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Two Distinct Types of Islet Abnormalities Associated with Endocrine Pancreatic Tumours

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Summary. Morphological and immunocytochemical studies of pancreatic material from eighteen patients with endocrine pancreatic tumours revealed two distinct types of islet abnormalities, both of which were associated with neoformation of endocrine cells from duct epithelium. Due to this phenomenon and the occurrence of greatly increased numbers of certain endocrine cell types the abnormalities were tentatively classified as hyperplasias. Hyperplasia type I was characterized by increases in number of all islet cell types. Increments in numbers of insulin and glucagon cells were most important quantitatively. Neoformation of islet cells from ducts (nesidioblastosis) was especially conspicuous with this type of hyperplasia. To some extent the islet cell neoformation resembled that seen in the human fetal pancreas. Hyperplasia type I was found in addition in two patients having hypergastrinemia due to achlorhydria. It is suggested that this type of abnormality results from a stimulatory effect of gastrin on the formation of pancreatic islet cells. Endocrine pancreatic tumours other than Zollinger-Ellison tumours were frequently, but not invariably, associated with hyperplasia type II, characterized by striking increases in frequency (and conceivably total mass) of pancreatic polypeptide (PP) cells. The variable occurrence of hyperplasia type II could not be related to any specific clinical abnormality. It seems possible that this hyperplasia may represent a non-specific response to pancreatic injury.

Key words: Endocrine pancreatic tumours — Nesidioblastosis — Islet hyperplasia — Islet hormones — Immunocytochemistry.

Introduction

Hormone-producing tumours are frequently associated with functional and morphological changes in other glandular tissues. To some extent this phenomenon—as in, for example, the syndromes of multiple endocrine adenomatosis (MEA)—may be attributed to the stimulatory or inhibitory effects of certain

hormones on other endocrine glands. Recently, gastrin-producing tumours have been noted to be associated with hyperplasia of the endocrine pancreas (Larsson et al., 1973; Creutzfeldt et al., 1975). While analyzing the specificity of this phenomenon I found that, depending upon the nature of the clinical syndrome, two distinct types of islet cell abnormalities occurred together with endocrine pancreatic tumours. In the following the two types are described and their physiological implications discussed.

Material and Methods

Pancreatic and tumour material was obtained at surgery. The material used is shown in Table 1. The cause for surgical intervention was usually extirpation of a pancreatic tumour or pancreatic resection. In the remaining cases pancreatic material was obtained during gastrectomy for gastric carcinoma. Control material from 10 cases without pancreatic illness was obtained at gastrectomy, splenectomy or autopsy.

The material was fixed in formalin or Bouin's fluid (5% acetic acid) or was frozen in melting Freon-22, freeze-dried and vapour-fixed in formaldehyde or diethyl-pyrocarbonate. Following embedding in paraffin, 5 μ sections were subjected to an indirect immunocytochemical method for the demonstration of insulin, glucagon, gastrin, somatostatin, pancreatic polypeptide and vasoactive intestinal polypeptide as described in detail elsewhere (Larsson et al., 1975a, 1976b). The site of antigen-antibody reaction was revealed either by fluorescein isothiocyanate-labelled goat anti-rabbit IgG (SBL, Stockholm, Sweden) or with the peroxidase-anti-peroxidase (PAP) method of Sternberger employing unlabelled sheep anti-rabbit IgG (cross-reacting with guinea-pig IgG, Anti-

Table 1. Clinical symptoms, localization and composition of tumours and type of islet cell hyperplasia. Note that most tumours contain more than one hormone-producing cell type

