Cytology of islet tumours and hyperplasias associated with the Zollinger-Ellison syndrome

C. CAVALLERO, E. SOLCIA, AND R. SAMPIETRO

From the Institute of Pathological Anatomy, the University of Pavia, Italy

EDITORIAL COMMENT These studies suggest that the tumours and the hyperplastic growths are not related to insulin-producing β cells or to glucagon-producing α cells but may originate from argyrophil-metachromatic or δ cells which may secrete gastrin.

Since Zollinger and Ellison described in 1955 a pathological entity consisting of multiple recurrent peptic ulcerations, gastric hypersecretion, and islet cell tumours of the pancreas, several reports have been published dealing with the clinical and biological aspects of this new syndrome (Priest and Alexander, 1957; Summerskill, 1959; Gregory, Tracy, French, and Sircus, 1960; Grossman, Tracy, and Gregory, 1961; Hallenbeck, Code, and Kennedy, 1961; Zollinger, Elliott, Endahl, Grant, Goswitz, and Taft, 1962; Selli, Baglioni, and Castrini, 1963; Sircus, 1964). Neoplastic or hyperplastic growths of the pancreatic islets have been considered to play an important pathogenetic role by secreting an 'ulcero-genic' factor very similar or, perhaps, identical with, the antral hormone gastrin (Gregory *et al.*, 1960; Gregory and Tracy, 1964).

Recent morphological studies have shown that in pancreatic islets of man and several vertebrate species, in addition to the α and β cells, there exists a third cell type with cytological and cytochemical characters which are quite different from those of other cell types. This cell has been labelled the α_1 cell (Hellman, Rothman, and Hellerström, 1962; Petersson, Hellerström, and Hellman, 1962) or, on the basis of its staining properties, the argyrophil-metachromatic cell (Cavallero and Solcia, 1963, 1964a, b). Previous researches from this laboratory suggest that δ cell, as described by Bloom (1931) and Thomas (1937) in pancreatic islets of man and vertebrates, is likely to be identical with our argyrophil-metachromatic cell (Solcia and Sampietro, 1965a).

On the basis of these data, it has seemed interesting to study pancreatic tumours and hyperplasias associated with the Zollinger-Ellison syndrome by applying the most reliable methods for islet cytology. The main purpose of this investigation has been to

ascertain a possible role for the argyrophil-metachromatic cell in the histogenesis of such neoplastic growths.

MATERIAL AND METHODS

Three islet carcinomas (cases 1, 2, 3), one multiple adenomatosis (case 4), and two islet hyperplasias (cases 5, 6) from patients with the Zollinger-Ellison syndrome were examined. Apart from case 3, being found at necropsy, the tissue samples were obtained from surgical specimens. The material was partly fixed in Bouin's fluid for 24 hours (cases 1, 4, 6), partly in formol for different times ranging from 24 hours to one year (cases 2, 3, 4, 6), partly in Bouin and subsequently stored in formol for several months (case 5); a formol-fixed specimen was refixed in Bouin before embedding (case 2). For comparison, surgical and necropsy material from unaltered human pancreases fixed in 10% formol, Bouin, 6% glutaraldehyde or in a glutaraldehyde-picric acid-sodium-acetate mixture, was also examined. Furthermore, Bouin-fixed samples of two islet β cell adenomas and two formol-fixed intestinal carcinoids were studied.

Paraffin sections of all tissues were stained with the aldehyde-fuchsin (Scott, 1952) and the pseudo-isocyanin (Schiebler and Schiessler, 1959) methods for islet β cells. Other sections were treated with Bodian's silver impregnation (McManus and Mowry, 1960) and dimethylamino-benzaldehyde (D.M.A.B.)-nitrite reaction for protein-bound tryptophan (Lison, 1960) on the grounds of the alleged specificity of these methods for α cells. For δ cells toluidine-blue metachromatic stain (Manocchio, 1960) and Davenport's silver impregnation (Hellerström and Hellman, 1960) were employed; sometimes methylation (Fisher and Lillie, 1954) or methylation followed by demethylation (Lillie, 1958) were performed before staining with toluidine blue. Phosphotungstic acid-haematoxylin (P.T.A.H.) stain (McManus and Mowry, 1960), Barnett and Seligman's (1958) method for protein carboxyl groups with or without the NaOH test according to Karnovsky and Fasman (1960) for distinguishing between side-chain and terminal carboxyl groups, and

