Metastatic Islet Cell Tumor With ACTH, Gastrin, and Glucagon Secretion

Clinical and Pathologic Studies With Multiple Therapies

JACOB LOKICH, MD,* ALBERT BOTHE, MD,† CARL O'HARA, MD,‡ AND MICHELINE FEDERMAN, PHD‡

A patient with metastatic islet cell carcinoma demonstrated multiple clinical syndromes simultaneously with secretion of ACTH, gastrin, glucagon, and serotonin. Hepatic arterial embolization resulted in an initial decrease in all secretory products, which was sustained for glucagon and serotonin. Recrudescence of the Cushings and Zollinger-Ellison syndrome was managed by surgical extirpation of the primary tumor and regional metastases as well as bilateral adrenalectomy. Electron microscopy and immunocytochemistry of the primary tumor and the metastatic lesions revealed (1) the presence of multiple types of granules within single cells and, (2) different patterns of secretory profiles in different tumor sites.

Cancer **59:2053–2058, 1987.**

ENDOCRINE TUMORS of the pancreas are rare, with less than 250 new cases estimated in the US annually for a prevalence of one per 100,000 population base.[1] In one of the largest series, 85% of islet cell tumors were "functioning" related to insulin secretion (60%); gastrin secretion (18%) or the secretion of other polypeptides, including vasoactive intestinal peptide (VIP) and adrenocortical hormone (ACTH) in 7%.[2] Cushings syndrome secondary to the secretion of ACTH from an islet cell tumor has been reported in 43 patients, 8 of which (19%) also demonstrated gastrin secretion with or without the Zollinger-Ellison syndrome.[3,4]

A patient presenting with the signs and symptoms of both Cushing's syndrome and Zollinger-Ellison syndrome was found to have a metastatic islet cell tumor that also secreted glucagon and serotonin. The clinical course and therapeutic management included the use of hepatic arterial embolization, chemotherapy, and surgical debulking with end organ ablation. Biochemical studies, including hormone monitoring and pathologic studies of both the primary and metastatic lesions, provided a basis for understanding the clinical course observed.

From the Departments of *Medicine (Section of Medical Oncology), †Surgery, and ‡Pathology, New England Deaconess Hospital, Harvard Medical School, Boston, Massachusetts.

Address for reprints: J. Lokich, MD, Section of Medical Oncology, The Cancer Center, Harvard Medical School, 125 Parker Hill Avenue, Boston, MA 02120.

Accepted for publication January 12, 1987.

Case Report

A 23-year-old woman presented in June 1985 with a facial swelling and rash. On subsequent evaluation, she was found to have a number of the clinical components of Cushing's syndrome, including mild hypertension (140/98 mmHg); glucose intolerance requiring insulin; and severe osteoporosis.

Initial laboratory values demonstrated an elevated AM cortisol level at 86 μg/dl (normal, 6–25 μg/dl) and an elevated ACTH level at 163 pg/ml (normal, 0–100 pg/ml). A computed tomography (CT) scan of the abdomen demonstrated an extensive tumor involving the retroperitoneum and pancreas that was contiguous with the left adrenal gland.

The patient previously had a history of ulcer symptoms for at least 2 years before presentation. A typical lesion was demonstrated by an upper gastrointestinal (GI) series in the previous year. Because an ACTH-secreting tumor was present in the retroperitoneum, other secretory products were sought. A serum gastrin level was 6260 pg/ml (normal, 0–100 pg/ml). The calcitonin level was normal. The glucagon level was 530 pg/ml (normal, 40–60 pg/ml), and the 24-hour urinary 5-hydroxyindol acetic acid level was elevated at 19.6 mg. Twenty-four-hour urinary free cortisol was greater than 16,000 μg/dl (normal, 35–120 μg/dl). Serum electrolytes were normal on initial presentation, but liver function tests revealed elevated serum glutamic oxaloacetic transaminase and lactate dehydrogenase levels as well as a decrease in serum calcium to 7.3 mg/dl and in serum phosphate to 1.8 mg/dl.

