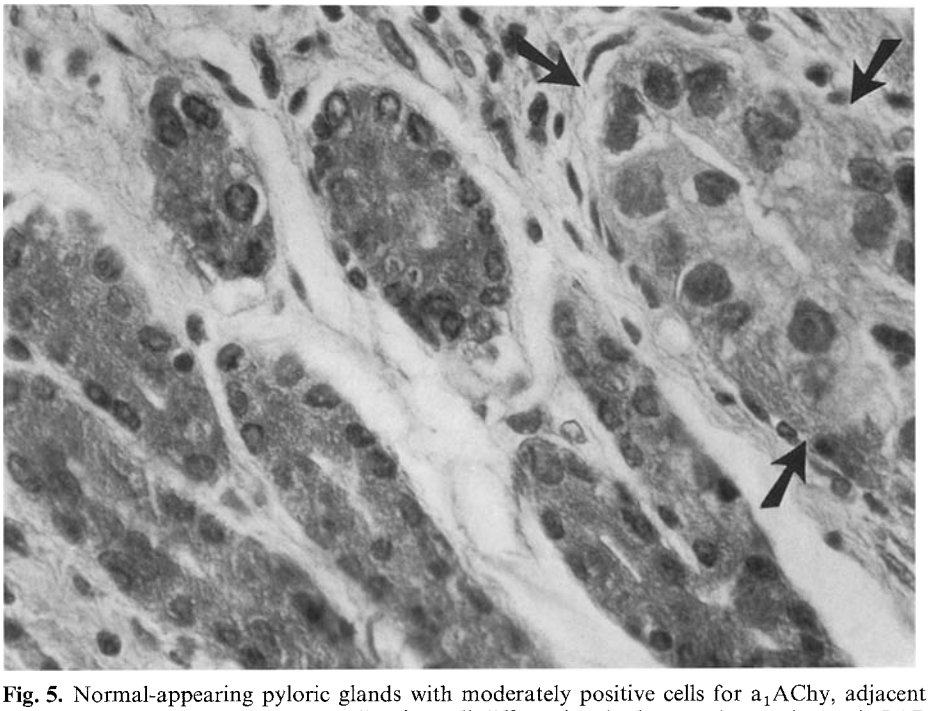

Fig. 5. Normal-appearing pyloric glands with moderately positive cells for a_1AChy, adjacent to completely negative malignant cells of a well differentiated adenocarcinoma (*arrows*). PAP ×400

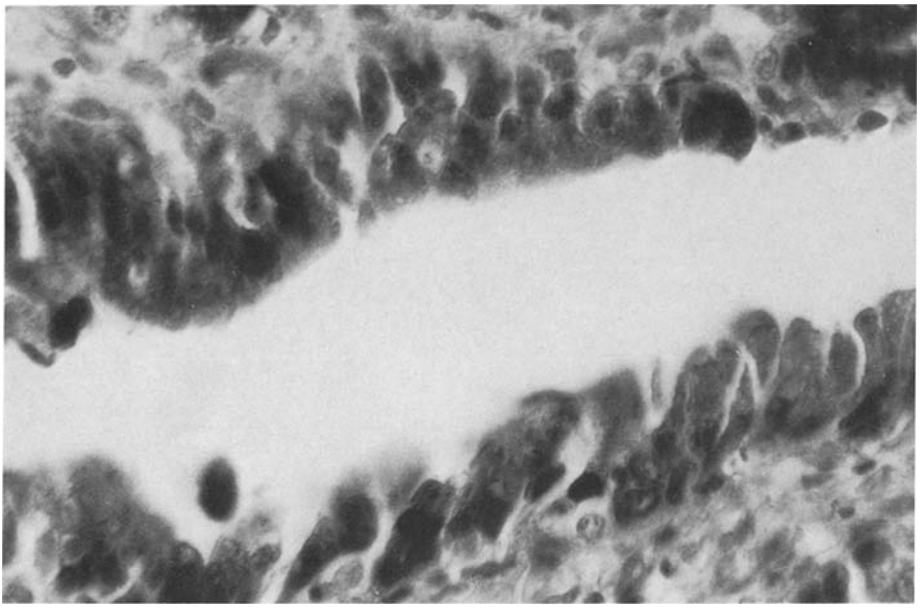

Fig. 6. The same case as in Fig. 3. Severely dysplastic epithelial cells of a gastric pit. Strongly positive cells for a_1AT. PAP ×1,000