Clinical condition	Tumour localization and composition	Hyperplasia type I	Hyperplasia type II
Zollinger-Ellison Syndrome	Antrum, gastrin	+	−
,,	Duodenum, gastrin	+	−
,,	Pancreas, gastrin/glucagon	−	−
,,	Pancreas, gastrin/glucagon/insulin	+	−
,,	Pancreas, gastrin	+	−
,,	Pancreas, gastrin/insulin	+	−
Pernicious anemia	−	+	−
Atrophic gastritis	−	+	−
Hyperinsulinemia	Pancreas, insulin	−	+
,,	Pancreas, insulin	−	+
,,	Pancreas, insulin	−	−
,,	Pancreas, insulin	−	−
,,	Pancreas, insulin/gastrin	−	+
,,	Pancreas, insulin/glucagon	−	+
,,	Pancreas, insulin/glucagon	−	−
,,	Pancreas, insulin/PP	−	−
,,	Pancreas, insulin/glucagon/gastrin/PP	−	+
Glucagonoma syndrome	Pancreas, glucagon/insulin	−	+
,,	Pancreas, glucagon/VIP	−	−
Diarrhoea, achlorhydria and defect glucose tolerance	Pancreas, somatostatin	−	+

+ = denotes presence of islet cell hyperplasia and − = absence of hyperplasia according to criteria presented in the text

bodies Inc., Ca.) as the second and PAP complex (Dacopatts, Copenhagen, Denmark) as the third layer (Sternberger, 1974). Controls were those recommended by Sternberger (1974) and included prior absorption of the antisera with corresponding as well as unrelated antigens. Immunofluorescence preparations were examined in a Zeiss standard 18 microscope equipped for epiilumination using an XBO 75 xenon lamp as light source and selective filters giving peak excitation at 490 nm. PAP preparations were analyzed either in a conventional light microscope or in a Zeiss inverted microscope with Nomarski interference contrast optics. Relative numbers of insulin, glucagon, PP and somatostatin cells were determined by counting the number of immunoreactive cells per 1000 islet cells. Differential counting was performed on immunocytochemically stained sections that were either slightly counterstained with haematoxylin and eosin or examined in the interference contrast microscope. For reasons that will become apparent from the text special emphasis was put on the diagnosis of PP cell hyperplasia. In the control material PP cells invariably represented less than 10% of the total islet cell population (usually between 0.5–2%). Thus, when the PP cell frequency exceeded this figure the diagnosis of PP cell hyperplasia was made.

Results

Morphological and immunocytochemical analysis revealed two distinct types of islet abnormalities depending upon the nature of the clinical symptoms. Both types were associated with budding of islet cells from duct epithelium. In addition, most cases were associated with an obviously increased islet cell mass. The abnormalities were hence classified as islet cell hyperplasias. The two types of hyperplasia differed above all with respect to the proliferating cell type(s).

Hyperplasia Type I

Of six cases of the Zollinger-Ellison syndrome, four were due to a pancreatic, one to a duodenal and one to an antral gastrin-producing tumour. Five of these cases had associated islet cell hyperplasia whereas no hyperplasia could be detected with one of the pancreatic tumours (Table 1). The hyperplasia varied in severity from signs of budding of islet cells from ducts (nesidioblastosis) to a virtual predominance of endocrine over exocrine elements. In the specimens investigated no signs of acute or chronic inflammation were detected, neither was evidence of duct obstruction present. Two more cases of chronic hypergastrinemia were investigated: one case of pernicious anaemia displaying moderate antral gastrin cell hyperplasia and one case of achlorhydria of unknown etiology with serum gastrin concentrations exceeding 600 pg/ml. In both of these cases a pronounced islet cell hyperplasia was noted, displaying the same features as those seen in the Zollinger-Ellison syndrome. This hyperplasia was characterized by proliferation of all islet cell types. However, the contribution of insulin and glucagon cells was quantitatively most important since in most cases the relative proportions between the different islet cell types were about the same as in normal islets. In the majority of cases, examination of routinely stained sections revealed an increased islet cell mass and also increases in the number of small and medium-sized ducts. In addition, some islets were of considerable size and showed very irregular contours (Fig. 1). A characteristic

