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Multihormonal, Amyloid-Producing Tumour of the Islets of Langerhans in a Twelve Year Old Boy

Clinical, Morphological and Biochemical Data and Review of the Literature

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Summary. Clinical, pathological and biochemical data from a twelve year old boy with hypoglycaemic attacks and hyperinsulinism are presented. A pancreatic islet cell adenoma was diagnosed by ultrasonic scanning and enucleated from the head of the pancreas. Histological study revealed a mixed cellular population consisting of A_1- and B-cells. Large amounts of extravascular amyloid deposited were found between the cell strands. The tumour extract produced a prompt elevation of gastric acid secretion in the rat gastrin bio-assay. It is concluded that this islet cell tumour was a combined gastrinoma-insulinoma. The possible pathogenesis of this variety of the multiple endocrine adenoma syndrome is discussed. Monocellular-multihormonal islet cell tumours might arise from biochemically multipotent endocrine stem cells, whereas the multicellular-multihormonal type could either be a regulatory neoplasia or be derived from different neuroendocrine cells.

Zusammenfassung. Bei einem übergewichtigen 12jährigen Knaben mit Hypoglykämieanfällen und erhöhtem Blutinsulinspiegel konnte durch Ultraschall ein Tumor des Pankreaskopfes dargestellt werden. Das exstirpierte Gewebe erwies sich histologisch als ein aus A_1- und B-Zellen aufgebautes Inselzelladenom mit ausgedehnten extravasculären Amyloidablagerungen. Ein Extrakt aus dem Tumorgewebe bewirkte eine sofortige Zunahme der Magensäuresekretion am perfundierten Rattenmagen. Diagnose: Insulinom-Gastrinom. Monocellulär-plurihormonelle Inselzelltumoren leiten sich möglicherweise von biochemisch pluripotenten Stammzellen der Langerhansschen Inseln her. Pluricellulär-plurihormonelle Adenome sind hingegen eher als Regulationsgeschwülste aufzufassen, könnten aber auch von verschiedenen neuroendokrinen Zellen abstammen.

Introduction

Among hormone producing tumours those of the islet cells of the pancreas are of special interest. The most common type is the insulinoma, generally easily diagnosed by the presence of the typical Whipple's triad. The less frequent gastrin producing tumours are closely connected to the syndrome of Zollinger-Ellison (Zollinger and Ellison, 1955; Gregory, Tracy, Agarwal and Grossman, 1969; McGuigan and Trudeau, 1968; Stremple and Meade, 1968). Glucagonomas are rare (Longmire, Brown, Buckberg, Cooke, Glober, Hanafee, Matsumoto, Plested, Rochlin and Wilkerson, 1968).

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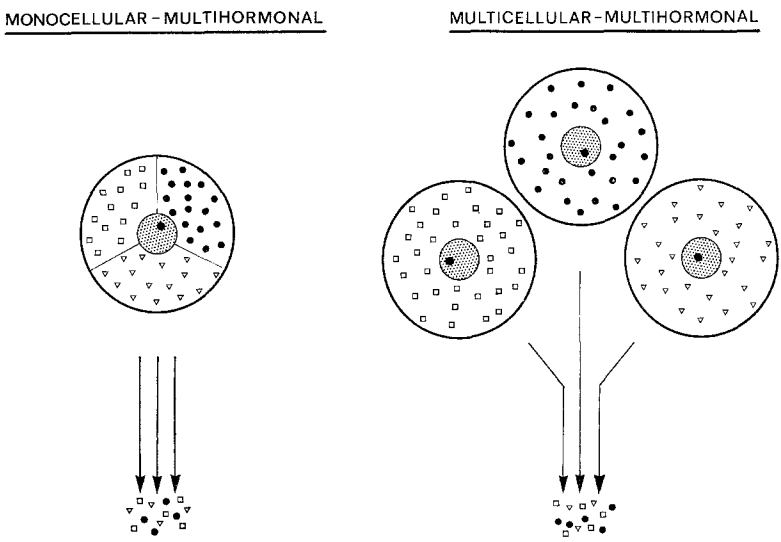

Fig. 1. Histological composition of endocrine tumours with multihormonal activity

Insulin and glucagon have been localized precisely in the B- and the A_2-cells respectively (Hellman and Hellerström, 1968; Lomský, Langr and Herout, 1967). Using a highly specific indirect immuno-fluorescence technic, gastrin has been detected only recently in the A_1-cells of normal pancreatic islets by Lomský, Langr and Vortel (1969). Indications are accumulating that at least one more active substance is synthesized in the islet cells: 5-HTA or serotonin (Cegrell, 1968).

