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Endocrine Tumors of the Pancreas Associated with Elevated Urinary Serotonin Excretion

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ABSTRACT

A review is presented of four cases of pancreatic endocrine tumors associated with elevated levels of blood serotonin or urine 5-hydroxyindoleacetic acid (5-HIAA). Clinical, light and electron microscopic observations are presented.

Introduction

Several studies of pancreatic neoplasms having elevated blood and urine serotonin levels have been recorded.[9,12,13,14,21,28] The precise mechanism of production of this biogenous amine within the pancreas has remained elusive, however. Of the four cases of pancreatic endocrine tumors associated with elevated levels of blood serotonin or urine 5-hydroxy indole acetic acid (5-HIAA), three cases were indistinguishable pathologically from carcinoid or islet cell tumors. A fourth case was morphologically identical to oat cell carcinoma of the lung. In one tumor ultrastructural study and comparison with the pulmonary carcinoid tumor aided in more precise classification than was possible by light microscopic techniques, alone.

Materials and Methods

In addition to routine hematoxylin and eosin stains, the formalin fixed material was stained with Schmorl's ferric ferricyanide, Masson-Fontana and Gomori's methenamine silver stains and indophenol reaction (argentaffin granules), Grocott's methenamine silver stain (argyrophil granules), Gomori's method for pancreatic islets (alpha and beta granules), and Gomori's aldehyde fuchsin stain (beta granules). Specimens for electron microscopy were fixed in gluteraldehyde for two hours and postfixed in osmium tetroxide. The epon embedded sections were double stained with uranyl acetate and lead citrate. Examination was done with an RCA EM U4A electron microscope. Analysis for blood serotonin, urinary 5HIAA, histamine, insulin, gastrin, adrenocorticotropic hormone (ACTH), cortisone, glucagon and aldosterone were also performed.

Patient Material

The clinical data of four patients with malignant endocrine tumors of the pancreas are summarized in table I. All patients were caucasian and were in their sixth decade. All cases complained of mid-epigastric abdominal pain which radiated to the back

TABLE I

CASE SUMMARIES

Patient	Clinical Causes	Physical Signs	5-Hydroxy-indole-acetic Acid (5-HIAA) Excretion	Outcome	Tumor Histology
A.P. 59 W/F	Diabetes mellitus, abdominal pain, weight loss diarrhea, fever, 1965. Pancreas biopsy showed "chronic pancreatitis", 1965. Celiac and superior mesenteric arteriogram showed pancreatic tumor and liver metastasis, 1970. Liver biopsy showed metastatic carcinoid or islet cell tumor, 11/70.	Abdominal mass. Left upper quadrant abdominal bruit. 1970	5-HIAA urine spot test positive. Quantitative urine 5-HIAA was 312-354 mg per 24 hr.	Lost to follow-up	Carcinoid-islet cell
R.M. 60 W/F	Weight loss, abdominal pain, 1966. Five hospitalizations between 1966 and 1970 for recurrent abdominal pain. Pancreas biopsy showed "chronic pancreatitis", 7/70. Diabetes mellitus, 1971. Left supraclavicular lymph node biopsy 1972 showed metastatic carcinoid or islet cell tumor. Revue of pancreas biopsy showed similar tumor.	Abdominal mass. Left upper quadrant abdominal bruit. 1971	5-HIAA urine spot test strongly positive X 5, suggesting 5-HIAA was >40 mg per 24 hr.	Living & well, taking 5 FU. 10/73	Carcinoid-islet cell
W.J. 58 W/M	Diabetes mellitus, abdominal pain, weight loss, 1971. Celiac and superior mesenteric arteriogram showed tumor of pancreas with liver metastasis 1971. Liver biopsy showed metastatic carcinoid or islet cell tumor 4/71. Diarrhea controlled with periactin.	Abdominal mass. Left upper quadrant abdominal bruit.	5-HIAA urine spot test positive X 2. Quantitative urine 5-HIAA was 220; 168 mg per 24 hr.	Died of disease 4/72	Carcinoid-islet cell
W.W. 56 W/M	Fatigue, weight loss, abdominal pain, 1968. Red blotches on legs, edema, cyanosis. Diabetes mellitus. Laboratory and liver biopsy showed metastatic oat cell carcinoma, 7/68. Died post-operatively. Autopsy: tumor of pancreas. No lung tumor.	Abdominal mass.	5-HIAA urine spot test positive. Quantitative urine 5-HIAA was 72-84 mg per 24 hr.	Died of disease 1968	Anaplastic (oat cell)