TABLE I

TUMOUR OR HYPERPLASTIC ISLET CELLS IN THE ZOLLINGER-ELLISON SYNDROME COMPARED WITH NORMAL ISLET CELLS

Material	Toluidine Blue (pH 5)	Davenport	Bodian	D.M.A.B.- nitrite	Aldehyde- fuchsin	Pseudo- isocyanin
<i>Normal pancreatic islets</i>						
α cells	—	—	+++	++	—	—
β cells	—	—	—	—	+++	+++
δ (argyrophil-metachromatic) cells	++	+++	+++	+	—	—
<i>Zollinger-Ellison tumours and hyperplasias</i>						
Case 1 (carcinoma)	++	++	++	—	—	—
Case 2 (carcinoma)	+	+	+	+	±	—
Case 3 (carcinoma)	+	+	+	±	—	—
Case 4 (adenomatosis)	+	++	+	+	—	—
Case 5 (hyperplastic cells)	++	++	++	—	—	—
Case 6 (hyperplastic cells)	++	++	+++	+	—	—

toluidine-blue stain at pH 5 after HCl and KOH treatment for 'masked' basophilia (Solcia and Sampietro, 1965c) were also employed. Finally, diazonium, Masson-Hamperl and Schmorl reactions (Pearse, 1960) were used as histochemical tests for argentaftin cells.

RESULTS

NORMAL PANCREATIC ISLETS As shown by Table I (upper part), non-neoplastic human islets appeared to be composed of three cell types with a different histological and histochemical reactivity. β cells were easily distinguished by their reactivity to the pseudo-isocyanin and aldehyde-fuchsin methods. The positivity to the Bodian and to the D.M.A.B.-nitrite methods was common for both α and δ cells, although the reactivity of δ cells for D.M.A.B.-nitrite was very weak. The morphological differentiation between these two cell types was achieved only on the basis of the selective positivity of δ cells for Davenport's silver impregnation and toluidine blue

red metachromasia at pH 5. δ cells were not stained by toluidine blue at pH 4 or after methylation of tissue sections; when alkali demethylation followed methylation the metachromatic stain at pH 5 was restored.

As regards the other methods employed, both α and argyrophil-metachromatic cells, differing from β cells, reacted positively to Barnett and Seligman's reaction, which was completely prevented by NaOH treatment according to Karnovsky and Fasman (1960), as well as to the method for 'masked' basophilia; α cells reacted more strongly than δ cells to the P.T.A.H. stain. Histochemical tests for argentaftin cells gave constantly negative results.

TUMOUR AND HYPERPLASTIC ISLETS As reported in Table I (lower part), tumour and hyperplastic cells from Zollinger-Ellison cases showed no cytological relationships to the islet β cells; in fact, they never reacted to the aldehyde-fuchsin stains (Figs. 1 and 2)

FIG. 1.

FIG. 2.

FIGS. 1 and 2. Normal islet showing high numbers of closely packed β cells (1) compared with a hyperplastic islet from a Zollinger-Ellison case showing scarce and irregularly scattered β cells (2). Aldehyde-fuchsin stain $\times 450$ and $\times 120$.

and to the more reliable pseudo-isocyanin method. A very weak staining of these cells by aldehyde-fuchsin in case 2 was of non-granular nature and in no way resembled that of specific beta-granules stored in the cytoplasm of β cells. Two insulinomas studied comparatively appeared to be composed partly of cells displaying aldehyde-fuchsin- and pseudo-isocyanin-positive granules, partly of agranular cells.

