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Immunohistochemical and Ultrastructural Studies on Rat Islet Cell Tumours Induced by Streptozotocin and Nicotinamide* ** ***

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Summary. Fourteen rat islet tumours induced by streptozotocin and nicotinamide were examined by light microscopy with indirect immunoperoxidase staining and by electron microscopy. Most tumours consisted predominantly of B cells, but over a half of the tumours examined showed mixed cellularity with considerable numbers of A cells and small numbers of D or PP cells. Only 4 tumours consisted exclusively of B cells. There was no positive reaction for any kind of specific islet hormone antibodies in 2 tumours. Ultrastructurally, most tumours were composed of cells containing numerous secretory granules with B cell properties, with great variation in size, shape and number. We often encountered enterochromaffin-like cells or atypical granular cells or cells containing non-beta secretory granules. We could not identify, however, the ultrastructural counterparts of A or D cells. The results suggested that the multiplicity of the endocrine cells of rat islet cell tumours might be an expression of the cellular dedifferentiation of tumour cells which could re-differentiate into the whole range of components of the endocrine pancreas.

Key words: Experimental islet cell tumour – Streptozotocin – Immunohistochemistry – Ultrastructure – Cellular dedifferentation

Introduction

With the recent progress in immunohistochemical procedures, the cell types producing peptide hormones can be specifically identified in tissue sections

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*** The term of gastroentero-pancreatic endocrine cells is used in this report according to the Lausanne classification 1977

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(Nakane and Pierce 1967). The results of immunostaining have given new insights into the pathology of functioning tumours, showing the endocrine tumours of the pancreas to be of mixed cellularity more frequently than previously expected (Larsson et al. 1975; Polak et al. 1976; Klöppel, Seifert and Heitz 1979).

Tumours of pancreatic islet origin can be produced in rats at a high rate either by the combined administration of streptozotocin and nicotinamide (Rakieten et al. 1971) or streptozotocin alone (Kazumi et al. 1978). These rat tumours were described as being composed almost exclusively of B cells, by electron microscopic observations (Volk, Wellmann and Brancato 1973). Our re-evaluation of rat islet cell tumours induced by streptozotocin and nicotinamide by means of immunoperoxidase methods and electron microscopy yielded different results, contrasting with their observations and other morphological studies on animal islet cell tumours (Grillo et al. 1967; Falkmer et al. 1969). In our series, the rat islet cell tumours have shown great variation of morphological features from case to case and were shown to be a valid model for human insulinomas.

Materials and Methods

The procedures for the production of an islet cell tumor were originally based on the methods of Rakieten et al. (1971). After an overnight fast, male Wistar strain rats 2 months of age, weighing 180–200 g were intravenously injected with streptozotocin (40 mg/kg, Upjohn Co., Kalamazoo, Michigan), preceding and following intraperitoneal injection with nicotinamide (350 mg/kg, Wako Ltd., Tokyo). Eleventh months after the treatment with streptozotocin and nicotinamide, the rats were sacrificed by decapitation.

The pancreases with or without visible tumors were dissected and prepared for histological investigations after laparotomy. In this study, 14 tumors found 17 surviving rats either macroscopically or under the light microscope were examined. For the light microscopic examinations, the specimens were fixed in Bouin's solution and embedded in paraffin. The sections were stained conventionally with haematoxylin and eosin. For the identification of discrete endocrine cells of the pancreatic islets, indirect immunoperoxidase staining was applied for paraffin sections of 4 μ thickness based on the methods of Nakane and Pierce (1967). All reagents were prepared in phosphate-buffered saline. Tissues were incubated with primary antisera for an hour at room temperature at the following dilutions; rabbit anti-monocomponent pork insulin, 1:32; rabbit anti-glucagon, 1:32; rabbit anti-somatostatin, 1:32; and rabbit anti-pancreatic polypeptide, 1:40. Anti-pancreatic polypeptide sera were kindly donated from Dr. R. E. Chance (Indianapolis, Indiana). Other anti-sera were raised in our own laboratory by immunization of rabbits using mono-component pork insulin (Novo Co., Copenhagen), crystalline glucagon (Sigma, St. Louis) and synthetic somatostatin (Peptide Institute, Osaka). Peroxidase-labelled anti-rabbit gamma globulin which served as a secondary antibody (Miles-Yeda Ltd., Israel) was used at 1:32 dilutions. Incubation of tissues with this antiserum was allowed to proceed for an hour at room temperature.