The phenomenon of *tumours with multiple hormone production* is of major interest. Two main tumour-types may be recognized: 1. tumours composed of a homogenous cell population synthesizing more than one hormonal substance, 2. and others composed of a heterogenous cell population in which different cells produce different hormones (Fig. 1).

At present, a precise statement concerning the origin of these tumours cannot be made. Close observation and analysis of these "experiments of nature" might provide an answer to such questions as correlative adaptation, multipotentiality of the endocrine cell and on connections with the multiglandular endocrine adenomatosis syndrome (Wermer, 1963).

Case report[1]

A 12 year old boy had suffered from spontaneous hypoglycaemic attacks for 15 months before his first hospitalization in the department of pediatrics of the University Hospital in Basel. Clinical examination revealed a boy apparently in good health with a body weight exceeding the average value (44 kg, normal: 37,5 kg). A two hours fasting period induced hypoglycaemia. ACTH-, epinephrine- and glucagon-tests were essentially normal, as was the fructose tolerance test. The fasting blood glucose levels were very low (27, 48 and 36 mg/100 ml respectively) while those of insulin (IRI) were significantly elevated (150, 90 and 80 μU/ml; normal range: 20–50 μU/ml). After intravenous injection of 250 mg/kg tolbutamide, the insulin levels rose up to 365 μU/ml.

1 Clinical and laboratory data, differential diagnosis and diagnostic procedures will be described in detail elsewhere (Egli, Kapp, Engelhart, Zahnd, Girard and Heitz, 1971).

(Determinations of immunoreactive insulin (IRI) were kindly done by Dr. G. Zahnd, Policlinique médicale Universitaire, Geneva).

These results pointed to pancreatic hyperinsulinism. Selective arteriography of the coeliac trunk as well as pancreas scintigraphy failed to localize a pancreatic tumour whereas by ultrasonic scanning (done by Dr. G. Engelhart, University Hospital of Basel) the presence of a rounded, negative area highly suspect for tumourous growth in the head of the pancreas was demonstrated. At operation, it was rather difficult to localize the suspected tumour, but finally a soft mass was discovered by palpation in the presumed region. It was enucleated without difficulty.

Postoperatively the patient was immediately free of symptoms. An examination 6 months after enucleation of the islet cell tumour revealed no more pathological clinical signs or laboratory data. There was an apparent weight-loss during this time.

Morphology

Gross Examination. The tumour was partly encapsulated, soft, measuring about 2:1,5:1 cm. The cut surface was grey, partly variegated.

One half of the specimen was immediately frozen for subsequent biochemical analysis. The remaining tissue was fixed in formalin, a small fragment of the latter was refixed and reembedded in Epon 812 for electron microscopic examination.

The following staining procedures have been used for light microscopic examination: H+E, van Gieson, silver-techniques of Hellerström-Hellman (1960) and Grimelius (1968), Victoria-blue (Ivič, 1959), a modification of Gomori's aldehyde-fuchsine technique (Müller, Runge and Ferner, 1956), Dominici's toluidin-blue and Congo red.

Microscopic Examination. The architecture of the surgically removed tissue was of typical endocrine appearance, arranged in islets, here and there in a basaloid pattern. There was a rich sinusoidal vascularization.

In routine H+E-sections two different cell populations were immediately recognized. There were groups of cells with a faintly eosinophilic cytoplasm, which was finely granulated and occasionally contained round, homogenous, eosinophilic inclusions. Nuclei were relativeley large, oval and always eccentric. They contained a conspicuous nucleolus. Between these cell groups were large ribbons consisting exclusively of cells with a basophilic, often granulated cytoplasm, which contained a few vacuoles. There were, in contrast to the first cell type, very distinct cellular borders. The nuclei were round and contained a dense chromatin network (Fig. 2a).