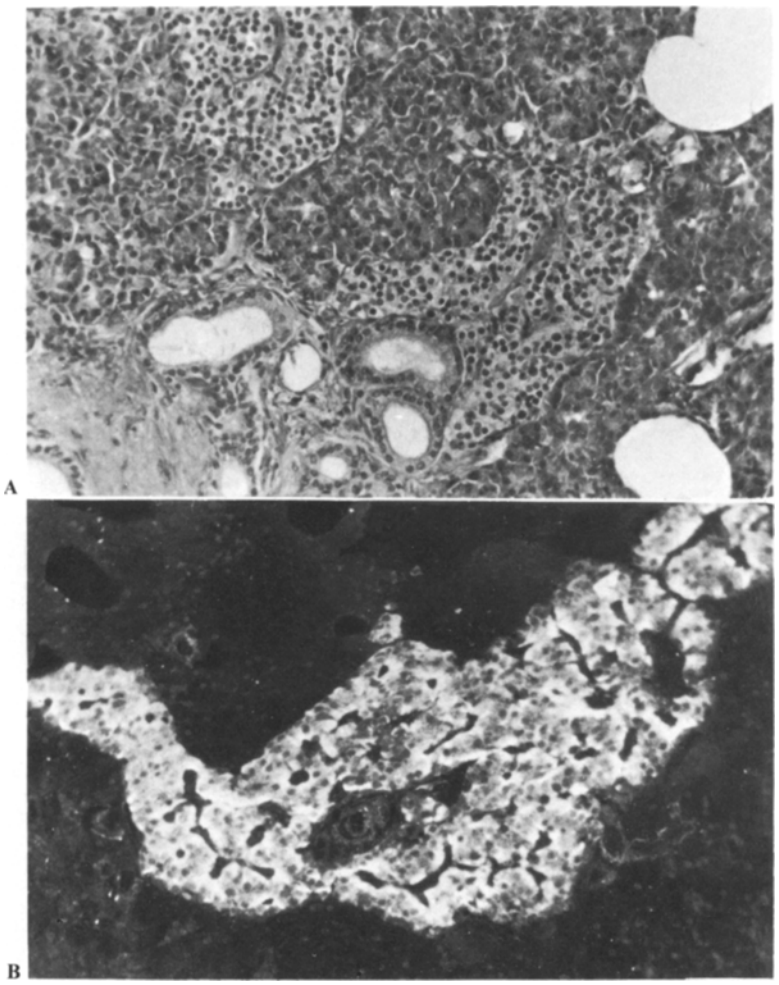

Fig. 1A and B. Pancreatic tissue from a patient with achlorhydria due to pernicious anemia. **A** Pancreatic islets are large, of irregular shape and occur in the vicinity of ducts, Haematoxylin-eosin staining (×160). **B** Insulin immunofluorescence in one of the large, irregular islets, the maximum diameter of which exceeds 600 μm. Note the incorporated duct in the center of the islet (×160)

and conspicuous feature was the presence of buds of clear cells that were connected with the epithelium of small and medium-sized ducts (Fig. 1). Immunocytochemistry showed such buds to consist mainly of glucagon and insulin cells (Figs. 3–5). In other ducts, part of the epithelium was replaced by a single layer of glucagon-immunoreactive cells (Fig. 4). Small ductular buds contained glucagon cells almost exclusively (Fig. 4) whereas larger buds usually contained a peripheral layer of glucagon cells and an inner core of insulin cells (Fig. 5). Sometimes, several buds were seen to be connected to a single duct (Fig. 5). Immunoreactive somatostatin and pancreatic polypeptide (PP) cells occurred scattered in the buds as well as in the duct epithelium. Islets were usually