Hormone-specific staining was finally accomplished by incubation of tissue sections with 0.05 M Tris-HCl buffer (pH 7.6) containing 50 mg/100 ml of 3,3'-diamino benzidine (Nakarai Chem. Ltd., Kyoto) and 0.33% H_2O_2.

Control incubations using excess antigen to immune serum as the first antibody and omitting the first antibody, resulted in the absence of hormone-specific staining. Thus the identification of tumour A, B, D, and PP cells was made on the serial tumour sections.

For evaluation of the percentage of endocrine cell types, four consecutive sections of the tumours stained with each specific islet hormone antisera were photographed. On the light micrographs, developed into a final magnification of × 1,000, positive immunoreactive cells were counted and their percentage of all the cells visible throughout the tumour on a single section was estimated.

For the electron microscopic observation, the specimens of 6 macroscopically visible tumours were fixed in 2.5% glutaraldehyde and postfixed with 1% osmium tetroxide. They were then dehydrated through an ascending series of ethanol and embedded in Epon 812. Ultrathin sections were obtained with LKB Ultrotome V and stained with uranyl acetate and lead citrate. The sections were observed under Hitachi HU 12 A electron microscope.

Results

On the sections stained with haematoxylin and eosin, the tumours consist of a proliferation of small polygonal or larger columner cells arranging in trabecular, ribbon-cord-like or tubular structures. They are clearly demarcated from the exocrine pancreas by thin or thick fibrous capsules, thus indicating that they arise from pancreatic islets (Fig. 1). The arrangement of tumour cells tends to become more regular as these are traced to the central from the marginal zones, where tumour islet cells form varying structures such as tubuli, single or duble cell columns and small clusters. Inflammatory cells such as lymphocytes, eosinophils or plasma cells are often encountered within some tumours.

By immunoperoxidase staining, the neoplastic cells of the trabecular or ribbon-cord like arrangement show diffuse positive immunoperoxidase reaction for anti-insulin antibodies showing dark-brown granular cytoplasm in most of the tumours (Fig. 2). In a few instances, the immunostainabilities of the neoplastic B cells are reduced in contrast with those of B cells in the non-neoplastic islets near the tumours but they are clearly distinguishable from negative backgrounds (Tumour No. 1, 3, and 4). In most case, the cells staining with anti-glucagon, anti-somatostatin, or anti-pancreatic polypeptide antibodies are much less than insulin-positive cells and preferentially locate in the peripheral parts of the tumours. However, in some tumours, the cells staining with anti-glucagon antibodies distribute diffusely throughout the the tumours arranging in ribbon-cord like structures (Fig. 3). In contrast with glucagon-positive cells, the cells staining with anti-somatostatin or anti-pancreatic polypeptide antibodies are very few and scatter between other tumour cells (Fig. 4). A few pancreatic polypeptide cells are often present in the interstitial tissues (Fig. 5). Two out of 14 tumours examined in this study are composed of cells that do not show any positive reaction to any kinds of specific islet hormone antibodies. Only 4 out of 14 tumours constitute exclusively of B cells and 8 tumours are of mixed cellularity even though the main component is B cells. The gross cell populations of neoplastic A, B, D and PP cells in each cases are summarized in Table 1.

Electron microscopically, the tumours consist of well granulated cells (Fig. 6). The majority of the tumour cells contain numerous secretory granules similar to the beta granules of normal islet B cells. However, these exhibit greater variation in shape, size and number. We can observe the primary lysosomes or pleomorphic electron dense lysosomal dense bodies near the Golgi complexes discernible from secretory granules. In the interstitium, there are cells, resembling PP cells, containing small secretory granules with electron dense core closely attached to the limiting membrane (Fig. 7). Thus, we can identify the tumour cells by their characteristic pictures of secretory granules. However, other islet