and shoulder and had associated weight loss. Diabetes mellitus was found in all cases. In three cases, a left upper quadrant abdominal bruit was present suggesting compression of the splenic artery by a pancreatic mass. Selective angiography clearly delineated the pancreatic mass and liver metastases in the two patients in whom it was employed. Elevated levels of urine 5HIAA or blood serotonin were observed in all cases. In addition, serum insulin, gastrin, ACTH, cortisone, glucagon, and aldosterone were normal in patient 2. All tumors demonstrated malignant behavior as evidenced at exploratory laparotomy or autopsy.

Pathology

In cases 1 and 3 were proven liver metastases, while in case 2 metastasis was demonstrated to a left supraclavicular lymph node. In case 4 there was a rapidly progressive course which, at autopsy, demonstrated metastatic deposits in the liver, adrenals, kidneys, gallbladder, thyroid and direct extention into the retroperitoneum. Histologically, cases 1, 2, and 3 were indistinguishable from either carcinoid or islet cell tumors. They were remarkably uniform, being highly vascular with nests of closely packed cells separated by finely vascular stroma. There was little variation in size and shape from cell to cell. Cytoplasmic borders were

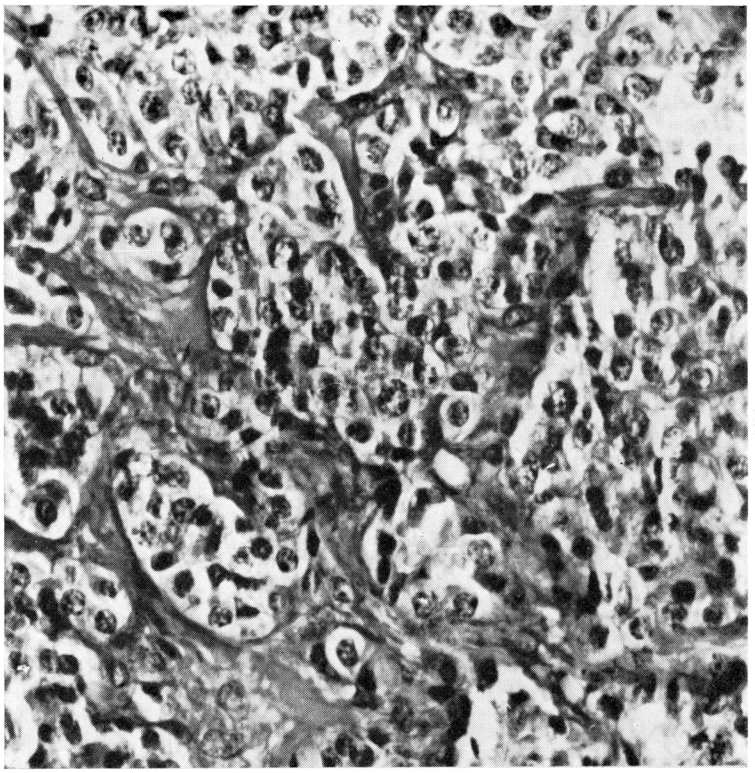

FIGURE 1. Case 1. Metastatic carcinoid-islet cell tumor in liver. (H and E × 250)

indistinct and the cytoplasm was finely granular. Mitoses were extremely rare or absent. Nuclei were regular showing peripheral clumping of nuclear chromatin as shown in figure 1. In contrast, case 4, was histologically indistinguishable from bronchogenic oat cell carcinoma. This tumor was arranged in sheets and ribbons lacking a well defined architecture. The cells were round or bean-shaped having little cytoplasm and sometimes demonstrating the characteristic "crush artifact" of oat cell carcinoma as shown in figure 2.