Differences between the two cellular components were even more striking in special stains. With Ivič's victoria blue and with the modified aldehyde fuchsine techniques the first cell type could be identified as partly degranulated B-cells. The toluidine blue stain revealed a few granula of Weichselbaum (Fig. 3). In the silver impregnation according to Hellerström and Hellman the cells of the described ribbons gave a strong argyrophilic reaction. They therefore had to be classified as A_1-cells (gastrin-producing cells of the pancreatic islets; Fig. 2b). The overall ratio of A_1- to B-cells was about 1:1, with little regional differences. No cellular polymorphism nor mitotic figures were noted.

The stroma of the tumour consisted of fine septa of connective tissue,which contained a few blood vessels and excretory ductules. There was an impressive number of mast cells and eosinophilic leukocytes and large extravascular deposits of amyloid between B-cells. This could be identified by Congo red stain and observation in polarized light, where it gave a bright green fluorescence. It was

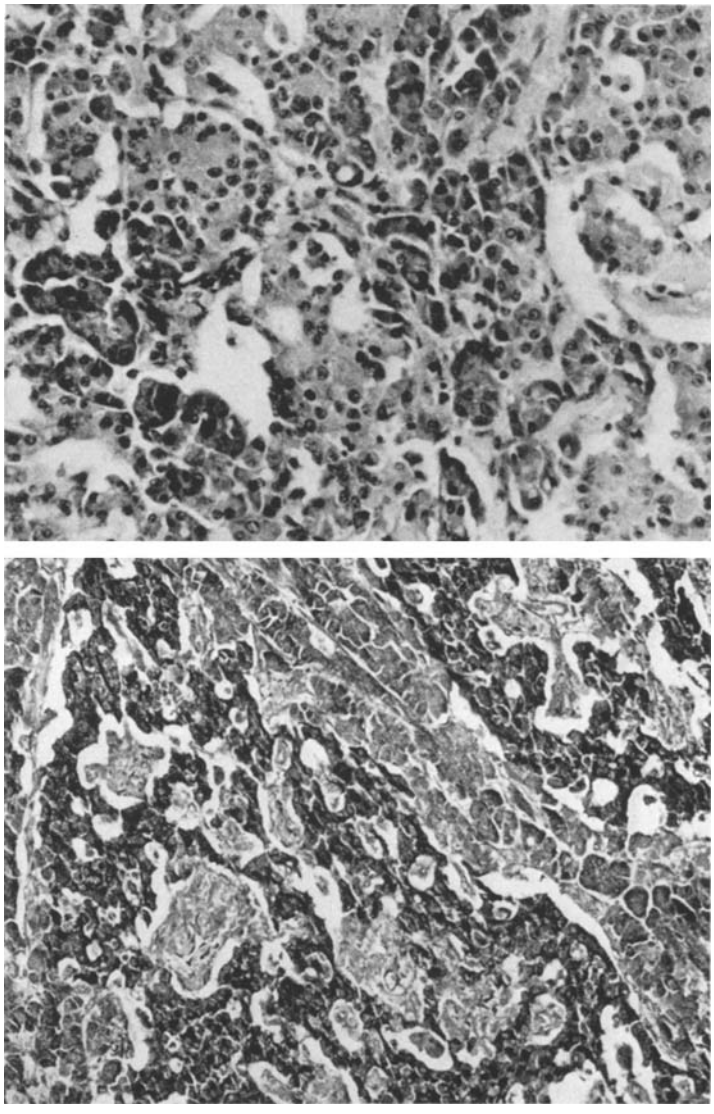

Fig. 2. a Tumour tissue with two distinct cellular populations. Cell groups with pale, eosinophilic cytoplasm and strands of cells with a strong basophilic cytoplasm. Hyalin substances in the stroma and relatively wide sinusoidal spaces. HE, 80:1. b Ribbons of A_1-cells with strongly argyrophilic cytoplasma (dark cell clusters) between strands of B-cells. Hellerström-Hellman's silver technique, 110:1

also shown electronmicroscopically despite a bad tissue preservation which unfortunately did not allow identification of tumour cells at this level (Fig. 4a, b).

Final Histological Diagnosis. Amyloid-producing islet cell adenoma with mixed cellular population (A_1- and B-cells).