In August 1985, the patient underwent abdominal exploration. An extensive tumor involving the pancreas and entire retroperitoneum was identified, as were multiple lesions of the surface of the liver. Biopsies were obtained, and revealed all of the histologic features characteristic of islet cell carcinoma. Initial studies of the tissue employing the immunoperoxidase technique

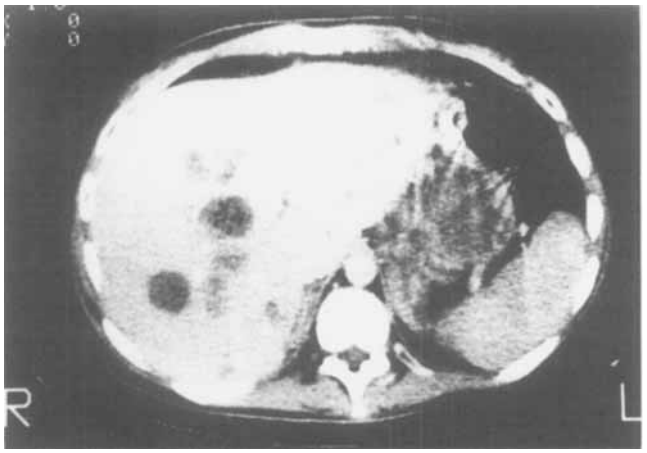

FIG. 1. Computerized tomographic of the liver after HAE, demonstrating gas within some of the hepatic metastases.

demonstrated the presence of pancreatic polypeptide, serotonin, gastrin, and cholecystakinin.

Approximately 1 month from the initial surgical exploration, the patient underwent hepatic arterial embolization for control of the tumor in the liver. Follow-up CT of the liver demonstrated air or gas within some of the lesions in the right lobe of the liver (Fig. 1).

After embolization, the patient experienced a marked reduction in gastrin, ACTH, and glucagon levels. Over the 3 months that followed, the patient received intermittent chemotherapy, initially with streptozotocin for two courses at 4-week intervals, subsequently with streptozotocin combined with 5-fluorouracil, and finally, with a 5-day course of DTIC.

In January 1986, the patient presented with recrudescent florid Cushing's syndrome with hypertension and insulin-dependent diabetes mellitus as well as persistent abdominal discomfort. The hormone parameters, including ACTH and gastrin levels, were persistently elevated and rising. Reevaluation using CT demonstrated a large cystic lesion in the pancreas in addition to the midbody lesion (Fig. 2). The hepatic lesions in the right lobe were diminished markedly, with a persistence of the left lobe lesions at a size comparable to that seen 4 months previously. The patient underwent surgical exploration, and the pancreatic tumor involved the body and tail of the pancreas with a 15-cm cystic lesion extending posteriorly. Cyst decompression by needle aspiration was followed by subtotal pancreatectomy leaving the head of the pancreas *in situ*. Bilateral adrenalectomy was performed following exploration of the retroperitoneum with removal of massively enlarged glands.

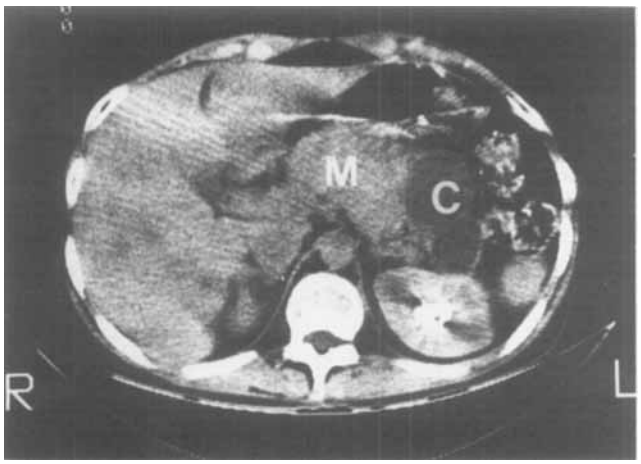

FIG. 2. Computerized tomographic scan of the upper abdomen demonstrating the pancreatic mass (M) in the body and a large cyst (C) in the tail.