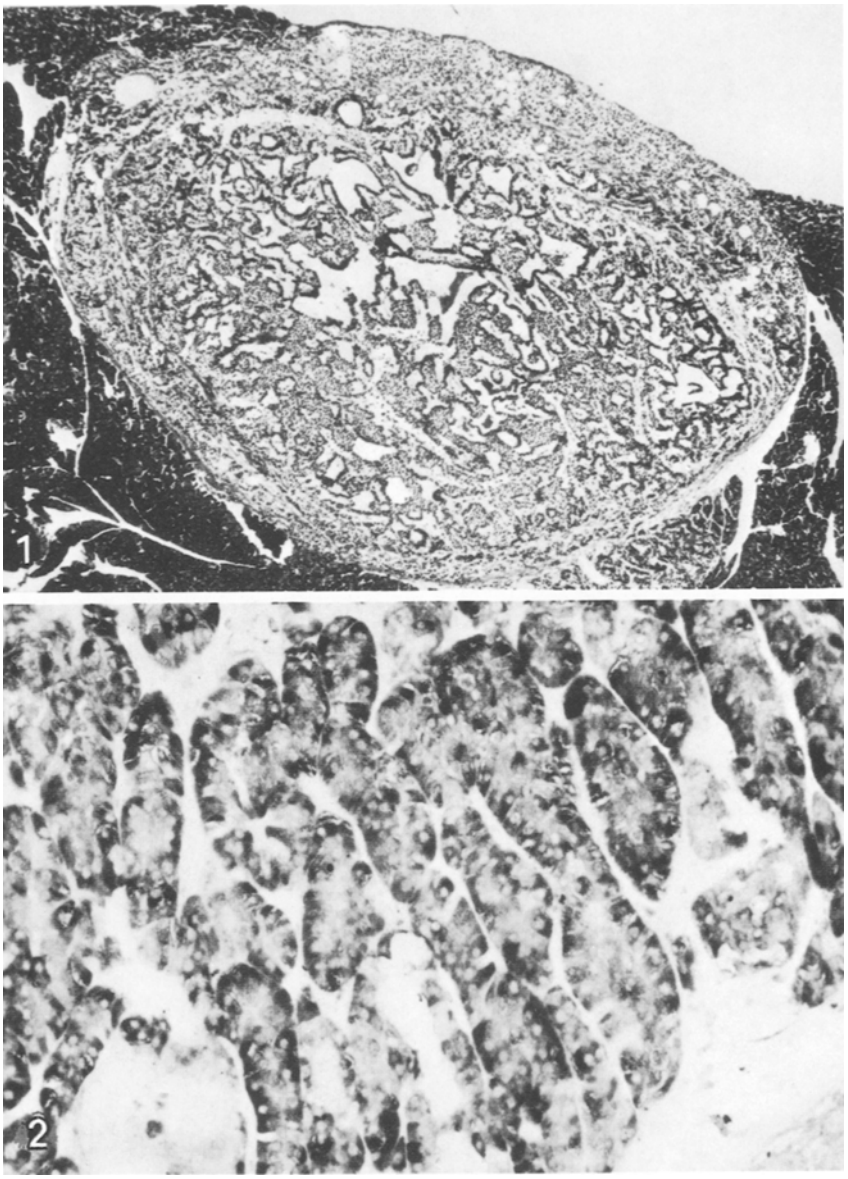


Fig. 1. Light microscopic view of an islet tumour. Haematoxylin and eosin stain. Mag. ×46

Fig. 2. Immunoperoxidase staining with anti-insulin antibody. Most of the tumour cells show positive reaction in contrast with negative backgrounds. Mag. ×230