Histochemistry

Argentaffin, argyrophil and stains for pancreatic alpha and beta granules were compared between all pancreatic lesions and the supraclavicular metastasis from case 2 and two ileal and two appendiceal carcinoids, as well as five carcinoid tumors of bronchial origin. The pancreatic tumor and supraclavicular metastasis from case 2, as well as the intestinal carcinoids stained positively with all argentaffin methods and were mildly argyrophilic as shown in figure 3. Four of five pulmonary carcinoids showed mild argyrophilia, while none had argentaffin granules. The pancreatic tumors from cases 1, 3 and 4 showed negative staining reactions to all methods. No tumor had pancreatic alpha or beta granules.

Electron-Microscopy

Specimens from the metastatic tumor in the supraclavicular lymph node from case 2 were examined ultrastructurally. The cells were of round to oval shape with smooth plasma membranes. No basement membrane was seen either between tumor cells or when these were in contact with sparse pericapillary connective tissue stroma. In regions of cell contact desmosomes were poorly formed and scarce. Most often the

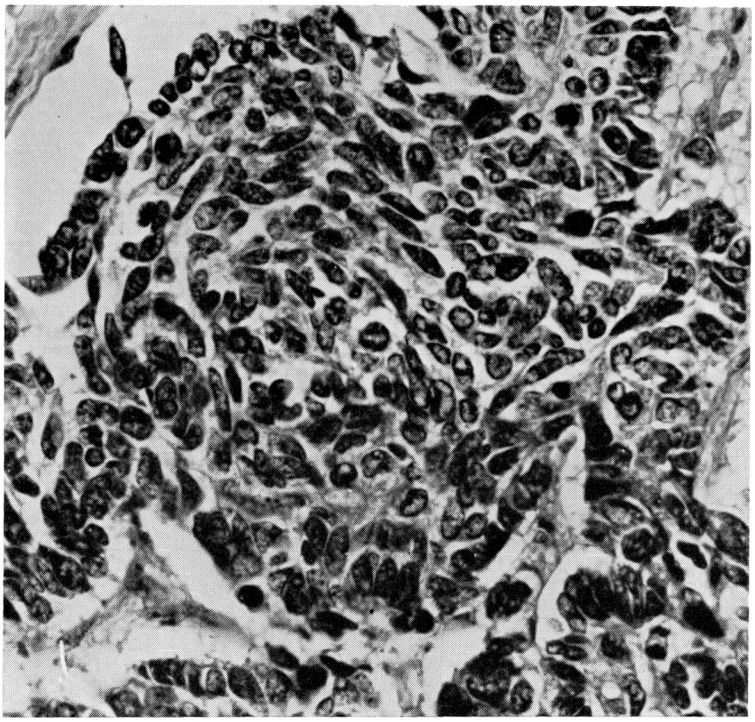

FIGURE 2. Case 4. Primary "oat cell" carcinoma of pancreas. (H and E × 250)

cell membrane of one cell directly abutted on the next without specialized junctions. Occasionally, complex interdigitating cell processes were seen. Infrequently, light cells were interspersed between more numerous cells of higher electron density. The organelles and secretory granules were of similar appearance in both cell types and only their concentration appeared to impart differences in electron density between the two cell types. The cytoplasm of the dark cells showed dilated cysternae of endoplasmic reticulum. Free ribosomes appeared more concentrated in the darker cells.

Numerous homogeneous dense granules of secretory type were present in all tumor cells. These were surrounded by a continuous limiting membrane with a clear halo interspersed between the membrane and dense core. They were of various shapes exhibiting a round, oval, cigar or dumbell appearance. The central core was markedly electron dense and homogeneous. These granules appeared concentrated in the basal pole of the dark cells, while they appeared more evenly distributed in the light cells. Occasionally, the granules appeared to be in close proximity to rough endoplasmic reticulum and present in sacs, suggesting origin from this organelle as shown in figure 4.

The Golgi apparatus was not prominent; where observed, however, numerous small, round, clear vesicles were seen in close proximity. Secretory granules showed no constant association with this organelle.