Sequential monitoring of ACTH, gastrin, and glucagon levels following embolization and in the immediate and extended postoperative periods is illustrated graphically in Figure 3. Circulating levels of gastrin, glucagon, and ACTH decreased promptly within 2 weeks of embolization with glucagon remaining normal throughout the subsequent clinical course. ACTH levels returned to normal, but gastrin levels were elevated persistently. Both rose to preembolization levels by the end of the 4th month. After partial pancreatectomy the ACTH and gastrin levels decreased acutely for a brief interval.

Results

Pathologic Condition

The tumor consisted of cords and solid sheets of small cells with round regular nuclei, inconspicuous nucleoli, and scant cytoplasm characteristic of an islet cell tumor. Minimal pleomorphism and infrequent mitoses were present, and fibrosis was prominent.

Immunohistochemistry

Paraffin sections were cut at 6 micra, deparaffinized, and subjected to a routine peroxidase–antiperoxidase (PAP) immunoperoxidase procedure. Sections were exposed to methanol peroxide and 2% swine serum as blocking reagents. Sections were then incubated overnight at 4°C with antisera to insulin, glucagon somatostatin, gastrin, VIP, (Dako, Santa Fe, CA) 1:500, 1:1000); anti-ACTH (Dako 1:100, 1:200) anti-calcitonin (Dako, 1:300, 1:600), anti-pancreatic polypeptide (Dr. Ron Chance, Eli Lilly, Indianapolis, IN, 1:2000, 1:5000). After appropriate washes in Tris buffer pH 7.6 the sections were incubated with swine anti-rabbit immunoglobulin (Dako, 1:60) and PAP (Cappel Laboratories, Cochranville, PA, 1:300). The antigens were localized with 3, 3-diaminobenzidine-tetramonohydrate (20 mg/10 ml in Tris buffer). A monoclonal antiserotonin (Dr. N. Penney, Miami University, FL, 1:100, 1:200) was followed by peroxidase-conjugated swine anti-rabbit immunoglobulin 1:60 (Dako). Normal pancreas (insulin, glucagon, somatostatin, and pancreatic polypeptide [PP]) appendix (serotonin), VIPoma (VIP), stomach (gastrin), pituitary (ACTH), and medullary carcinoma of thyroid (calcitonin) were used as positive controls. Tris buffer was substituted for primary antisera for