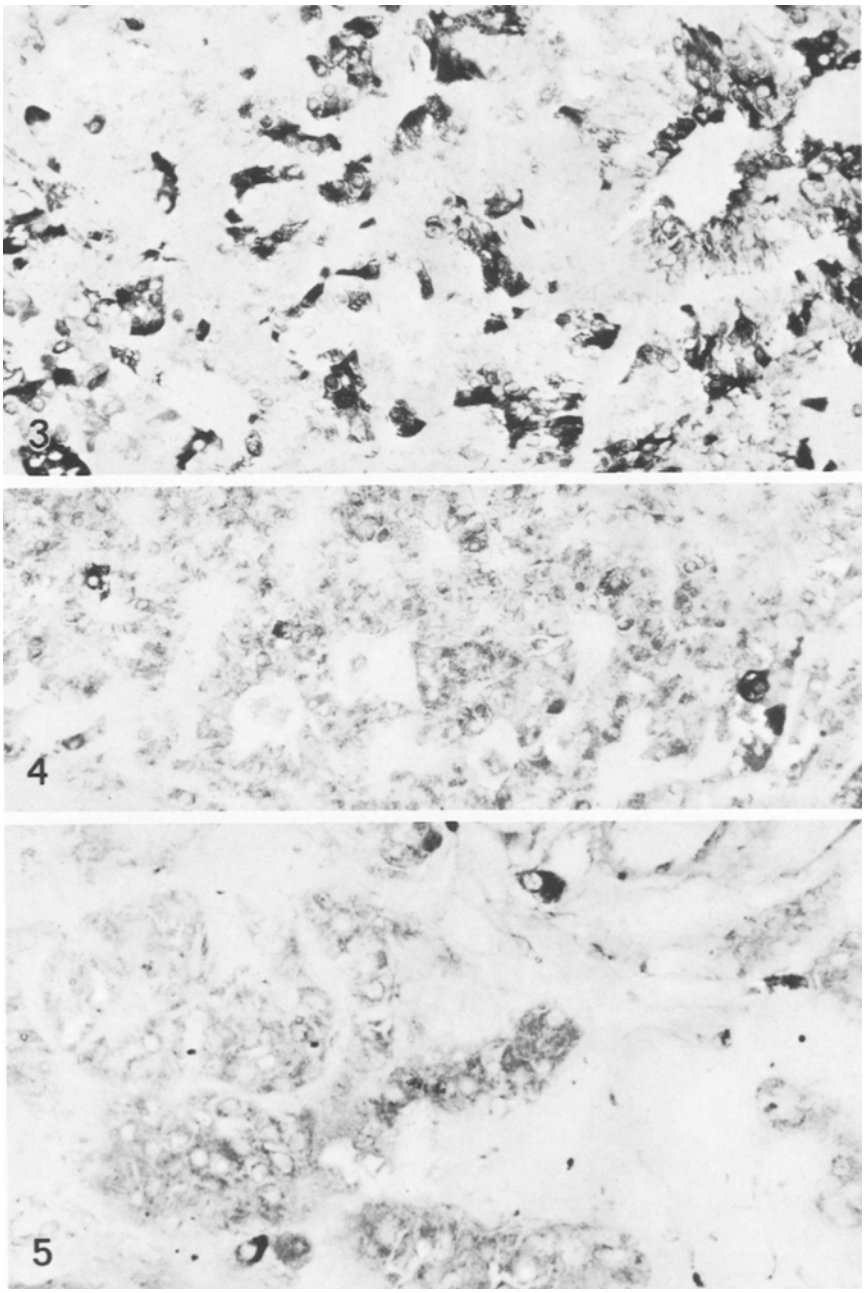


Fig. 3. Immunoperoxidase staining with anti-glucagon antibody. Many immunoreactive glucagon-containing cells distribute throughout the tumour showing ribbon-cord like structures. Mag. ×230

Fig. 4. A few immunoreactive somatostatin-containing cells are located preferentially in the peripheral parts of the tumour. Mag. ×230

Fig. 5. A few immunoreactive pancreatic polypeptide-containing cells are seen in the interstitium of part of the tumour. Mag. ×460

Table 1. Cell population of endocrine cell types in experimentally-induced islet cell tumours[a]

Tumor no.	Counted cells[b]	A Cell	B Cell	D Cell	PP Cell
1	(n=1204)	15.2 (%)	73.6 (%)	1.2 (%)	0.2 (%)
2	(n= 965)	13.3	76.6	2.0	0.8
3	(n=1200)	8.4	78.2	0.9	1.1
4	(n= 720)	4.2	63.5	1.4	0
5	(n=1425)	4.0	0	0.3	0.3
6	(n=2122)	2.1	86.8	0.7	0.4
7	(n= 881)	1.6	68.3	0.4	0
8	(n=1922)	1.5	95.2	0	0
9	(n= 961)	0	96.4	0	0
10	(n=1550)	0	92.0	0	0
11	(n=1102)	0	90.4	0	0
12	(n=1645)	0	86.4	0	0
13		0	0	0	0
14		0	0	0	0
Mean ± S.D. (n=14)		3.59 ± 5.12	64.81 ± 36.45	0.49 ± 0.65	0.20 ± 0.35

