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Formaldehyde-Ozone-Induced Fluorescence in Gastrin-Producing Tumours

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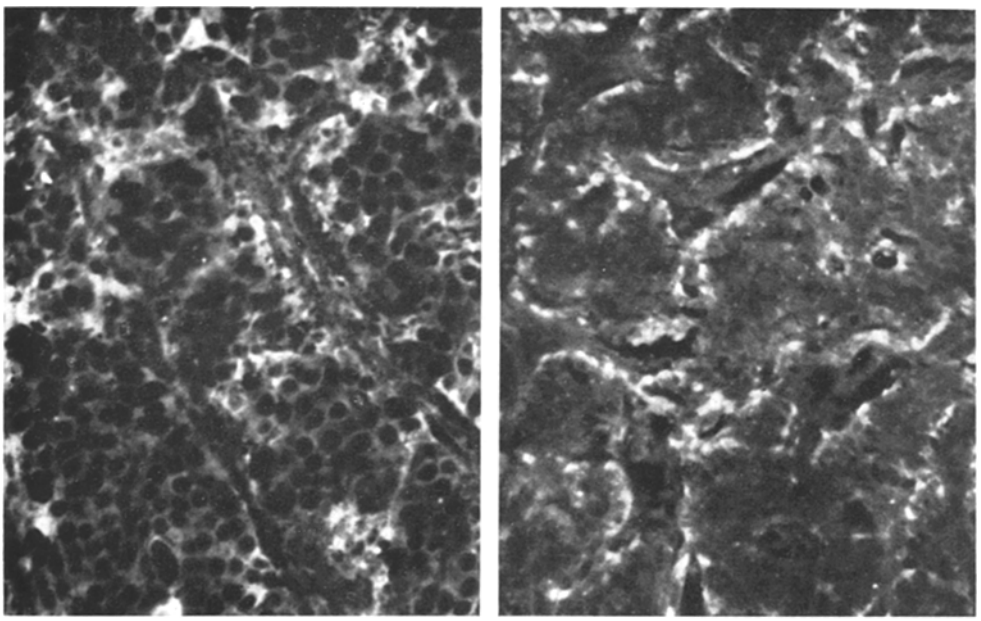
Summary. Material from eight peptide hormone-secreting tumours, extirpated from the pancreas or from the antrum-duodenum region, was examined. Four of the patients had the clinical manifestations of the Zollinger-Ellison syndrome, two showed the features of an insulin-secreting tumour and one had a glucagonoma. Gastrin-producing cells, identified by immunohistochemistry, were found in five of the tumours. These cells displayed a varying degree of formaldehyde-ozone-induced fluorescence. This agrees with previous observations on the gastrin cell of human antral and duodenal mucosa. From model experiments, formaldehyde-ozone-induced fluorescence is thought to reflect the presence of peptides having tryptophan in the NH_2-terminal position. The nature of this peptide in gastrin-producing cells is unknown.

Introduction

Recently, the gastrin cell of the human antropyloric and duodenal mucosa was found to emit intense formaldehyde-ozone-induced fluorescence (Larsson, Håkanson, Sjöberg and Sundler, 1975b). The pancreatic A_1 cells, which have been claimed to store gastrin (Lomsky, Langr and Vortel, 1969; Greider and McGuigan, 1971; Polak, Stagg and Pearse, 1972), did not display such fluorescence (Larsson *et al.*, 1975b). In contrast, the glucagon-producing A_2 cells gave intense fluorescence. From histochemical model experiments it has been suggested that formaldehyde-ozone-induced fluorescence (excitation max. 370, emission max. 510 nm) indicates the presence of peptides with NH_2-terminal tryptophan (Håkanson and Sundler, 1971a, b). It seemed of interest to examine gastrin- and glucagon-producing tumours for formaldehyde-ozone-induced fluorescence. Peptide hormone-producing tumours from eight patients were investigated. Four of these had the clinical manifestations of the Zollinger-Ellison syndrome, two showed the features of an insulin-secreting tumour, and one had a glucagon-secreting tumour.