The mitochondria were elongated or round, having poorly formed, short cristae. The endoplasmic reticulum was sparse and mostly of granular type, usually arranged in parallel arrays. Numerous free and aggregated ribosomes were present in the cytoplasm. Occasional cells contained structures which appeared to form from coalescence of the rough endoplasmic reticulum. These structures showed loss of bound ribosomes and the reticulum membranes

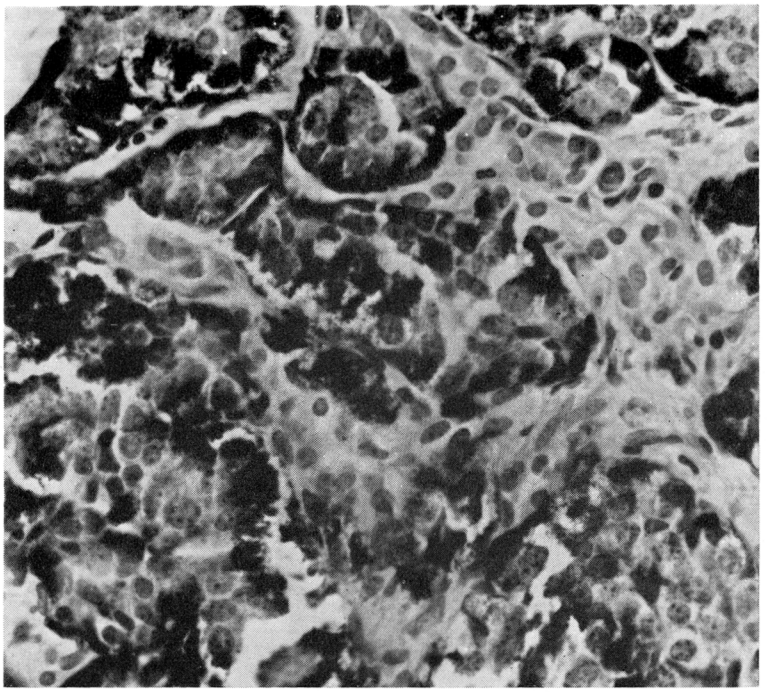

FIGURE 3. Case 2. Primary pancreatic tumor showing positive Fontana-Masson stain for argentaffin granules. (× 250)

were in parallel fashion resembling abbreviated annulate lamellae. Similar structures have been observed in bronchial carcinoids.[3] Occasional cells had dense lysosomes containing lipid material. Intracytoplasmic microfibrils, usually in a perinuclear distribution, were occasionally observed.

The nuclei were round to oval in shape exhibiting irregular, clumped chromatin. Single large nucleoli were occasionally seen but were frequently difficult to distinguish from the coarsely clumped chromatin. The nuclei of the darker cells had a more evenly distributed chromatin pattern.

Ultrastructure of Bronchial Carcinoids

Five carcinoid tumors removed surgically from the lungs or bronchi were compared ultrastructurally to the supraclavicular metastasis from case 2. The basic structure of these pulmonary tumors differed little from one another and, therefore, they will be discussed together.

The cells were oblong or oval and there was little intercellular stroma. Where cytoplasmic membranes were in direct apposition, rare desmosomes were seen. In general, these were somewhat more numerous than in the pancreatic lesion. Occasionally, opposing cell borders formed complex interdigitating structures. Microvilli were frequently present, projecting into a luminal space formed by the confluence of two or more tumor cells. These structures were not observed in the pancreatic tumor. Cells demonstrating clear electron-lucent cytoplasm were interspersed between those which were more electron dense. In the latter cells, the mitochondria were more numerous than in the light variety, and the rough endoplasmic reticulum showed prominent cysternal dilatation. In two tumors round secretory-type granules could be seen in close association to the endoplasmic reticulum, as seen in the pancreatic lesion in figure 5. Previous studies have suggested that these granules may form in association with the Golgi apparatus;[16] however, Toker has suggested origin from the endoplasmic reticulum.[26]