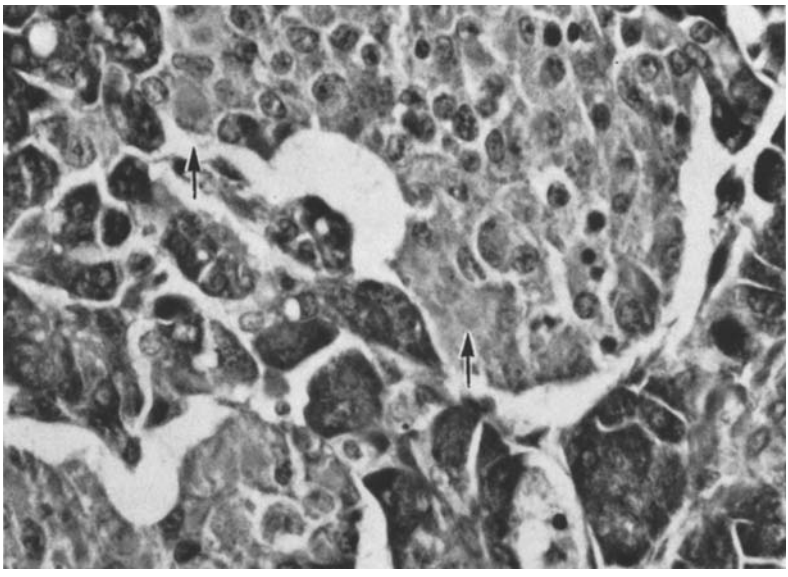

Fig. 3. High-power field of tumour tissue. At bottom a strand of A_1-cells. At top and on the right B-cells with partly granulated, partly homogenous cytoplasm. In few cells round, faintly eosinophilic, homogenous cytoplasmic inclusions (arrows). For comments see text. Ivič's Victoria blue, 520:1

Biochemical Analysis of the Tumour

The tumour was kindly submitted to the gastrin extraction procedure by Prof. R. A. Gregory, Physiological Laboratory, University of Liverpool (Gregory and Tracy, 1964). The activity of this extract was measured in our laboratory by a modified perfused rat stomach preparation (Halter, Kohler and Smith, 1970). This assay combines three recent improvements in the original Gosh and Schild method (Parsons, 1969; Lawrence and Smith, 1969; Smith, Lawrence, Colin-Jones and Schild, 1970). The perfusate is recirculated so that the acid secreted is integrated. The dead space in the stomach is reduced as much as possible by special intragastric canulae.

The secreted acid has been measured by conductivity which improves the linearity and reproducibility of the measurement.

Threshold doses for pentagastrin and human gastrin I, are as low as 0.5–5 ng in a sensitive animal. The response starts within one to two minutes after intravenous injection of gastrin or histamine. With low doses not exceeding 62 ng of gastrin, it is terminated within eight to ten minutes. After insulin injection the response is delayed by fifteen to twenty minutes.

In the present case, the lyophilized tumour extract was dissolved in 1 ml of distilled water adjusting the pH to 7 by adding some drops of HCl. It was injected over a period of two minutes. This resulted in a significant rise of the conductivity of the perfusate, starting immediately at the end of the intravenous injection (Fig. 5). The response was terminated eight minutes later. Its magnitude is approximately equivalent to 30 ng of gastrin I, if compared to the previous dose response curve.

It is most likely that the increased acid output in the gastric perfusate of the rat was due to gastrin or a gastrin-like substance in the tumour extract. It can not be due to insulin as the pattern of an insulin response is very different in our preparation.