Tumour and hyperplastic islet cells reacted to the Bodian silver impregnation (Figs. 3 and 4) and the D.M.A.B.-nitrite test which have been considered as specific stains for α cells; however, as noted before, these methods stain normal δ cells too. In our cases, the reactivity of tumour cells for D.M.A.B.-nitrite was more comparable to the weak reactivity of δ cells, than to the moderate positivity of α cells. As shown in Table I, both tumour and hyperplastic cells in Zollinger-Ellison cases were metachromatically stained by toluidine blue (Figs. 5 and 6) and silver impregnated by Davenport's method (Figs. 7 and 8), although marked differences were observed in the degree of stain from one case to another and, in the same case, from one cell to another. Different fixation of various samples might account in part for these differences; Bouin-fixed tissues reacted far better than formol-fixed ones. On the other hand, a different degree of anaplasia might explain the variable reactivity of the cells observed in the same tissue section. Some sections previously stained with toluidine blue were drawn or photographed and subsequently silver impregnated; thus, it was ascertained that cytoplasmic granules showing metachromasia are also argyrophil and that there is a good correspondence between the amount of metachromatic material and the degree of silver impregnation.

FIG. 3.

FIG. 4.

FIGS. 3 and 4. Low numbers of silver-impregnated cells in a normal pancreatic islet (3) compared with a hyperplastic islet from a case of the Zollinger-Ellison syndrome showing interlacing cords of reactive cells (4). Bodian's silver impregnation. $\times 350$ and $\times 120$.

It should be noted that tumour and hyperplastic cells in Zollinger-Ellison cases, like normal δ cells, stained metachromatically with toluidine blue at pH 5 both directly or after HCl and KOH 'unmasking' treatments; further, no staining was observed with toluidine blue at pH 4 or after methylation, although alkali demethylation restored their stainability. These histochemical data indicate that carboxyl groupings are involved in the metachromatic reaction. A positive reaction for the Barnett and Seligman method, which was completely prevented by the NaOH treatment, confirms this hypothesis and suggests that we are dealing with side-chain carboxyl groups of proteins. Some weak positivity was observed for the P.T.A.H. method. By using the histochemical methods for argentaftin cells, highly positive reactions were found in carcinoid tumours, whereas Zollinger-Ellison tumours and hyperplasias gave constantly negative results.

DISCUSSION

In islet-cell pathology, the existence of insulin-producing β cell tumours or insulinomas is fully accepted, while only few glucagon-producing α cell tumours have been reported and their endocrine activity is widely questioned. Till now, there is no agreement as regards the histogenesis of the Zollinger-Ellison tumours. A possible origin from glucagon-producing α cells was originally suggested (Zollinger and Ellison, 1955; Greider and Elliott, 1964), but several findings do not support such a hypothesis, (Zollinger and Craig, 1960; Rawson, England, Gillam, French and Stammers, 1960; Marks, Selzer, Louw, and Bank, 1961; Martin and Potet, 1962; Brown and Crile, 1964). It is now generally believed

FIG. 5.

FIG. 5. *Islet hyperplasia. Cords of hyperplastic metachromatic cells adjacent to a residual non-hyperplastic islet. Toluidine blue stain at pH 5. $\times 250$.*

FIG. 6.

FIG. 6. *Islet carcinoma showing various amounts of metachromatic granules in the cytoplasm of tumour cells. Toluidine blue stain at pH 5. $\times 600$.*

FIG. 7.

FIG. 8.

FIGS. 7 and 8. *Silver-impregnated tumour cells in islet carcinomas. Davenport silver impregnation. $\times 250$ and 700 .*

that no relationships exist between the Zollinger-Ellison tumour and islet β cells.