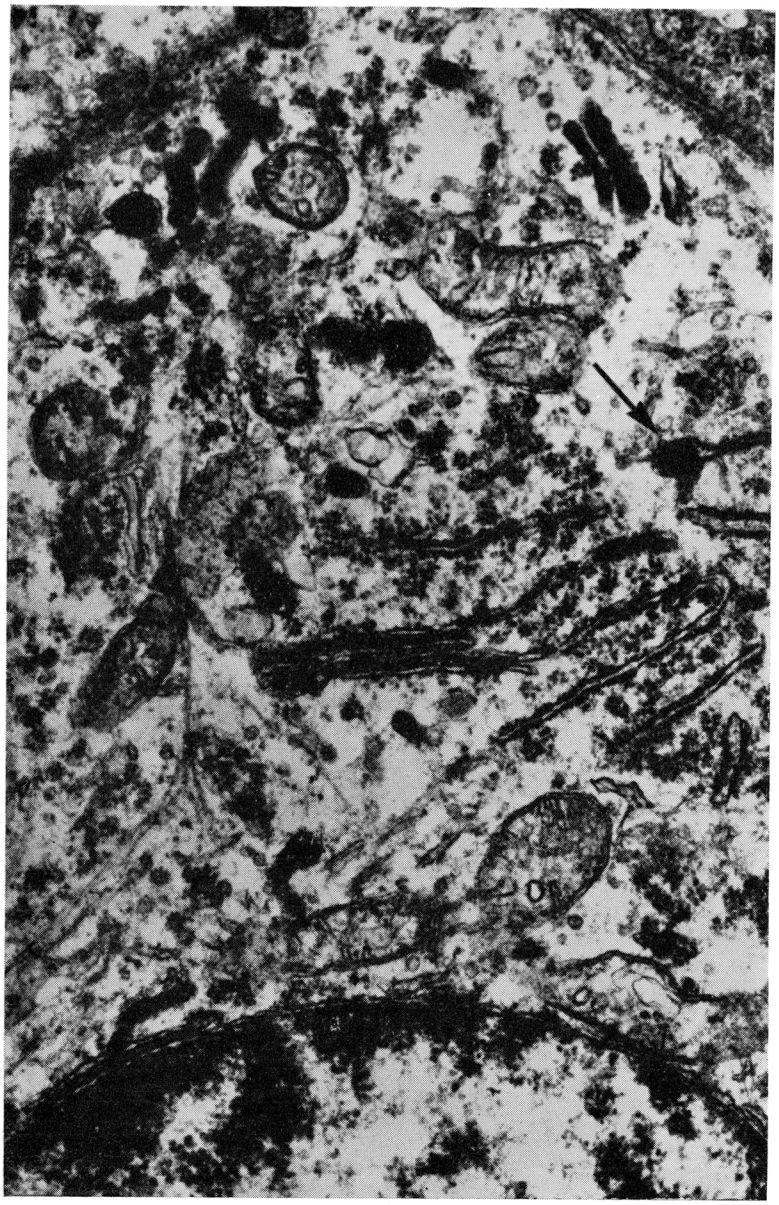

FIGURE 4. Case 2. Supraclavicular metastasis. Dense core pleomorphic secretory granules surrounded by a limiting membrane are seen. One granule is present within the endoplasmic reticulum (arorw). Parallel formation of membranes continuous with the rough endoplasmic reticulum is seen in center. Microfilaments and free ribosomes are also present. (× 43,700)

Secretory granules were more numerous in the darker than the lighter cells. In four of the five tumors, these were of uniform round shape and had a clearly defined continuous limiting membrane separated from the dense core by a clear halo. Little variation in shape was apparent, although differences in size and electron density were seen, both within the same tumor and among the group. In one pulmonary tumor, the secretory granules were identical in size and configuration to those observed in the pancreatic lesion. Indeed, in this tumor there were few ultrastructural differences between it and its pancreatic counterpart.