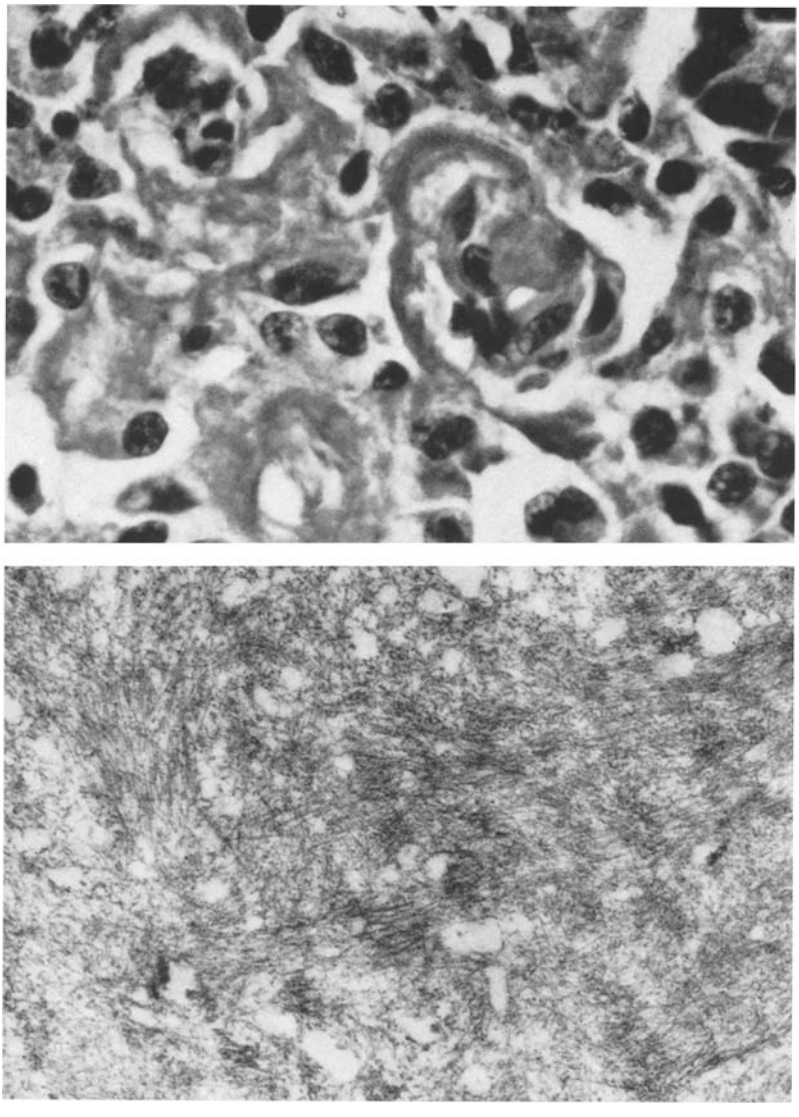

Fig. 4. a High-power view of a B-cell group. Between the cells there is a large quantity of amyloid, which is deposited outside of the blood vessels. In polarized light this material gives a bright-green fluorescence. Congo red, 500:1. b Electron microscopically densely packed interlacing bundles of amyloid fibrils in the stroma between B-cells. Material fixed in formalin, embedded in paraffin, subsequently reembedded in Epon 812. Ruthenium red stain, 7000:1, orig. (Photomicrograph by Dr. Rohr, Institute of Pathology, University of Basel)

Discussion

The outstanding features of this case are the low age of the patient, amyloid deposits, tissue eosinophilia and mastocytosis in the tumour as well as the combined production of insulin and gastrin.

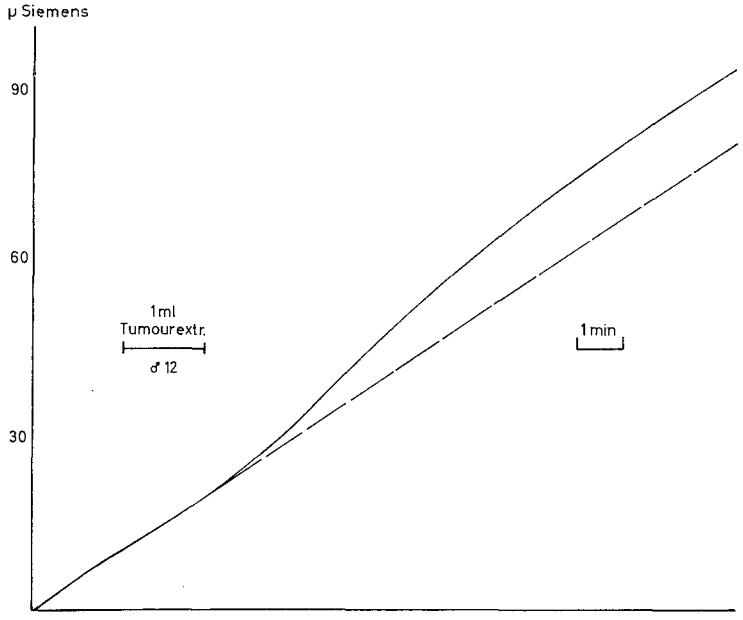

Fig. 5. Reperfusion of urethanized rat stomach. Conductivity change due to intravenous injection of one ml of tumour extract. Ordinate: Conductivity in μ Siemens. Abscissa: Time. (---) extrapolated basal secretion

Islet cell tumours are *rare in children:* Only 3 out of 95 insulinomas were found in children by Scholz, ReMine and Priestley (1960), only 6 insulinomas have hitherto been reported in newborns (Salinas, Mangurten, Roberts, Simon and Cornblath, 1968; Garces and Kenny, 1968; Grant and Barbor, 1970).