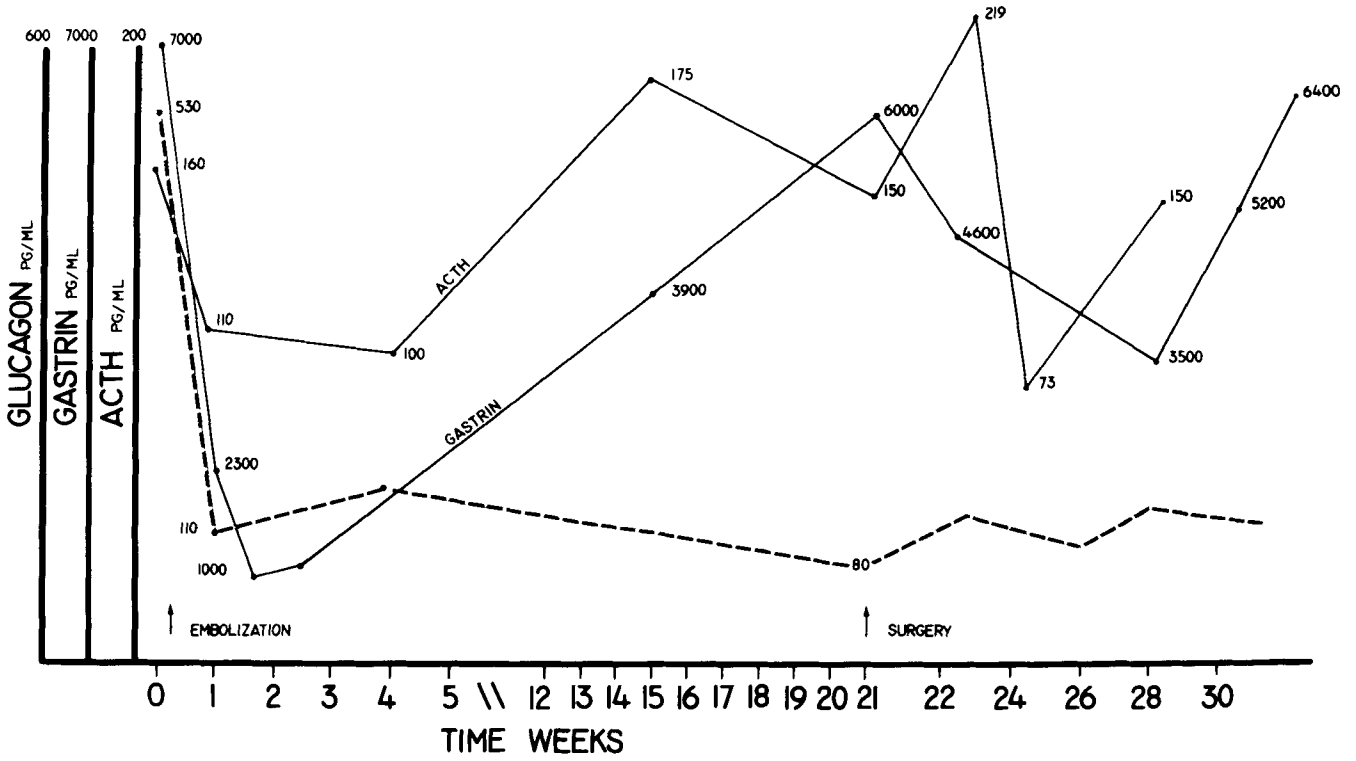

FIG. 3. Sequential levels of ACTH, glucagon, and gastrin during the clinical course and treatment employing hepatic arterial embolization, chemotherapy, and palliative surgical resection.

negative controls. All slides were counterstained with hematoxylin.

Separate specimens were examined from the primary pancreatic tumor nodal metastases and the liver metastases. Tissue was fixed in formalin, alcohol zinc formol, Bouin's solution, and B5. Immunoperoxidase studies on paraffin sections of the primary tumor revealed positive staining of occasional cells in the primary lesion for glucagon and insulin and rare cells stained for pancreatic polypeptide and serotonin. The major staining was for gastrin and approximately 5 to 10% of the cells were positive. No staining was obtained for somatostatin, VIP, or calcitonin. Despite repeated attempts in three different laboratories no staining was obtained for ACTH.

In contrast, the liver metastases revealed positive staining of only occasional clusters of cells for gastrin and VIP with rare cells positive for serotonin and pancreatic polypeptide. No staining was obtained for insulin, glucagon, somatostatin, ACTH, or calcitonin. A different pattern of staining was observed for the nodal metastases (Table 1) in which gastrin and VIP were identified but stains for other hormones were negative.

Electron Microscopy

The most striking ultrastructural feature of the cells examined was the variety of granules within the cytoplasm. The contents of the membrane bound granules were heterogeneous, ranging from uniform, very electron dense cores to grainy, pale secretory material. The granules also ranged in size from approximately 100 nm to 500 nm, with the majority falling in the 200 to 250 nm range (Fig. 4). The heterogeneity of the granules was seen within single cells and adjacent cells demonstrated different

TABLE 1. Comparison of Histochemical Staining for Secretory Products in Separate Tumor Sites

	ACTH	Gastrin	Glucagon	Insulin	PP	VIP	Serotonin	Somatostatin calcitonin
Primary Tumor	No	5–10%	Occasional	Occasional	Rare	No	Rare	No
Liver Metastases	No	Occasional	No	No	Rare	Occasional	Rare	No
Lymph Node Metastases	No	5–10%	No	No	No	5–10%	No	No

ACTH: adreno cortical hormone; PP: pancreatic polypeptide; VIP: vasoactive intestinal peptide.