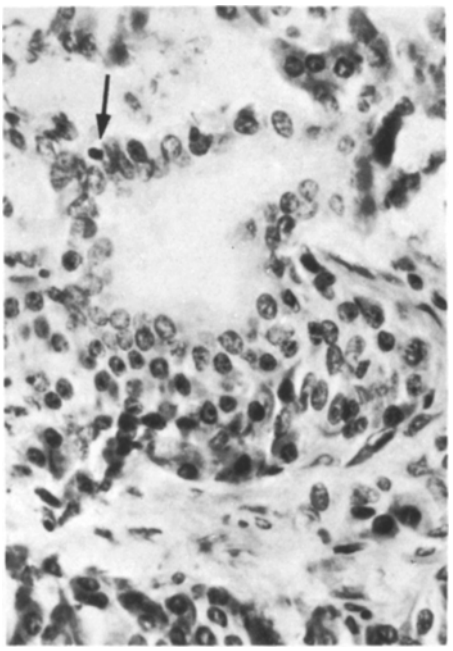

Fig. 2. Budding of cells from a small pancreatic duct. Patient with Zollinger-Ellison syndrome due to a pancreatic tumour. With haematoxylin and eosin staining the bud is seen to be composed of clear cells and a peripheral zone of acidophil cells. The latter were by immunocytochemistry shown to represent glucagon cells. The arrow indicates a mitosis in the duct epithelium ($\times 450$)

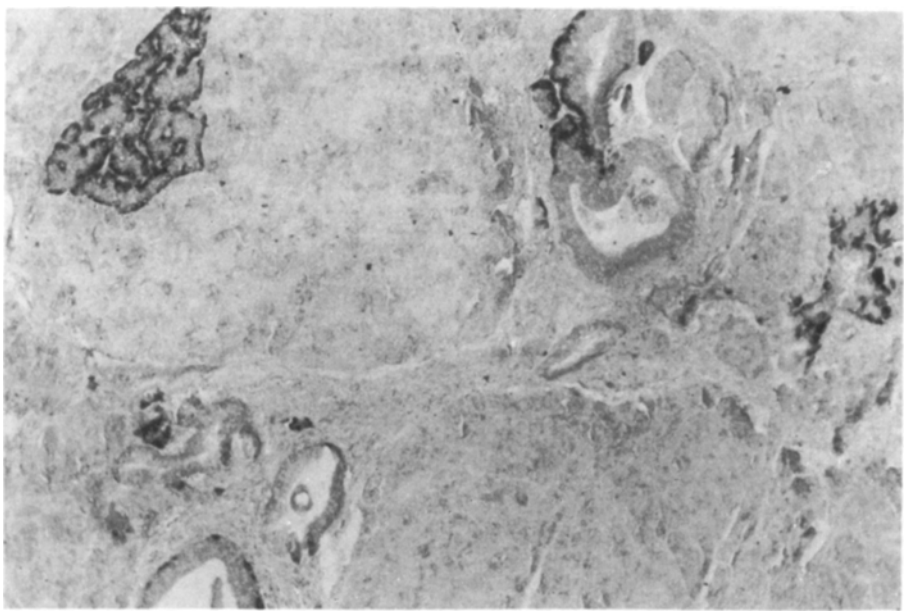

Fig. 3. Pancreatic tissue from a patient with Zollinger-Ellison syndrome due to a pancreatic tumour. Immunocytochemical (PAP) staining for glucagon. In this low power photomicrograph is seen an apparently normal islet in the upper left corner, scattered ductal glucagon cells in the lower left corner and different stages of budding of glucagon cells from ducts in the upper right corner ($\times 110$)