Amyloid deposition in the stroma of functioning endocrine tumours, typical in calcitonin-producing thyroid cancer, is found sometimes in insulinomas (Porta, Yerry and Scott, 1962). Two different localizations may be distinguished: extravascular deposits around tumour cells and subendothelial deposits within the vascular wall (Steiner, 1969). The above mentioned homogenous, round, eosinophilic intracytoplasmic inclusions in B-cells (Fig. 3), already described by Seifert and Berdrow (1958), might represent newly formed intracellular amyloid, which is found as well in the calcitonomas.

The presence of many mast cells in the tumour tissue is an interesting finding, which is not yet understood (Steiner, 1969). It may be interesting, that in experimentally induced slight hyperglycamia in syrian hamsters the number of mast cells is significantly elevated and that they disappear in severe hyperglycemia induced by subtotal pancreatectomy and alloxan administration (Steiner, 1968, unpublished data). Already in 1955 Cottenot observed extreme degranulation of the tissue mast cells in diabetic subjects. Therefore, the existence of a relation between the mast cells and the insulin dependent metabolic reactions is very probable. However, the mechanisms are unknown with only few exceptions (Chakravarty, 1968).

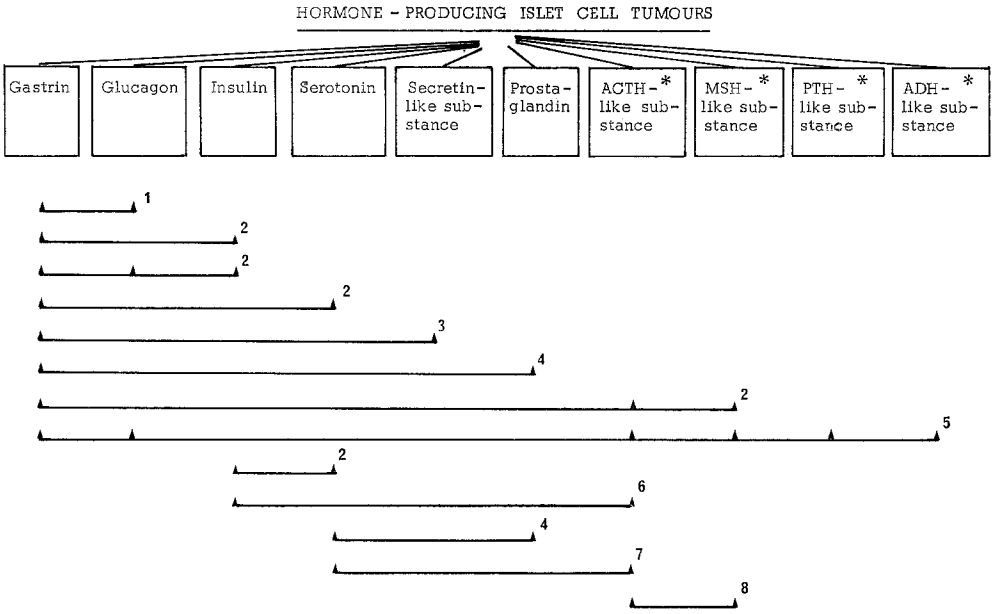

Fig. 6. Demonstrated or presumed combinations of hormonal substances elaborated simultaneously by a single islet cell tumour. *1* Histologically multicellular tumours, no biochemical analysis. *2* Demonstration of hormonal substances by biochemical analysis or immunoassay (Law, Liddle, Scott and Tauber, 1965). *3* Evidence of secretin-like and gastrin-like activity by bio-assay (Sircus, Brunt, Walker, Small, Falconer and Thomson, 1970; Cleator, Thomson, Sircus and Coombes, 1970). *4* Elevated levels in the blood of these hormonal substances (Sircus, 1969). *5* ACTH$^+$, MSH$^+$, gastrin and glucagon present in tumour extract, PTH$^+$-like and ADH$^+$-like activity in the blood probable (hypercalcaemia, hyperosmolarity of urine compared to serum; O'Neal, Kipnis, Luse, Lacy and Jarret, 1968). *6* Blood insulin elevated, clinical picture of Cushing's syndrome (Balls, Nicholson, Goodman and Touchstone, 1959). *7* Clinical picture of Cushing's syndrome and qualitatively elevated 5-HIAA$^+$ in urine (no quantitative analysis: Sayle, Lang, Green, Bosworth and Gregory, 1965). *8* Substances present in blood of patient not defined with precision (Hallwright, North and Reid, 1964). $^+$*ACTH* Adrenocorticotrophic hormone; *MSH* Melanocyte stimulating hormone; *PTH* Parathormone; *ADH* Antidiuretic hormone, vasopressine; *5-HIAA* 5-hydroxyindoleacetic acid

The *large amount of eosinophilic granulocytes* present in this tumour is still less well understood. The number of these cells is in general also increased in embryopathia diabetica where elevated insulin synthesis takes place.