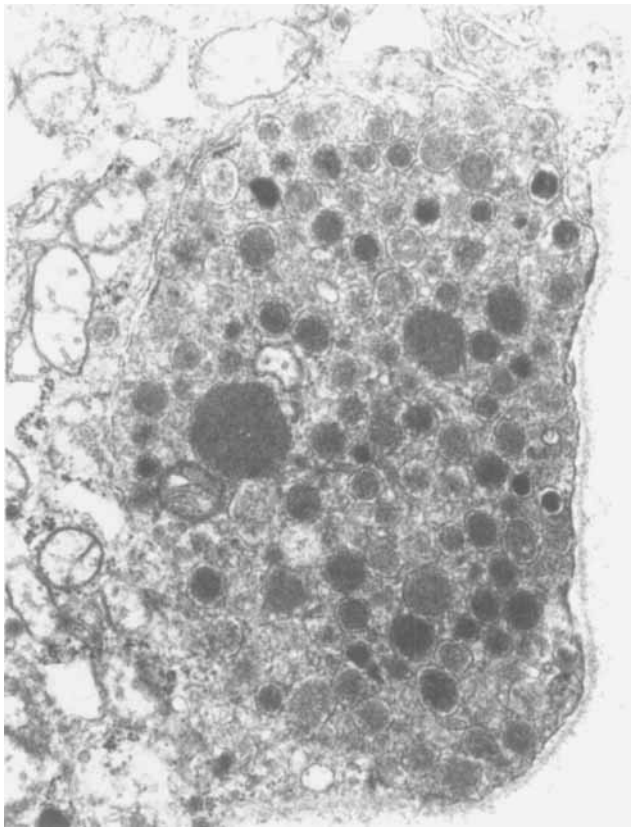

FIG. 4. Electron micrograph shows heterogenous granule population in same cell crowding out all other organelles. Granules range in size from 100 nm to 500 nm (×25,000).

granule characteristics (Fig. 5). In some cells, the entire cytoplasm was replete with granules to the almost total exclusion of other organelles, while in other cells few granules were seen (Fig. 6). Other ultrastructural features included the presence of an external lamina surrounding groups of cells, desmosomes, and filaments.

Discussion

Although 85% of islet cell tumors are functioning, the proportion with ACTH secretion is small representing only three of 168 and four of 94 patients in two reported series.[2,5] Gastrin secretion with the Zollinger-Ellison syndrome is much more common, representing 18% of all islet cell tumors as reported by Kent *et al.*[2] Although the production of multiple hormones is increasingly recognized as a consequence of more sophisticated immunoperoxidase studies, the concurrent development of clinical Cushing's and Zollinger-Ellison syndromes is rare, with only eight patients previously reported (Table 2).[3,4,6–11] Five of the eight patients were female, with an age range of 19 to 68 years. Adrenocorticotropin hormone ACTH levels were only slightly elevated in four of the five patients in whom the information was available. In contrast, gastrin levels were enormously elevated in all but one patient. Using immunohistochemistry, a variety of additional ectopic hormones were identified in patients studied. Median survival was 18 weeks (range, 1 day–12 years). The 5-year survival of the 42 patients reported with Cushing's syndrome secondary to islet cell tumor is 16%.[3]

The present case report is distinctive from previous reports of the combined syndromes (Cushing's and Zollinger-Ellison) in that the clinical manifestations included additional syndromes (hypocalcemia and glucagon related cutaneous eruption). In addition, therapeutic management employing hepatic arterial embolization for initial tumor control and tumor "debulking" and end organ ablation (adrenalectomy) successfully controlled the hormonal manifestation of the tumor. Finally, detailed immunohistochemistry studies and electromicroscopy of the primary tumor and metastatic sites in the liver, as well as in the lymph nodes demonstrated the heterogeneity of the tumor and the totipotential secretory function of individual tumor cells.

Although chemotherapy, particularly streptozotocin, 5-fluorouracil, and DTIC,[12,13] is effective in islet cell tumors with response rates up to 60% or greater, our patient did not demonstrate a substantive effect from such therapy.