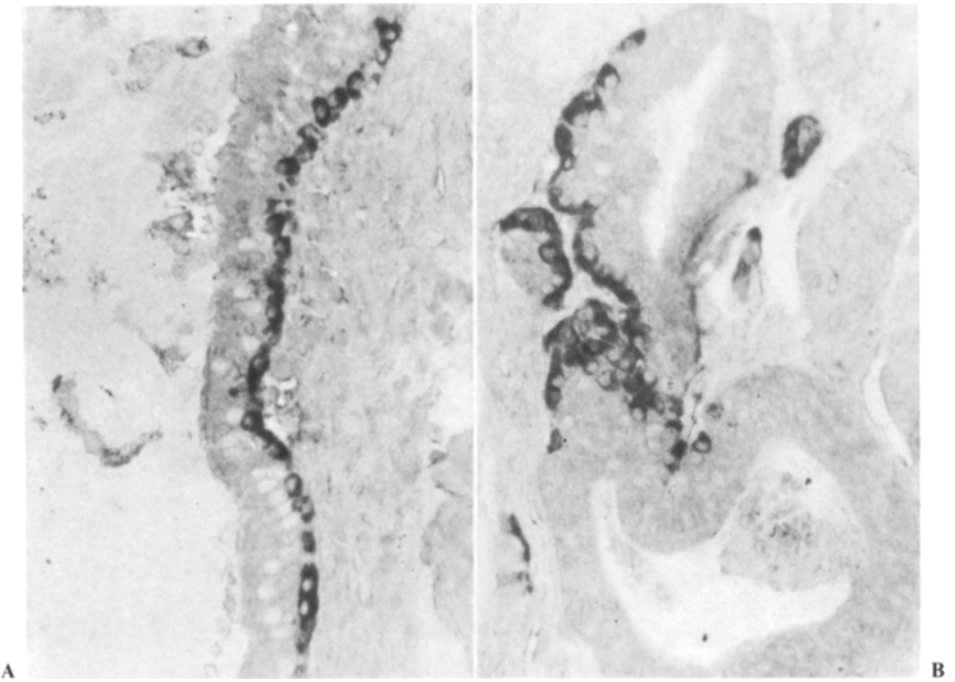

Fig. 4A and B. Pancreatic tissue from the same patient as in the preceeding figure. Immunocytochemical (PAP) staining for glucagon. **A** Row of glucagon cells in the epithelium of a medium-sized duct (×290). **B** Buds of glucagon cells emanating from another duct (×290)

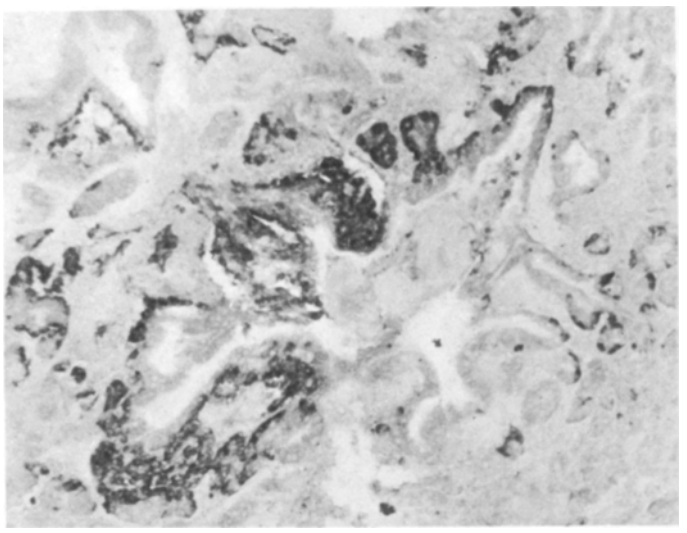

Fig. 5. Pancreatic tissue from a patient having achlorhydria and serum gastrin levels exceeding 600 pg/ml. Immunocytochemical (PAP) staining for glucagon. A duct is seen to contain several buds in different stages of formation. Parallel sections revealed that the larger buds also contained insulin, somatostatin and PP cells (×110)

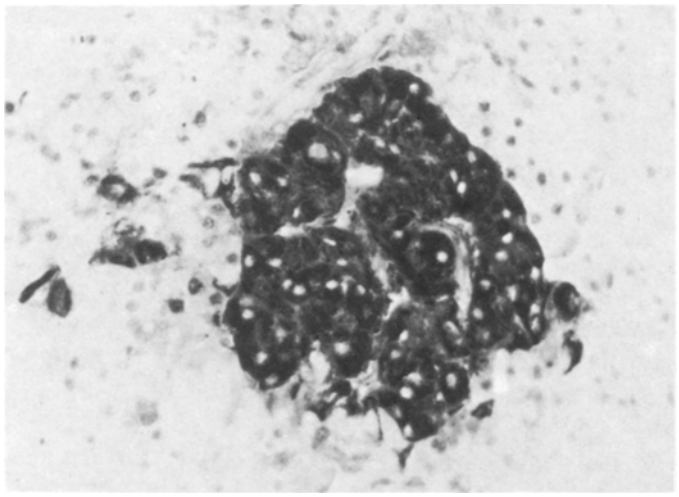

Fig. 6. Pancreatic islet from a patient having symptoms of hyperinsulinism due to an insulin-producing pancreatic tumour. Immunocytochemical (PAP) staining for pancreatic polypeptide (PP). The islet is seen to be almost entirely composed of PP cells. To the left a few extrainsular PP cells are seen (× 360)