[a] Cell population is evaluated by estimating the percentage of positive immunoreactive cells to all the tumour cells on four consecutive immunostained sections

[b] Number of cells counted on a single immunostained section of the tumour

cells such as A or D cells cannot be identified with certainty in the tumours, in contrast to the results of immunostaining. Some enterochromaffin-like cells containing comma-shaped or dumb-bell-shaped granules (Fig. 8) or the cells containing large electron dense granules (atypical granular cell) are also observed infrequently (Fig. 9).

The tumour cells are relatively rich in cytoplasmic organelles. Mitochondria are abundant in number, relatively large, but varied in size, and oval or spherical in shape. Rough and smooth surfaced endoplasmic reticula are present in great quantity, forming mainly small vesicles and occupying extensive areas of the cytoplasm with electron dense contents within their cisterns. Rarely, fine parallel fibrillar structures are seen within the widened cisternae of rough endoplasmic reticulum (Fig. 10). Well developed Golgi complexes are numerous throughout the cytoplasm. Centrioles or the mitotic figures of the cells are rarely seen. Intranuclear fibrillary structures are also identified (Fig. 11). Within the tumours, nerves showing degenerated myelin are also seen (Fig. 12).

Discussion

The present immunohistochemical study confirmed the mixed cellularity of most of the rat islet cell tumours induced by streptozotocin and nicotinamide. By electron microscopic observations, we often encountered cells which contained secretory granules resembling other than granules of B cell type. These variations of secretory granules suggested multiplicity of the types of endocrine cells of

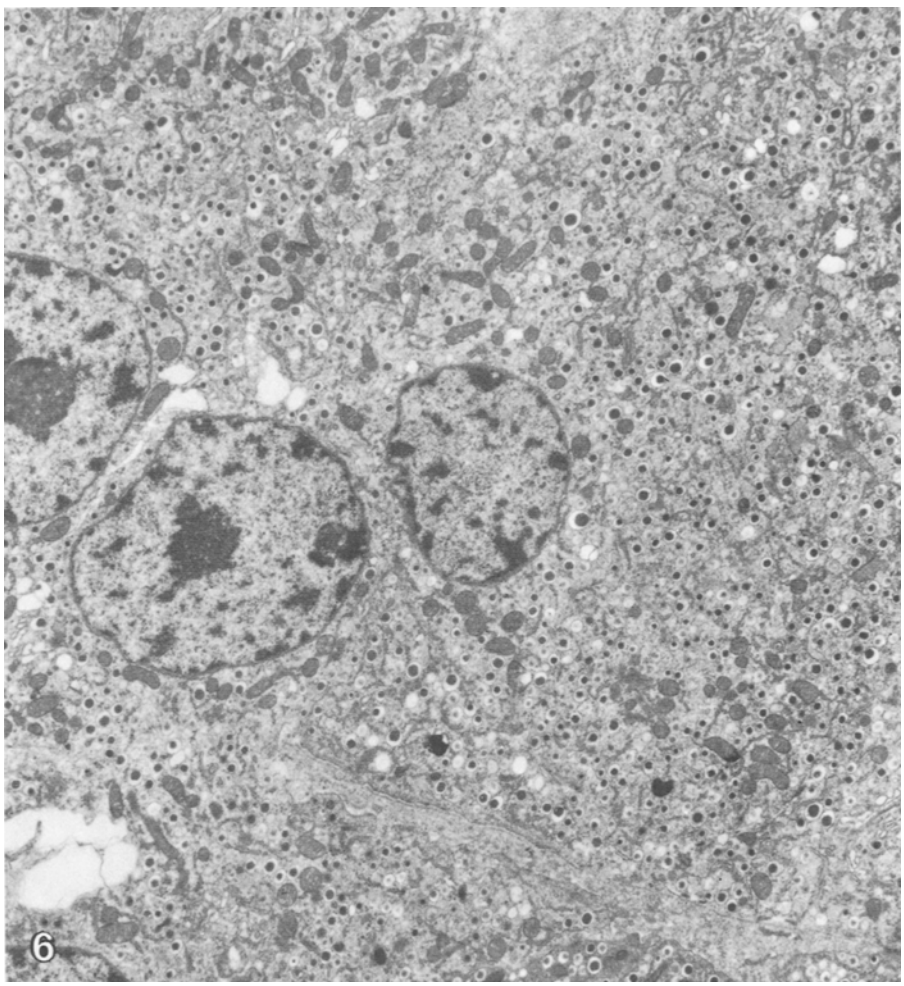
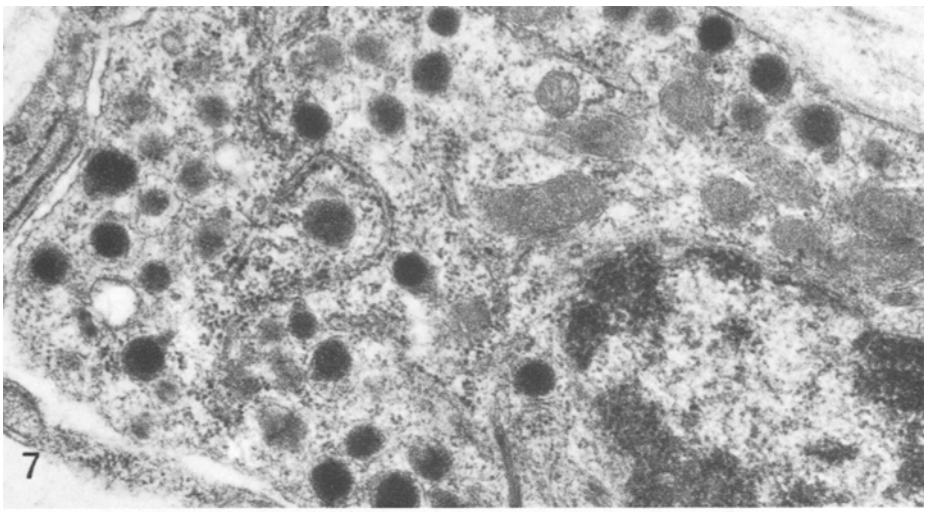