Materials and Methods

All tumour material was obtained at surgery (with the exception of Cases No. 7 and 8; autopsy), fixed in 10% neutral formalin for varying lengths of time and embedded in paraffin. The paraffin-embedded tumour material was obtained from hospitals in Gothenburg (Sweden), Pavia (Italy) and Uppsala (Sweden). Of the eight cases presented No. 1, 3 and 5 have previously been described immunohistochemically (Larsson, Grimelius, Håkanson, Rehfeld, Stadil, Holst, Angervall and Sundler, 1975a; Cases No. 2, 1 and 5, respectively). Sections were cut at 5 μ and deparaffinized whereafter some were treated with formaldehyde gas in the presence



a b

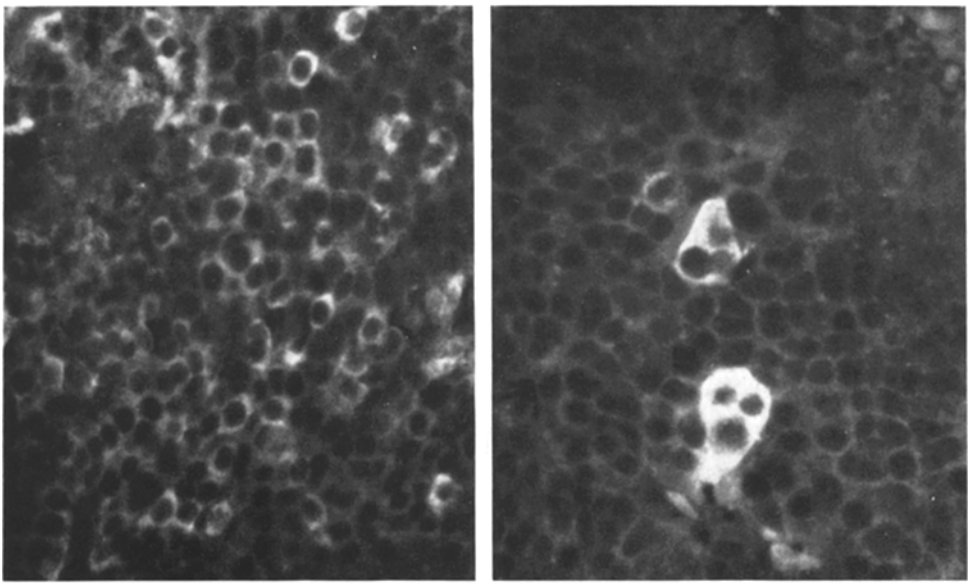
Fig. 1a and b. Case No. 1. Sections from a lymph node metastasis. a) Gastrin immunofluorescence of cells at the marginal parts of the tumour trabecules. (× 250). b) Formaldehyde-ozone-induced fluorescence with a similar localization. (× 150)

of ozone as described in detail elsewhere (Håkanson and Sundler, 1971a) and others were subjected to an indirect immunofluorescence method for the demonstration of gastrin, insulin, glucagon or ACTH (for details see Larsson *et al.*, 1975a). The sections were examined in a Leitz Orthoplan fluorescence microscope equipped with a Ploem illumination system. The formaldehyde-ozone-induced fluorescence was analyzed microspectrofluorometrically as described by Björklund, Ehinger and Falck (1968).

Results

Case No. 1. Woman, 24 years, with symptoms of hypergastrinemia, such as gastric hypersecretion and recurrent duodenal ulcers. At surgery (1972) no tumour could be found in the pancreas. Several enlarged peripancreatic lymph nodes were removed and a total gastrectomy was performed. One lymph node metastasis showed the growth pattern of an islet cell tumour. Moderate gastrin immunofluorescence was observed in some tumour cells (Fig. 1a), with a characteristic localization to the margin of the tumour trabecules. Formaldehyde-ozone-treatment induced moderate to strong fluorescence (370/510 nm) in some marginally located tumour cells (Fig. 1b). The tumour cells did not display insulin, glucagon or ACTH immunofluorescence.