very numerous and often occurred in association with ducts. Some islets showed normal distribution of the four cell types and may represent preexisting islets (Fig. 3). Other islets showed a more or less bipolar arrangement of insulin and glucagon cells and may represent large buds in the process of leaving their connection with the ducts. In some instances duct remnants with preserved lumina were detected inside islets. In two of the Zollinger-Ellison cases the number of insulin cells per islet was lower than usual whereas glucagon cells were more frequent than in normal islets.

Hyperplasia Type II

A different type of islet cell abnormality was found associated with other types of endocrine pancreatic tumours. Thus, islet cell hyperplasia type II was found in association with five out of nine cases of hyperinsulinemia and with one out of two cases of the glucagonoma syndrome. Since most of the tumours of this group were multihormonal and thus contained more than one hormone-producing cell type the results are summarized in Table 1. Type II hyperplasia was characterized by an increase in number of PP cells, usually with only slight or no disturbance of the frequency of other islet cell types. The degree of PP cell hyperplasia was very variable. In two cases (insulinoma patients) islets almost entirely composed of PP cells (Fig. 6) were regularly detected besides apparently normal islets. In most cases PP cells were of frequent occurrence in ductal epithelium. Occasionally buds of PP cells were encountered, suggesting a ductal origin of the hyperplastic cells. In addition to the insular PP cell hyperplasia, extrainsular proliferation of such cells was sometimes observed,

but usually, insular PP cells showed the most conspicuous numerical increase. In one case of a tumour producing somatostatin (Larsson et al., 1977b), however, the extrainsular PP cells showed extensive hyperplasia whereas insular PP cells were only slightly increased.

Discussion

The unequivocal diagnosis of islet cell hyperplasia in a surgical material represents a difficult task. Regional variations in the distribution of islets as well as unavailability of the entire pancreas makes a definite morphometric diagnosis impossible. However, in the present cases, a tentative diagnosis of islet cell hyperplasia seems justified due to the observations of active islet cell neoformation from ducts and the abundance of islet tissue.

Hyperplasia type I seems to be associated with hypergastrinemia. Previous observations made on patients having the Zollinger-Ellison syndrome indicate that islet cell hyperplasia and nesidioblastosis are frequently associated with gastrin-producing tumours (Larsson et al., 1973; Creutzfeldt et al., 1975). Interestingly, in most cases the islet cell neoformation appears to follow a specific pattern (cf. Figs. 3–5). At first endocrine progenitor cells of the ducts (nesidioblasts) proliferate and differentiate into glucagon cells. Buds consisting primarily of glucagon cells (and a few somatostatin and PP cells) form and invade the surrounding parenchyma. Subsequently insulin cells may appear between the advancing edge of glucagon cells and the ductal epithelium. With continued growth the buds may either lose their connection with the duct or may incorporate it. It appears that one single duct may give rise to several buds and also that some stimulation of ductal growth may occur. Newly formed islets are frequently bipolar with a predominance of glucagon cells at one pole and insulin cells at the other. Importantly, this type of hyperplasia seems to be a feature of hypergastrinemia rather than of pancreatic tumours per se, since it was also detected in patients having extrapancreatic Zollinger-Ellison tumours or achlorhydria associated with much elevated serum gastrin concentrations. To some extent hyperplasia type I seems to mimic the formation of pancreatic islets in the human fetus. Thus, fetal islets have been noted to form from ductular buds and have been described to be bipolar (Robb, 1961; Like and Orci, 1972). In accordance with these observations, preliminary studies have shown that gastrin stimulates growth and ^{3}H-thymidine-incorporation in cultivated rat islets (Larsson et al., 1976a). Furthermore, patients with the Zollinger-Ellison syndrome or with pernicious anemia have been found to show exaggerated plasma insulin (Rehfeld et al., 1976; Rehfeld, 1976) and glucagon (J.J. Holst and J.F. Rehfeld: personal communication) responses to stimulation. If gastrin proves to be the pathogenetic factor behind hyperplasia type I the variable degree of nesidioblastosis seen in hypergastrinemic patients could be due to differences in gastrin concentrations, component patterns and duration of illness.