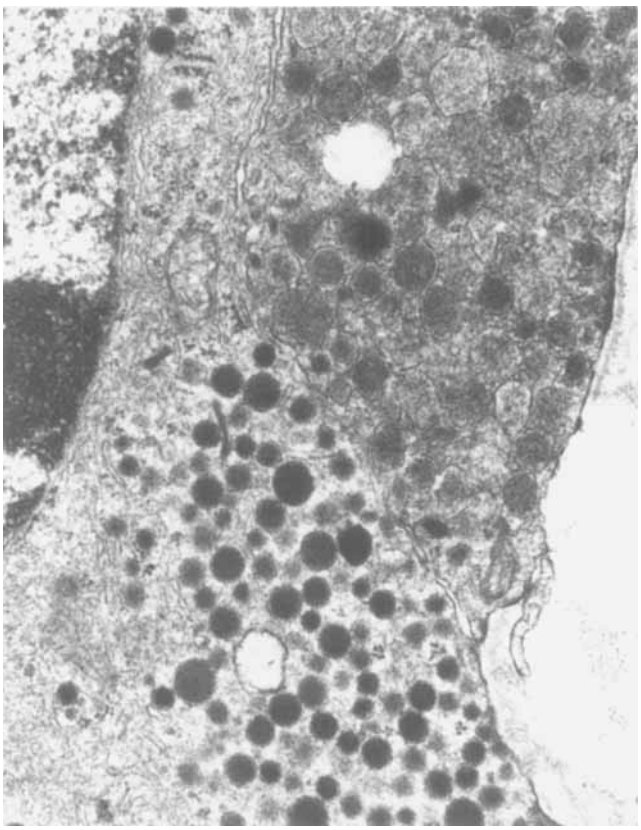

FIG. 5. Electron micrograph to two adjacent cells with granules of different densities and size (×25,000).

The initial decrement in ACTH and gastrin presumably was related to the hepatic arterial embolization in that the tumor in the right hepatic lobe was the only area to demonstrate measurable regression by sequential CT scanning. Hepatic arterial embolization (HAE) to control hormonal syndromes including hypercalcemia[14] and the carcinoid syndrome[15] have been reported. Hepatic embolization also has been reported in four patients with islet cell tumors producing gastrin, ACTH, or both. In all four patients, a decrease in the size of metastasis or circulating levels of the hormones was achieved.[16] HAE obviously effects only the portion of the tumor mass in the liver and not the primary tumor. Pharmacologically active mediator substance was acutely reduced in the patients, which implies that the metastasis was the predominant producer of the hormones. The rationale for embolization is largely that such tumors are extraordinarily hypervascular and the ability to achieve acute thrombosis of the arterial supply by the use of gel foam pledgets or metal coils provides an expeditious method for immediate and complete initial control of the clinical syndrome.

End organ ablation is another means of dealing with the hormonal syndromes secondary to metastatic islet cell tumors. Prior to the availability of H2 blockers total gastrectomy for the gastin mediated Zollinger-Ellison syndrome was commonly employed. Adrenalectomy as a means of dealing with the Cushing's syndrome has been relatively uncommon, related in part to the greater frequency of malignancy in the ACTH-producing tumors and the relatively short survival of these patients. A variety of medications to block steroid synthesis are available including mitotane, aminoglutethamide, and ketoconazol. The extraordinary hypertrophy of the adrenal glands and the massive production of corticosteroids under the stimulus of the autonomous production of ACTH by the malignancy, however, more than likely precludes the ability to deliver a sufficient dose of those enzyme blockers to effect sufficiently production of the steroids.

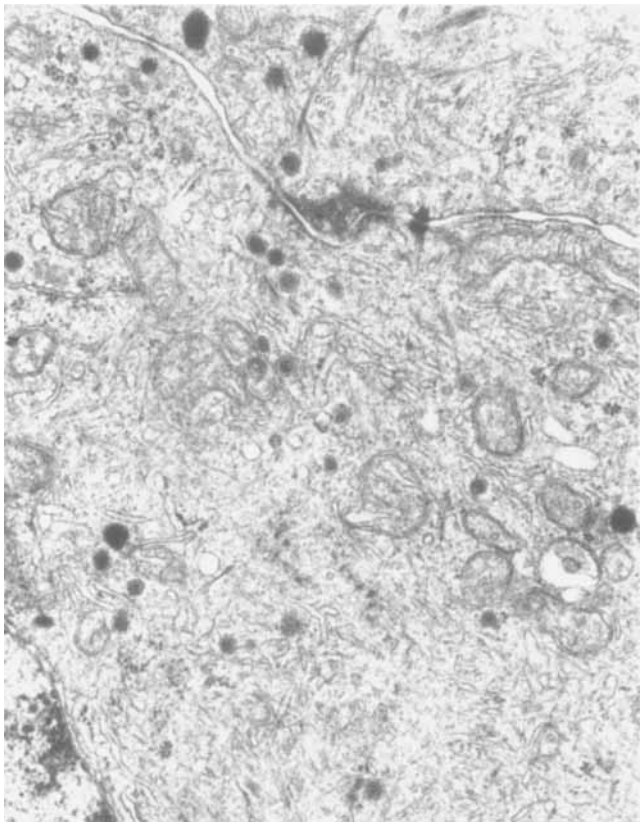

FIG. 6. Electron micrograph showing portion of two cells (1, 2) with few small granules measuring approximately 100 nm (×25,000).