The rough endoplasmic reticulum of the light cells was sometimes arranged in parallel arrays and numerous free ribosomes

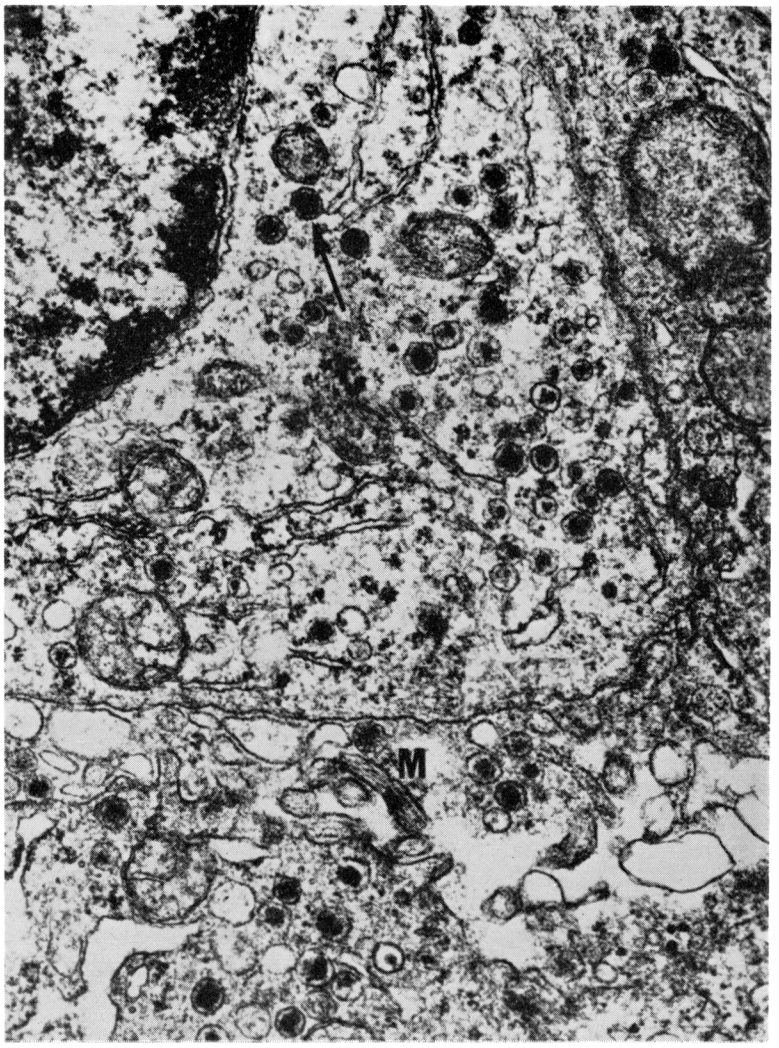

FIGURE 5. Pulmonary carcinoid. Dense, round secretory granules are seen similar to carcinoids of foregut origins. Occasional granules are adjacent to endoplasmic reticulum (arrow). Microvilli are present at M. (× 32,000)

were present. Membrane-bound lysosomes were sparsely seen in both cell types.

The Golgi apparatus was not prominent and the mitochondria were round to oblong with well formed cristae. Occasionally intracytoplasmic microfilaments were observed.

The nuclei of most tumors were round to oval and showed finely dispersed chromatin which was relatively increased in the dark cells imparting a more dense quality.

Discussion

The clinical, biochemical, histochemical and ultrastructural evidence has demonstrated the existence of a carcinoid tumor of the pancreas, indistinguishable from that of bronchial origin and clearly separable from islet cell tumor. The elevated levels of serotonin and 5 HIAA in the blood and urine, the argentaffin reaction of the tumor cells and the comparable ultrastructural morphology of the tumor in case 2 to the bronchial carcinoid are similar to those features of carcinoid tumors of the enterochromaffin cells of the gastrointestinal tract and lung and unlike those of normal pancreatic islets or islet cell tumors.[4,16,17,19,23,25] The features that enable separation of these