The *combination of insulin and gastrin production* by the tumour of our patient is evidenced histologically. Both cell types showed hormonal activity as insulin was found in excess in venous blood and gastrin was present in a tumour extract. The outstanding feature of the clinical picture were recurrent hypoglycaemic attacks and therefore proof is lacking that gastrin was released into the blood stream. This tumour may therefore be classified as *multicellular-multihormonal.*

Although reports of functioning multihormonal islet cell tumours are far from numerous, an impressive spectrum of various combinations of simultaneously produced hormones already exists (Fig. 6). Shieber (1963) first reported a complete

biochemical analysis of a tumour containing gastrin, insulin and traces of histamine. The clinical picture in this case was that of a Zollinger-Ellison syndrome.

Far more frequent are reports of tumours with a *heterogenous cell population.* Unfortunately biochemical analysis of tumour extracts failed in several cases due to technical reasons (Gepts, Desneux and Henrotin, 1960; Sieracki, Marshall and Horn, 1960).

On the other hand *clinical syndromes* combining features of Zollinger-Ellison syndrome as well as hypoglycaemia have been reported. In these cases heterogenous cellular population of islet cell tumours has not always been demonstrated unequivocally and biochemical data very often are lacking (Wegmann, Zollinger and Markoff, 1964). In a series of 132 insulinomas Laroche, Ferris, Priestley, Scholz and Dockerty (1968) observed 21 patients with hyperinsulinism as well as gastrointestinal ulcers. Among these patients five furthermore suffered from multiple endocrine neoplasia. These findings suggested the occurence of multihormonal islet cell tumours as a part of this latter syndrome (Type I according to Steiner, Goodman and Powers, 1968).

Another mechanism leading to mixed functioning islet cell tumours might be a compensatory proliferation of an actively secreting cell population as a neutralizing reaction versus an antagonistic hormone secreted in excess. This is suggested by an observation of a patient with a malignant islet cell tumour by Murray-Lyon, Eddleston, Williams, Brown, Hogbin, Bennett, Edwards and Taylor (1968). Selective destruction of B-cells by streptozotocin, a specif B-cytotoxic substance, stopped excessive insulin as well as gastrin and glucagon-production of the tumour. Moreover, islet cell tumours with sequential secretion of three different peptide hormones over a period of several years have been reported (Vance, Kitabchi, Buchanan, Stoll, Hollander and Wood, 1968; Sircus, 1969). Indeed several authors describe an inhibitory effect of gastrin exerted on insulin release by B-cells (Jarret and Cohen, 1969; Lernmark, Hellman and Coore, 1969), but this is questioned by others (Creutzfeldt, Feurle and Ketterer, 1970).

On the other hand it is conceivable that some mixed tumours are really mixed from the beginning of the proliferation and arise from cells normally present in loco. The occurence of several cell types probably may be explained by the common origin of endocrine cells of the gastro-intestinal tract and of pancreatic endocrine stem cells (Greider, Bencosme and Latta, 1970).