Pathologic studies of the primary tumor and metastatic lesions in the liver were of particular interest. Electromicroscopy identified secretory granules of various configurations in the tumor and some cells contained more than one type of granule. Immunocytochemical analysis for a variety of peptides identified gastrin, pancreatic polypeptide, serotonin, and glucagon. ACTH was not identified in the tumor, and probably was related to the inadequacy of the assay system. Electronmicroscopic studies suggested

TABLE 2 Published Reports of Combined ACTH and Gastrin Secretion From Islet Cell Carcinoma

Patient no.	Age/sex	Ref. no.	Peptide levels (pg/ml)		Histochemistry	Survival
			ACTH	Gastrin		
1	48/F	7*	ND	ND	Glucagon, MSH	36 mo
2	35/F	10	ND	ND	ND	1 d
3	59/M	6	ND	ND	ND	12 yr
4	41/M	9	200	300	ND	3 mo
5	68/F	8	115	1700	Calcitonin, somatostatin, endorphin	20 mo
6	19F	11*	136	1676	ND	3 d
7	45/F	3	1160	939	ND	33 mo
8	67/M	4	447	1100	HCG	18 w
9	23/F	Present case*	173	7000	Insulin, PP	Alive, 6 mo

ACTH: adrenocortical hormone; PP: pancreatic polypeptide; MSH: melanocyte-stimulating hormone; HCG: human chorionic gonadotrophin. ND: no data reported.

* Adrenalectomy

that whether multiple hormones were being produced by a single cell using a double labeling technique, although it was not possible to study. A quantitative estimate of hormone production in the primary tumor and in tumor from metastatic sites was suggested that the metastatic lesions contains substantially greater amounts of gastrin and VIP compared to the primary and different metastatic sites demonstrated different patterns of hormone production.

The analysis of the pathologic condition may partly explain the patterns of change in circulating hormone levels in relationship to therapy. The rapid fall in ACTH, gastrin, and glucagon following HAE suggests that at that time the predominant site of production for these hormones was the liver metastases. Subsequently, the primary tumor and lymph node metastases represented the major source of gastrin and ACTH production and glucagon was not secreted. The ACTH decreased promptly following surgical resection of those tumor bearing areas and gastrin levels remained elevated possibly because the residual disease was in the liver where gastrin was prominent on immunocytochemical analysis.

Immunochemistry has helped clarify some but not all of the clinical pathologic aspects of islet cell tumors of the pancreas. Many and possibly all islet cell tumors produce multiple hormones. Mukai *et al.* 26 found that of 77 islet cell tumors (33%) demonstrated multiple hormones in the tissue.[17] The clinical syndromes, however, usually are related to only one substance.[18]

The simultaneous development of two or more clinical syndromes is most common with gastrin, which has been reported in association with the Werner–Morrison syndrome and vasoactive intestinal polypeptide production[19] as well as with Cushing's syndrome. Insulinoma and glucagonoma have not been reported yet as part of multiple syndromes, although islet cell tumors often contain either or both of these hormones. PP was present in the tumor in our patient, and is commonly identified in functioning as well as nonfunctioning islet cell tumors but it does not produce an identifiable clinical syndrome.[20] The current case report of multiple ectopic hormone production with multiple mainfest syndromes is unique, and additional unknown substances also may have been produced. The correlation of pathologic findings with hormonal monitoring and the clinical course during a variety of therapeutic maneuvers was imperfect but provided some insight into the utility and limitations of present technology.