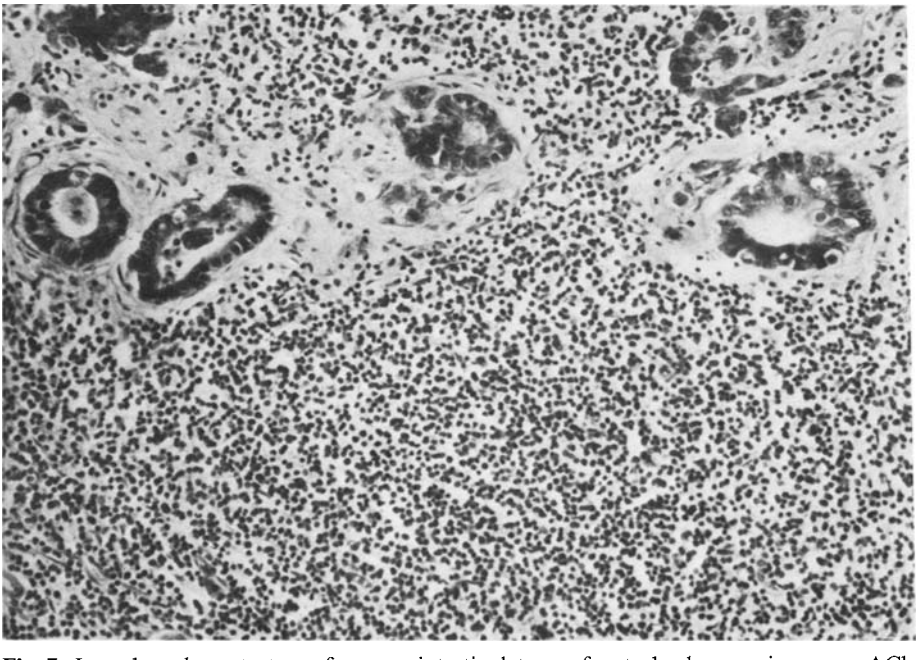

Fig. 7. Lymph-node metastases from an intestinal type of antral adenocarcinoma. a_1AChy positive malignant cells. PAP ×170

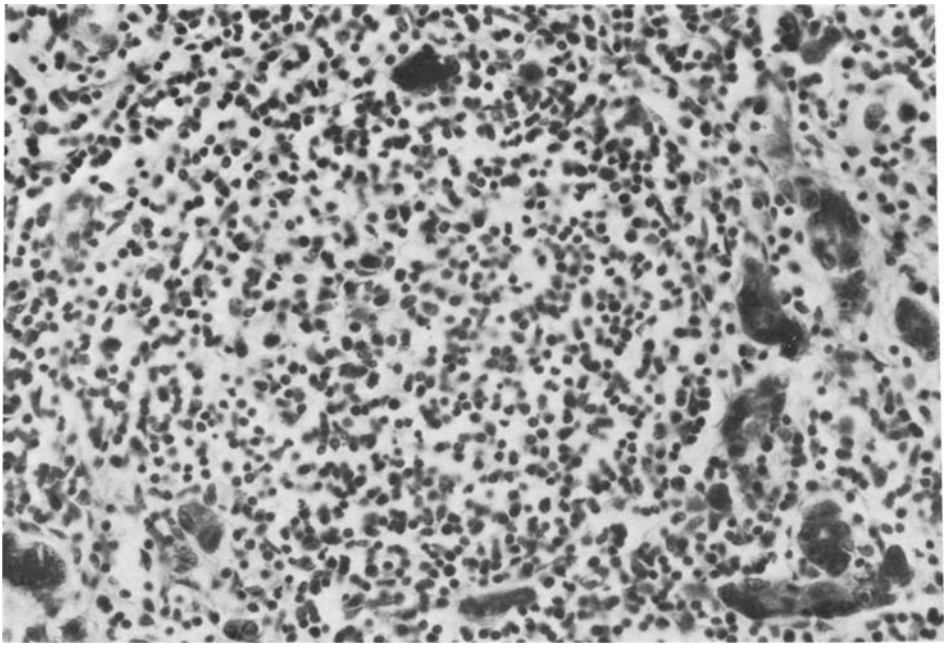

Fig. 8. Lymph-node metastases from a diffuse type of cardiac adenocarcinoma. Lysozyme-positive malignant cells. PAP × 340

malignant tissue. Thus, morphologically normal pyloric glands with positive cells for one of the tested substances could be seen in the vicinity of completely negative malignant cells (Fig. 5). On contrast, the immunostaining reaction of severely dysplastic areas was always similar to that of the adjacent tumour (Fig. 6). The latter observation also applied in intestinal-like metaplastic epithelium bordering adenocarcinomas of the stomach but only if severely dysplastic cells were present. Otherwhise the metaplastic epithelium was negative for all the tested enzymes apart of occasionally seen, normal-appearing, lysozyme-positive Paneth cells.