In this context the existence of bronchial carcinoids (carcinomas) secreting serotonin, insulin or glucagon might be recalled. These findings could be explained 1. by dystopic localization of entodermal cells during embryological development (Shames, Dhurandhar and Blackard, 1968), 2. by an inappropriate secretion of peptide hormones by neuroendocrine cells located in all organs derived from the primitive alimentary tract (Weichert, 1970), and 3. by trapping or stocking of the substances (so-called sponging) by these tumours (Unger, de Lochner and Eisentraut, 1964).

In few instances *serotonin* has been found to be produced by pancreatic tumours. This substance has been reported to be associated with *insulin* (Gloor, Pletscher and Hardmeier, 1964; Aronsen, Boquist, Falkmer, Hägerstrand, Steiner and von Studnitz, 1970; in the latter case a monocellular-multihormonal tumour was suggested by ultrastructural observation) and with *gastrin* hypersecretion

(Sircus, 1969). From the histological point of view it is indeed striking to find quite frequently typical carcinoid structures in islet cell tumours, sometimes mixed with islet structures.

Recently a simultaneous production of a secretin-like and a gastrin-like substance as demonstrated by bio-assay was reported in two cases (Sircus, Brunt, Walker, Small, Falconer and Thomson, 1970; Cleator, Thomson, Sircus and Coombes, 1970; Fig. 6). This rises the question if secretin-producing cells exist in the islets of Langerhans or if they are ectopic in the reported tumours.

A further reported combined secretion of substances by islet cell tumours is that of gastrin and prostaglandin E_2 (Sircus, 1969). It is probable, that the occurence of prostaglandins in endocrine tumours is not at all specific to these neoplasias, because of the presence of these substances in many different tissues (Bergström, Carlson and Weeks, 1968).

Besides the above-mentioned substances, which are considered to be *orthotopical* secretion of *ectopic hormones* by islet cell tumours has been reported. Substances such as PTH (Kofstad, Frøshov, Gjone and Blix, 1967), ACTH (Geokas, Youl Chun and Beck, 1965), ADH as well as MSH have been detected by biochemical analysis or their secretion could be presumed by the clinical picture (for details see Fig. 6).

Pathogenesis

Different hypotheses may provide an explanation for the occurence of these most intriguing tumours.

The occurence of *neoplasias of the monocellular-multihormonal type* might be explained by the concept of a biochemically multipotent endocrine stem or precursor cell (Laidlaw, 1938; Boquist, 1968; Weichert, 1970).

Pathogenesis of *multicellular-multihormonal tumours* may be explained in different ways.

1. A hormonal substance produced by an originally monocellular tumour could stimulate the proliferation of a second cell type, thus leading to a *regulatory hyperplasia* and later on a *regulatory neoplasia*. Moreover a regulatory neoplasia could arise by much more complex hormonal interactions.

2. Mixed islet cell tumours have been observed as part of the syndrome of multiple endocrine neoplasia. Solitary mixed islet cell tumours therefore might be a variant of this syndrome restricted to the islets of Langerhans.

3. According to the hypothesis put forward by Weichert (1970) endocrine cells of the alimentary tract are neuroendocrine cells of ectodermal origin, which migrate to the primitive gut during embryogenesis. Afterwards part of these cells are carried to the various derivatives of the primitive alimentary tract, where they mature into polypeptid producing cells (APUD-series of Pearse, 1968). If several hormonally active cell types derived from percursor cells with multiple biochemical potencies become neoplastic, histologically mixed endocrine tumours with simultaneous production of more than one peptide hormones may arise in any organ derived from the primitive gut. *Ectopic hormone production* might equally be explained by this theory. According to this hypothesis multiple endocrine neoplasia may be a dysplasia of neural ectoderm.

Reports on multihormonal tumours should be interpreted with caution. If such a tumour is diagnosed clinically and said to contain a hormone normally

produced by islet cells a coexisting islet cell neoplasia should be excluded. Furthermore storage of a substance circulating in the blood in normal amounts by the tumour has to be taken into consideration. Understanding of pathogenesis of multihormonal tumours can only be achieved by systematic and precise clinical, morphological and biochemical analysis. Clinical diagnosis often is very difficult because clinical appearance can be variegated and change with time. Precise histological examination for several cell types and checking of blood samples as well as tumour extracts for several hormones are required in every suspected case. In this way combined activity of endocrine tumours will be detected more frequently.

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