Case No. 2. Woman, 44 years, with perforated peptic ulcer. At surgery (1968) a large tumour in the pancreatic head was removed together with several liver metastases. A fair number of tumour cells showed gastrin immunofluorescence;



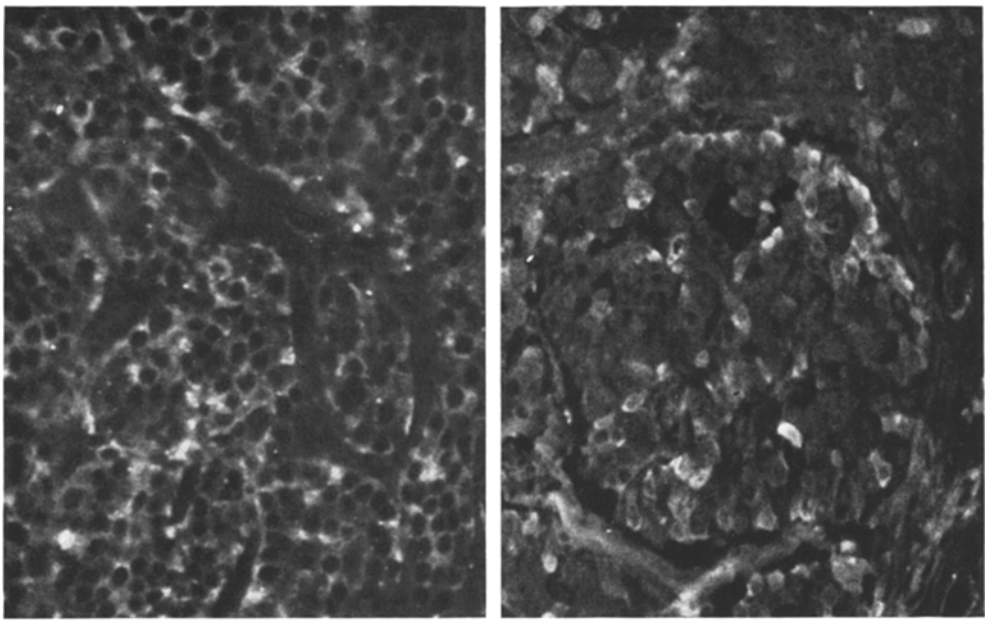
a b

Fig. 2a and b. Case No. 2. Sections from hepatic metastasis. a) Gastrin immunofluorescence of low intensity in cells located centrally in a tumour nodule. b) Intense gastrin immunofluorescence in cells that give the impression of being multinucleated. (× 400)

those situated at the margins of the tumour trabecules showed strong immunofluorescence, whereas the more centrally situated cells displayed weak to moderate immunofluorescence (Fig. 2a). Occasionally tumour cells with multinuclear appearance showing strong immunofluorescence were encountered (Fig. 2b). Formaldehyde-ozone treatment induced typical fluorescence of moderate intensity in the tumour cells, particularly in those situated at the margins of the tumour trabecules. No cells giving glucagon, insulin or ACTH immunofluorescence were observed. The metastases were not examined by histochemistry.

Case No. 3. Woman, 31 years, having the Zollinger-Ellison syndrome and a pancreatic tumour (removed 1961), found to contain gastrin bioactivity (Angervall, Dotevall, Lehmann and Norberg, 1963). There was weak to moderate gastrin immunofluorescence in numerous tumour cells. As noted in the previous cases, the cells showing strong immunofluorescence were situated in the peripheral parts of the tumour nodules and trabecules. A few, scattered cells displayed glucagon immunofluorescence. Weak insulin immunoreactivity was found in a number of cells, predominantly located at the tumour periphery. In the peripheral parts of the tumour a few scattered cells showed intense ACTH immunofluorescence. Formaldehyde-ozone-treatment induced weak fluorescence chiefly in cells located at the periphery of the tumour nodules with a localization corresponding to that of the gastrin cells.

Case No. 4. Woman, 36 years, with gastric hypersecretion and recurrent peptic ulcers. Extensive gastric resection in two seances. A small tumour was



a b

contain peripherally located cells showing weak to moderate gastrin immunofluorescence. (× 400). b) Weak formaldehyde-ozone-induced fluorescence in similarly located cells. (× 350)

found in the duodenal submucosa. Histologically, it displayed the features of an endocrine pancreatic tumour. No tumour cells showed glucagon or insulin immunofluorescence, whereas a large number of cells situated at the periphery of the tumour trabecules gave strong gastrin immunofluorescence. Formaldehyde-ozone-treatment induced weak to moderate fluorescence in numerous cells in the same location.