Fig. 6. Low-magnification electron micrograph of a part of the tumour. The tumour cells contain numerous secretory granules with characteristic electron dense central cores. Mag. ×4,900

rat islet cell tumours. Gross distribution of each endocrine cell varied case by case in the tumours examined. On the histochemical sections stained with Grimelius silver, considerable numbers of intensively-stained argyrophil cells were found in human insulinoma (Woodtli and Hedinger 1976; Capella et al. 1977). Using specific antisera to islet hormones, immunohistochemical studies have also shown that human endocrine pancreatic tumors are often multihormonal (Larsson et al. 1975; Polak et al. 1976; Nieuwenhuijzen Kruseman et al. 1978; Klöppel, Seifert and Heitz 1979) as seen in our studies.

In our series, most of the tumours consisted mainly of neoplastic B cells and thereby should be classified as insulinoma. Some tumours did not react with any kinds of specific islet hormone antibodies. The physiological reaction



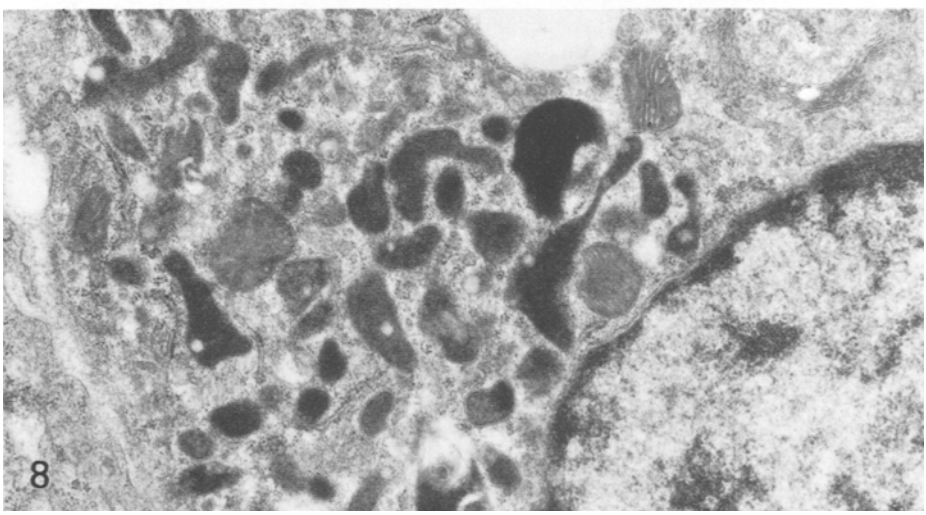


Fig. 7. PP-like cells containing small electron dense core seen in the interstitium of part of the tumour. Mag. ×28,800

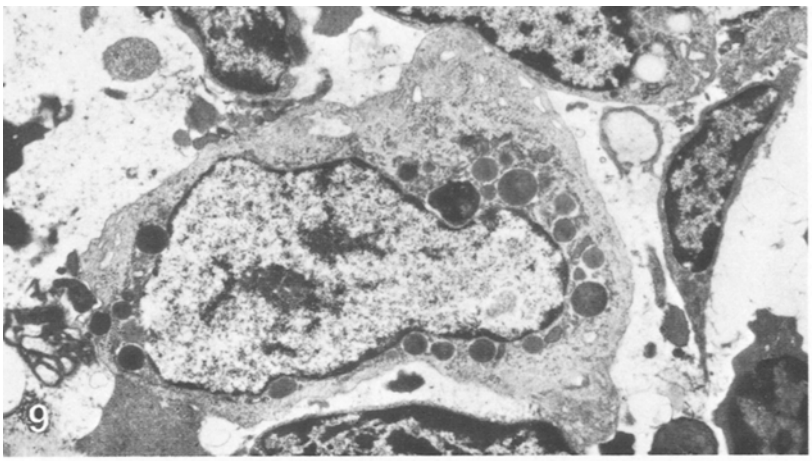
Fig. 8. Enterochromaffin-like cells containing pleomorphic electron dense granules seen in a part of the tumour. Mag. ×16,800

Fig. 9. Atypical granular cell containing relatively electron-dense granules seen in a part of the tumour. Mag. ×5,000

Fig. 10. Fine parallel fibrillar structures within the cisterns of granular endoplasmic reticulum. Mag. ×61,200