All lymph-node metastases showed identical staining to that of the primary tumour (Figs. 7 and 8).

All adenocarcinomas of the large intestine and their lymph-node metastases were completely negative for lysozyme, a_1AChy and a_1AT.

Discussion

It has been previously shown that lysozyme is localized in Paneth cells throughout the G.I.T., in cells of the cardiac and pyloric glands of the stomach and in epithelial cells of the Brünner glands of the duodenum (Klockars and Reitamo 1975; Junqueira and Carneiro 1980). Additionally, a_1AChy and a_1AT have been demonstrated in gastric-pyloric and duodenal-Brünner glands as well as in absorptive epithelial cells of the small intestine,

while they are absent from any epithelial cell of the large intestine (Geboes et al. 1982; Kittas et al. 1982).

These observations are in agreement with the findings of the present study concerning the morphologically normal mucosa adjacent to gastric adenocarcinomas. Thus, we have immunohistochemically demonstrated a_1AChy, a_1AT and lysozyme in normal pyloric glands while lysozyme alone has been detected in normal appearing cardiac glands.

We have also found that lysozyme, a_1AChy and a_1AT can be identified by an immunoperoxidase technique in malignant cells of adenocarcinomas of the stomach but not of the large intestine. Another interesting observation is that severely dysplastic areas bordering adenocarcinomas show identical immunohistochemical reaction to that of the invasive tumours. This finding supports the close histogenetic relation of severe dysplasia and invasive adenocarcinoma of the stomach.

In an analysis of all immunoperoxidase-positive tumours in respect to their site of origin, it appears that the immunohistochemical profile of adenocarcinomas partly reflects the distribution of a_1AChy, a_1AT and lysozyme in the normal gastrointestinal epithelium. Thus, a_1AChy and a_1AT have been found in our study in carcinomas of the antrum, where those substances are also known to be present in normal glands. In contrast, they have not been detected in malignant tumours of the cardia of the stomach and the various parts of the large intestine, where a_1AChy and a_1AT are known to be absent from the normal mucosa. Moreover in the present work, lysozyme has been demonstrated in tumours of the gastric cardia and antrum, areas in which epithelial cells of normal glands are known to contain this substance. Since lysozyme has been previously occasionally seen in Paneth cells of normal large intestinal mucosa and neoplastic Paneth cells have been found in colorectal carcinomas (Sandow and Whitehead 1979; Shousha 1979), a positive-immunohistochemical reaction for this enzyme was expected to be seen in, at least, some of the tested large-intestinal adenocarcinomas of our study. Lysozyme has been previously found by others in an adenocarcinoma of the stomach, rich in neoplastic Paneth cells (Heitz and Wegmann 1980). However, we have failed to demonstrate lysozyme in malignant cells of any of our fifteen cases of large-intestinal adenocarcinomas. It is possible that the percentage of neoplastic Paneth cell-containing tumours of the large intestine is very low.

As an exception to the above stated observations, we have seen malignant cells positive for lysozyme and a_1AChy in tumours of the body of the stomach. These enzymes and a_1AT are known to be absent from the normal mucosa of this area of the stomach.

Considering the theory of Soga et al. (1971) that all gastric neoplasms derive from a common progenitor, we may offer an explanation for the immunostaining reaction of malignant cells in relation to the region of development of the tumours of our study. It is possible that the neoplastic cells may have originated from a more primitive progenitor cell which is capable of differentiation under the influence of an unknown stimulus, towards cellular types with characteristics of the cells of the region of origin.

We have also found that in all tested cases of adenocarcinomas either of the stomach or the large intestine, the lymph-node metastases showed identical immunoreactivity to that of the primary tumour. Since large intestinal tumours are completely negative for a_1AChy and a_1AT, we suggest that the immunohistochemical demonstration of these enzymes in sections from lymph node metastases of an adenocarcinoma of unknown origin, indicates a gastric rather than intestinal and possible extra-intestinal primary tumour.

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