Case No. 5. Male, 52 years, with symptoms of an insulin-secreting tumour. At surgery (1970) a pancreatic tumour was removed together with several liver metastases. The tumour was predominantly scirrhous with areas of necrosis. As evidenced by immunohistochemistry it consisted of a mixture of gastrin, insulin and glucagon cells. The glucagon cells, which exhibited intense immunofluorescence, were very numerous. The gastrin cells were less numerous and gave weak to moderate immunofluorescence. The insulin cells, which displayed moderate immunofluorescence, were few in number and were concentrated to a peripheral portion of the tumour. The tumour displayed a high degree of autofluorescence, which made it difficult to evaluate if formaldehyde-ozone-induced fluorescence was present or not. A liver metastasis showed a less scirrhous growth pattern. The majority of the cells showed intense gastrin immunofluorescence but a few displayed only weak immunofluorescence. A fair number of cells showed moderate to strong glucagon immunofluorescence. Insulin immunofluorescence was absent.

Many of the cells in the metastasis showed strong formaldehyde-ozone-induced fluorescence.

Case No. 6. Woman, 61 years, with symptoms of an insulin-secreting tumour. At surgery (1971) a pancreatic tumour was removed. Immunohistochemical examination of the tumour revealed moderate insulin immunofluorescence in the majority of cells. There was no gastrin or glucagon immunofluorescence. ACTH-immunofluorescence was not tested. No formaldehyde-ozone-induced fluorescence was observed.

Case No. 7. Woman, 62 years, with a pancreatic insuloma (autopsy 1968) as well as metastases in the liver and in the regional lymph nodes. The tumour was found to contain large amounts of glucagon measured by radioimmunoassay. This case has been described previously (Grimelius, Petersson, Lundquist, Dahlgren and Parrow, 1971; Larsson, Sundler, Grimelius, Håkanson and Holst, 1973a). No gastrin- or insulin-immunofluorescence was observed. ACTH immunofluorescence was not tested. Nearly all cells of the tumour and metastases displayed intense glucagon immunofluorescence. No formaldehyde-ozone-induced fluorescence was observed.

Case No. 8. Male, 65 years, with gastric carcinoma. At autopsy (1974) a small nodule was found in the duodenal submucosa, having the histological appearance of pancreatic endocrine tumours. Many cells of this nodule showed fairly intense gastrin immunofluorescence; no cells displayed glucagon or insulin immunofluorescence. The majority of the tumour cells showed strong formaldehyde-ozone-induced fluorescence.

Discussion

Gastrin-producing tumour cells of pancreatic as well as gut origin were found to display a varying degree of formaldehyde-ozone-induced fluorescence. This agrees with previous observations on the gastrin cell of human antral and duodenal mucosa (Larsson, Sundler, Håkanson, Grimelius, Rehfeld, and Stadil, 1974b; Larsson, Håkanson, Sjöberg and Sundler, 1975b). It has been suggested that the pancreatic A_1 cells represent the parent cell of pancreatic gastrinomas (Thiery and Bader, 1966; Cavallero, Solcia and Sampietro, 1967). However, it should be noted that the A_1 cells of human pancreas do not display formaldehyde-ozone-induced fluorescence (Larsson *et al.*, 1975b). Other workers have expressed doubts as to whether pancreatic gastrinomas were indeed A_1 cell neoplasms, based on their ultrastructural appearance and on the fact that many of them fail to stain with the Hellerström-Hellman silver impregnation method (Creutzfeldt, Creutzfeldt and Perings, 1969).

In human pancreas, the glucagon-producing A_2 cells exhibit formaldehyde-ozone-induced fluorescence (Larsson *et al.*, 1975b). It could therefore be expected that tumours, totally or partly composed of glucagon-producing cells, should display such fluorescence. However, in the cases investigated the glucagon-producing tumour cells showed only very weak or no formaldehyde-ozone-induced fluorescence.

The present results show that the previously demonstrated association between gastrin immunoreactivity and formaldehyde-ozone-induced fluorescence

(Larsson, Sundler, Håkanson, Rehfeld and Stadil, 1973b; Larsson *et al.*, 1975b, 1974a, b) is a feature also of gastrin-producing tumour cells. From model experiments, formaldehyde-ozone-induced fluorescence is thought to reflect the presence of peptides having tryptophan in the NH_2-terminal position (Håkanson and Sundler, 1971a and b). The nature of this peptide in gastrin-producing cells is unknown.

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