Fig. 11. Intranuclear filaments seen in part of a tumour cell. Mag. ×10,600

Fig. 12. Nerve showing degenerated myelin figure, in the interstitium of part of the tumour. Mag. ×4,900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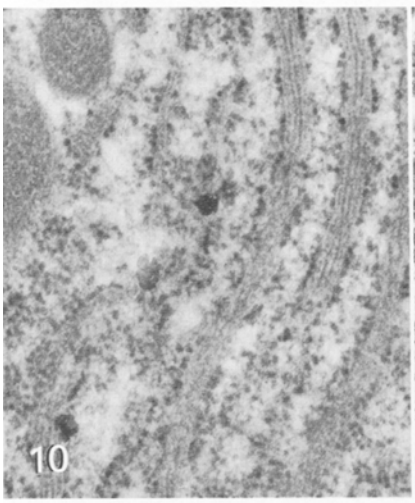
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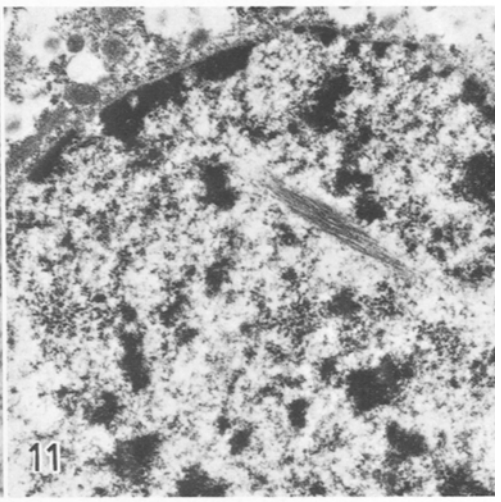
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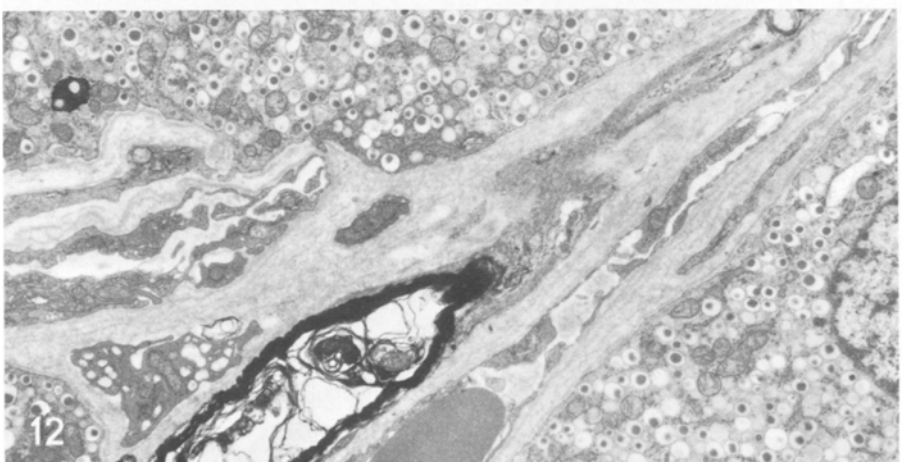
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to a glucose load in the rats bearing tumours induced by streptozotocin and nicotinamide was reported to be divided into two types: some tumour-bearing rats showed fast response of an increase in blood glucose to glucose load, the others showed sluggish response (Dixit and Bauer 1976) permitting a biological response to be used as a method of classification of the tumours.

The distribution of each endocrine cell type showed rather intriguing patterns in this study. The insulin-positive cells were distributed throughout the tumours in most cases. The cells reactive to anti-glucagon antibodies were also distributed throughout some tumours showing ribbon-cord like arrangement. However, the cells reactive either to anti-somatostatin or to anti-pancreatic polypeptide sera were preferentially located in the peripheral parts of the tumours. In cases of human insulinoma, Woodtli and Hedinger (1976) reported that well differentiated trabecular adenoma showed regular immunofluorescence to insulin but the less differentiated medullary adenomas did not. Furthermore, it has been indicated that ribbon-like structures of two or more layers of the cells tended to show positive reactions to either insulin, gastrin or glucagon, and were not preferentially stainable for one of the hormones (Nieuwenhuijzen Kruseman et al. 1978).

The basic proliferative patterns of endocrine tumour cells in the human insulinoma mentioned above were also well recognized in our rat tumours. However, these patterns were usually mixed each other within a single tumour and could not be simply classified in our series. The immunostainabilities of insulin-positive cells were often reduced when compared with those of non-neoplastic islets as seen in cases of human insulinoma (Arnold et al. 1972). Ultrastructurally, many tumour cells contained variable immature forms of secretory granules suggesting the production of immature forms of peptides such as proinsulin, that has also been detected in the tissues of human insulinoma (Creutzfeldt et al. 1973). Therefore, the variation of immunostainability may be ascribed to the difference in cellular differentiation of tumour cells.

The ultrastructure of tumour cells in our study suggested cellular immaturity of the endocrine pancreas. Intranuclear filaments were noticed in the cells in the sequence leading to the cellular differentiation of embryonic islet tissues (Munger 1959) or the cells of human insulinoma (Bencosme et al. 1969; Suzuki and Matsuyama 1973). The appearance of enterochromaffin-like cells or atypical granular cells might also suggest the dedifferentiation of islet cells.

From pharmacological studies, streptozotocin is considered to act selectively on pancreatic islet B cells as a cytotoxic or tumorigenic agent by virtue of its chemical structure (Rakieten et al. 1971; Karunanayake et al. 1976). However, the multiplicity of the endocrine cell types or great variation in the ultrastructure of this tumour cannot can explain the selective tumorigeneity of streptozotocin to islet B cells. It may rather express cellular dedifferentiation of islet cells, or suggest the presence of latent totipotential stem cells which can differentiate into any kinds of endocrine cells of the pancreatic islets.

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