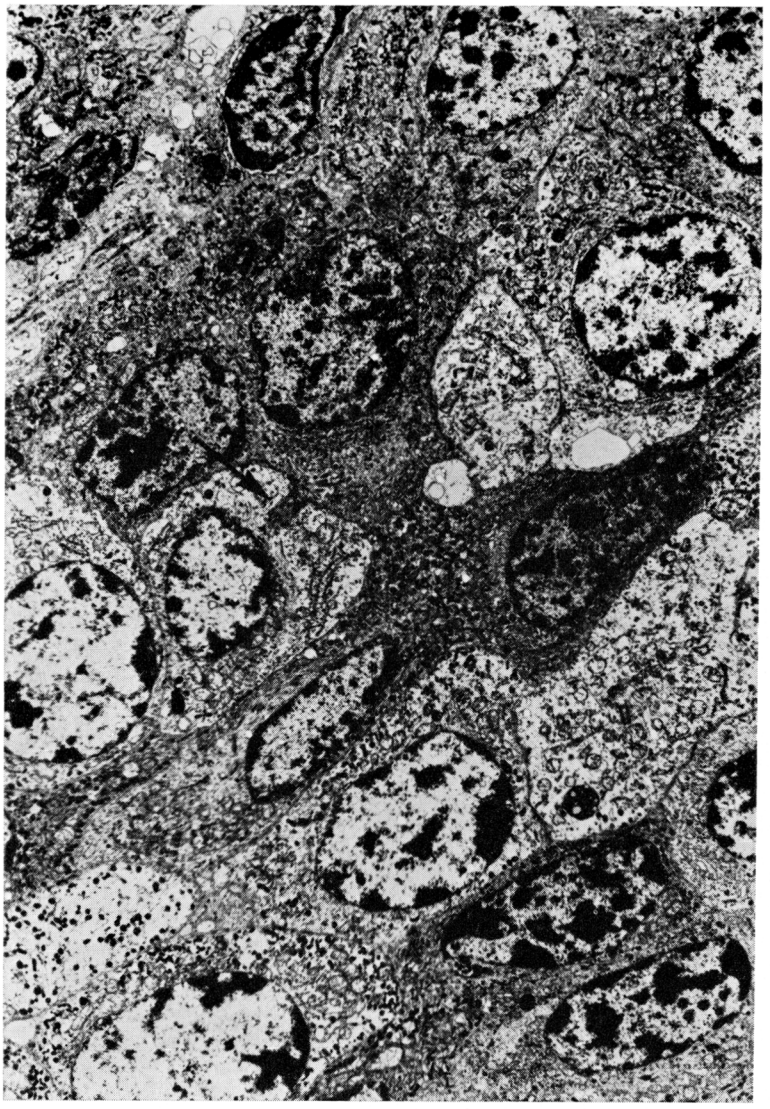

FIGURE 6. Pulmonary carcinoid. Pleomorphic enterochromaffin type secretory granules identical to those of pancreatic tumor are seen in both light and dark cells. A portion of a swollen endothelial cell with large vesicles is present. (× 12,300.)

histologically similar neoplasms are based mainly on the ultrastructural morphology of their secretory granules. Most electron microscopic studies of pancreatic islet cell tumors have shown the secretory granules to correspond to the cell types present in the normal pancreatic islets and to correlate with the known physiological activity of the parent cell or origin. Thus, the granules of insulin,[25] gastrin,[23] and glucagon[18] secreting tumors are round or crystaloid and of fairly uniform type compared to the granules observed in some carcinoid tumors of the small intestine,[4,16] which may have pleomorphic bananna or cigar shaped granules as were observed in the current case. Islet cell tumors have not been observed to contain similar secretory granules.

Similarities Between the Bronchial Carcinoids and Pancreatic Tumor

These similarities are of interest and support a common cell of origin. The general cellular features of these neoplasms were

quite similar. Both the lung and pancreatic lesions were composed of light and dark cells having complex interdigitating plasma membranes and desmosomes. The configuration of the mitochondria, rough endoplasmic reticulum, abundance of free ribosomes, sparse golgi apparatus, microfilaments, presence of secretory granules and nuclear morphology indicate a closely allied structure. Both tumors had abundant granules of secretory type. In four of the five pulmonary lesions these granules were round and regular in shape corresponding to those previously observed in bronchial carcinoids. One case, however, showed pleomorphic granules of identical size and shape as the pancreatic tumor. This finding again suggests that the latter lesion was derived from precursor cells similar or identical to those of the bronchial neoplasm and, therefore, were of enterochromaffin origin.[2] This is further substantiated by recent ultrastructural studies showing enterochromaffin like cells similar to those found in the intestines to be present in the pancreas of several species[6,20] and man[6] and the presence of hydroxy-indoles in the pancreatic islets by ultrastructure cytochemical,[15] spectrophotofluorimetric,[7] biochemical[8] and radioautographic methods.[10]