REFERENCES

1. Maldower RE, Connelly RR. Epidemiology of pancreatic cancer in course. *Gastroenterology* 1968; 55:677–686.
2. Kent RB, Van Heerden JA, Weiland LH. Nonfunctioning islet cell tumors. *Ann Surg* 1981; 193:185–190.
3. Clark ES, Carney JA. Pancreatic islet cell tumor associated with Cushing's Syndrome. *Am J Surg Pathol* 1984; 8:917–924.
4. Allison MC, Renfrew CC, Webb WJS, Chappel ME, Pounder RE. Neuroendocrine islet cell tumor producing gastrin and ACTH in a patient with calcifying chronic pancreatitis. *Gut* 1985; 26:426–428.
5. Heitz PO, Kasper M, Polak JM, Kloppel G. Pathology of the endocrine pancreas. *J Histochem Cytochem* 1979; 27:1402–1407.
6. Geokas MC, Chun JY, Dinan JJ, Beck IT. Islet-cell carcinoma (Zollinger-Ellison syndrome) with fulminating adrenocortical hyperfunction and hypokalemia. *Can Med Assoc* 1965; 93:137–143.
7. O'Neil LW, Kipnis DM, Luse SA, Lacy PE, Jarrett L. Secretion of various endocrine substances by ACTH-secreting tumors: gastrin, melanotropin, norepinephrine, serotonin, parahormone, vasopressin, glucagon. *Cancer* 1968; 74:1219–1232.
8. Asa SL, Kovacs K, Killinger DW, Marcon N, Platts M. Pancreatic islet cell carcinoma producing gastrin, ACTH, alpha-endorphin, somatostatin and calcitonin. *Am J Gastroenterol* 1980; 74:30–35.
9. Kyriakides GK, Silvis SE, Ahmed M, Vennes JA, Vogel SB. Gastrinoma associated with common bile duct obstruction and the ectopic production of ACTH. *Am J Surg* 1979; 137:800–803.
10. Law DH, Liddle GW, Scott HW, Tauber SD. Ectopic production of multiple hormones (ACTH, MSH and gastrin) by a single malignant tumor. *N Engl J Med* 1965; 76:729–733.
11. Lyons DF, Eisen BR, Clark MR, Pysher TJ, Welsh JD, Kem DC. Concurrent Cushings and Zollinger-Ellison syndromes in a patient with islet cell carcinoma. *Am J Med* 1984; 76:729–733.
12. Moertel CG, Hanley JA, Johnson LA. Streptozocin alone compared with streptozocin plus fluorouracil in the treatment of advanced islet-cell carcinoma. *N Engl J Med* 1980; 303:1189–1194.
13. Kessinger A, Foley JF, Lemon HM. Therapy of malignant APUD cell tumor effectiveness of DTIC. *Cancer* 1983; 51:790–794.
14. Attali P, Houssin D, Roche A, Buffet C, Bismuth H, Etienne JP. Hepatic arterial embolization for malignant hypercalcemia in hepatocellular carcinoma. *Dig Dis Sci* 1984; 29:466–469.
15. Moertel CG, May GR, Martin JK, Rubin J, Schutt AJ. Sequential hepatic artery occlusion (HAO) and chemotherapy for metastatic carcinoid tumor and islet cell carcinoma. *Proc ASCO* 1985; 4:80.
16. Carrasco CH, Chuang VP, Wallace S. Apudomas metastatic to the liver: treatment by hepatic artery embolization. *Radiology* 1983; 149:79–83.
17. Mukai K, Greider MH, Grotting JC, Rosai J. Retrospective study of 77 pancreatic endocrine tumors using the immunoperoxidase method. *Am J Surg Pathol* 1982; 6:387–399.
18. Heitz PU, Kasper M, Polak JM, Kloppel G. Pancreatic endocrine tumors: immunocytochemical analysis of 125 tumors. *Hum Pathol* 1982; 13:263–271.
19. Barragry TP, Wick MR, Delaney JP. Pancreatic islet cell carcinoma with gastrin and vasoactive intestinal polypeptide production. *Arch Surg* 1985; 120:1178–1181.
20. Tomita T, Friesen SR, Kimmel JR. Pancreatic polypeptide-secreting islet cell tumor: A follow-up report. *Cancer* 1986; 57:129–135.