Bloom (1931) and Thomas (1937) have reported that a third granular cell type, named δ cell, takes part in the composition of islet tissue both in man and several vertebrate species. Recent morphological investigations using more reliable methods, such as Davenport's silver impregnation (Hellman *et al.*, 1962; Cavallero and Solcia, 1964a), metachromatic reaction with basic dyes (Solcia and Sampietro, 1965a, b) and electron microscopy (Caramia, Munger, and Lacy, 1965; Munger, Caramia, and Lacy, 1965) confirm this. From the above results it appears that, on properly fixed human islet tissue, both argyrophilia with Davenport silver impregnation and toluidine blue metachromasia are characteristic

staining reactions for a cell type different from both α and β cells. Thus, the possibility of a third non-insulin nor glucagon-producing islet tumour should be considered.

Our morphological investigation clearly indicates that tumour and hyperplastic cells from Zollinger-Ellison cases are not related either to α or to β cells but display the cytological and cytochemical aspects of the argyrophil-metachromatic islet cells, as suggested before (Solcia and Bertoli, 1964; Cavallero and Solcia, 1965; Solcia, 1966). Accordingly, Franksson, Hellström, Hultquist, and Perman (1960), Goulon, Mercier, Reilly, and Laporte (1960), Martin and Potet (1962), Miehler, Hartsock, Geokas Ballard, and Frame (1962), Martini, Strohmeyer, Haug, and Gusek (1964), and Wegmann, Zollinger,

and Markoff (1964) have noticed various degrees of argyrophilia in tumour cells; for example, positive silver impregnations were obtained by Potet, Martin, Thiery, Bader, Bonfils and Lambling (1965) who employed Hellerström's and Hellman's modification of Davenport's method, just as we did. No morphological relationships appear to exist between the argyrophil cells of these tumours and the argentaffin cells of intestinal carcinoids.

Since Gregory *et al.* (1960) and others have found that tumour extracts from cases of the Zollinger-Ellison syndrome show a gastrin-like activity, the question arises as to whether argyrophil-metachromatic cells in normal pancreatic islets also are concerned with the production and release of a gastrin-like substance. In this connexion, it should be recalled that argyrophil-metachromatic cells morphologically similar to the ones of pancreatic islets have been observed in the antro-pyloric mucosa of the stomach (Solcia and Sampietro, 1965b, c) where gastrin is normally present.

On histochemical grounds it may be suggested that the secretory granules in normal and tumoural argyrophil-metachromatic cells store an acid protein with several side-chain carboxyl groups closely arranged so as to give basophilia of metachromatic type. These histochemical data appear to be consistent with the chemical structure of purified gastrin as determined by Gregory, Hardy, Jones, Kenner, and Sheppard (1964) and by Bentley, Kenner, and Sheppard (1966).

SUMMARY

Cytological and cytochemical studies of six tumours and hyperplasias of the pancreatic islets associated with the Zollinger-Ellison syndrome have been performed by employing the most suitable methods for islet morphology. The results obtained suggest that the tumour and hyperplastic growths are not related either to insulin-producing β cells or to glucagon-producing α cells, but originate from the argyrophil-metachromatic or δ cells. It was found that both normal and tumour argyrophil-metachromatic cells store in their cytoplasm an acid protein. On histochemical grounds a possible relationship between this protein and the hormone gastrin is discussed.

This work was supported by a grant from the Consiglio Nazionale delle Ricerche, Roma (N. 115/1139/991).

We are greatly indebted to Professor R. M. Zollinger, Department of Surgery, the Ohio State University College of Medicine, Columbus, U.S.A., to Professor P. Stefanini, Istituto di Patologia Chirurgica, Università di Roma, Italy, to Professor C. Krauspe, Pathologisches Institut

des Universitätskrankenhauses, Hamburg, Germany, to Professor A. Tardini, Istituto di Anatomia Patologica, Università di Parma, Italy, to Professor S. Donati, Clinica Chirurgica, Università di Pavia, Italy, and to Professor A. Trivellini, Clinica Chirurgica, Università di Milano, Italy, for supplying surgical and necropsy material from Zollinger-Ellison cases and insulinomas.

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