The studies of Forssman et al have shown that the rat gastrointestinal tract contains several different types of endocrine cells each having their own site specificity within the intestine.[11] Similar studies in man by Black have shown that intestinal enterochromaffin cells vary in morphology depending on whether they are located in the embryological derivatives of the fore, mid, or hindgut.[4] Black furthermore supports the concept of Williams and Sandler that carcinoid tumors arising in any of these subdivisions recapitulate the morphologic characteristics of the parent cell of origin.[28] Thus tumors of foregut origin usually contain round dense-core secretory granules while those of midgut origin contain pleomorphic oblong, cigar or dumbell shaped granules. Both studies have shown, however, that cells usually more numerous in the midgut may be found in the derivatives of the embryological foregut. The structure of the secretory granules in the present pancreatic tumor as well as its pulmonary counterpart were of the pleomorphic type described as enterochromaffin type II cells,[20] which are usually found in carcinoid tumors of the midgut. The remainder of the bronchial carcinoids had round secretory-like granules similar to those usually observed in foregut tumors.[1,2,4,26]

While it is difficult to draw firm conclusions based solely on morphologic observations of the fine structure of secretory granules, taking into account possible variations in maturity, secretory activity and neoplastic transformation, previous observations in man that pleomorphic granules of secretory type are seen in intestinal enterochromaffin cells and carcinoids and not pancreatic islet cells strongly suggest an enterochromaffin origin of the pancreatic tumor. While round enterochromaffin granules, usually seen in the foregut,[4,11] may resemble those of pancreatic alpha cells and the granules of some beta cell tumors, the pleomorphic structure of the granules in the present case is against their being of islet cell origin. This evidence supports the presence of enterochromaffin cells associated with the exocrine ducts in the human pancreas.[6]

Relationship Between the Argentaffin System and the Pancreas

An intimate relationship is suggested by direct and circumstantial evidence. Embryological studies have shown that argentaffin cells are present along the entire foregut and migrate into the bronchi and pancreatic ducts and islets.[27] The carcinoid syndrome accompanied by hypoglycemia has been associated with bronchial carcinoids[24] and pancreatic islet cell tumors.[14]

Tumors of the pancreas showing argentaffin,[22] as well as both argentaffin and alpha and beta granules have also been reported.[12] Elevated levels of serotonin have been recorded in the Zollinger-Ellison syndrome. Indeed, it has been suggested that the islet cell and argentaffin cell are derived from the same neuroectodermal precursor.[27]

In man, few instances of direct tissue analysis, or specialized ultrastructure cytochemistry and radioautography have been recorded. It is difficult, therefore, to exclude tumors of complex derivation, tumor storage rather than actual production of humoral substances, or associated hyperfunction of adjacent islet tissue, in most cases. Circumstantial and direct evidence suggest, however, that both serotonin and insulin may be produced and stored in the islets of Langerhans.[10,15]

The cell of origin of the oat cell tumor is more speculative than that of the better differentiated lesions. However, the authors believe that this lesion is also derived from pancreatic enterochromaffin cells. This tumor was morphologically identical to oat cell carcinoma of the lung, and the clinical course was similar to this highly malignant tumor. Bronchogenic oat cell carcinoma has been known to be hormonally active, and the carcinoid syndrome has been reported with this tumor. Electron microscope examination of these lesions has shown neurosecretory granules identical to those seen in carcinoid tumors of the bronchus.[2] This evidence suggests that oat cell carcinoma of the lung and bronchial carcinoid are histogenetically related tumors, one the anaplastic variant of the other. The presence of this type of tumor in the pancreas, also of foregut derivation, suggests a relationship to carcinoid tumors of the pancreas.

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