It is noteworthy that of all pancreatic tumours examined, only those giving rise to symptoms of the Zollinger-Ellison syndrome were associated with islet

cell hyperplasia type I. Other tumours that in addition to insulin and glucagon cells contained gastrin cells, but produced symptoms of hyperinsulinemia, were associated with hyperplasia type II rather than with type I (Table 1). Previously it has been emphasized that multihormonal or mixed endocrine pancreatic tumours produce symptoms characteristic of overproduction of only one of the involved hormones (Larsson et al., 1975a, 1976b; Arnold et al., 1976). Whether this is due to hypersecretion of only one of the tumoral products or to defect end organ sensitivity to the other hormones is currently unknown. At any rate the absence of hyperplasia type I under these conditions seem to correlate with the absence of other features of the Zollinger-Ellison syndrome.

Hyperplasia type II was evident in some cases of endocrine pancreatic tumours other than those causing the Zollinger-Ellison syndrome. Usually the PP cells made up much more than 10% of the total islet cell population and in some cases were the predominating cell type in most islets. A disadvantage of the present method of diagnosing hyperplasia type II is that the PP cell number varies with age (Larsson et al., 1975b, 1976c). Thus, the use of a large body of age-matched control material would permit the diagnosis of even more cases of PP cell hyperplasia. It should be noted that the frequency of occurrence rather than the total mass of PP cells was determined. In view of the fact, that the quotient between endocrine and non-endocrine cells was unchanged or even increased, however, the term PP cell hyperplasia seems justified. Furthermore, signs of active PP cell neoformation from ducts were encountered. Radioimmunochemical studies by Floyd and co-workers have indicated an important increase in serum PP concentrations in insulinoma patients (Floyd et al., 1975, 1976). In contrast to recently published studies by Polak and colleagues (1976) our results indicate that the increased PP levels are caused by the extratumoral pancreatic tissue. Provided that tumours causing the WDHA syndrome are excluded,PP cells are not more frequent in multihormonal endocrine tumours than are other islet cell types (Larsson et al., 1976b). These results are in accord with those of Floyd et al. (1975, 1976) showing PP concentrations to be persistently elevated even following succesful removal of insulinomas. Furthermore, these workers showed that most of the extirpated tumours were devoid of PP (Floyd et al., 1975, 1976).

The frequent association between PP cell hyperplasia and various types of endocrine pancreatic tumours raises the possibility that hyperplasia type II may represent a non-specific response to pancreatic disorders. Recently, Gepts and co-workers reported that the pancreas of some, but not all, juvenile diabetics showed a striking PP cell hyperplasia (Gepts et al., 1977). Furthermore, recent studies have indicated an increase in total PP cell mass in alloxan-diabetic rats (Sundler et al., 1977), whereas the PP cell frequency has been shown to decrease considerably in hyperinsulinemic obese Zucker rats (Larsson et al., 1977a). In the present material no juvenile diabetics were included, nor could correlations between hyperplasia type II and serum insulin levels be obtained. The variability of the PP cell hyperplasia is currently unexplained. If this hyperplasia reflects the degree of pancreatic injury it may possibly be related to the size or position of the tumours. However, our Zollinger-Ellison patients with pancreatic tumours did not display hyperplasia type II. In addition, most

Zollinger-Ellison patients have normal plasma PP concentrations (T.W. Schwartz: personal communication). Studies on patients with non-endocrine pancreatic tumours are needed to establish whether hyperplasia type II really reflects a pancreatic injury or whether it is due to a more specific disorder common to diabetics and patients with certain types of endocrine pancreatic tumours.

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