

## Brief Report

## MALABSORPTION DUE TO CHOLECYSTOKININ DEFICIENCY IN A PATIENT WITH AUTOIMMUNE POLYGLANDULAR SYNDROME TYPE I

CHRISTOPH HÖGENAUER, M.D., RICHARD L. MEYER, M.D.,  
GEORGE J. NETTO, M.D., DIANA BELL, M.D.,  
KATHERINE H. LITTLE, M.D., LAURA FERRIES, M.D.,  
CAROL A. SANTA ANA, B.S., JACK L. PORTER, M.S.,  
AND JOHN S. FORDTRAN, M.D.

**A**UTOIMMUNE polyglandular syndrome type I is an autosomal recessive inherited disease caused by mutations in the autoimmune regulator gene.<sup>1</sup> Its hallmarks are the failure of multiple endocrine glands due to an autoimmune process, susceptibility to chronic candida infection because of a T-cell defect, and dystrophy of ectodermal tissues.<sup>2</sup> The most common endocrine manifestations are hypoparathyroidism and adrenal failure. Hypogonadism, hypothyroidism, type 1 diabetes mellitus, and hypopituitarism may also occur. Nonendocrine manifestations include enamel hypoplasia, nail dystrophy, keratoconjunctivitis, and pernicious anemia.<sup>2,3</sup> Except for candidiasis, patients with this syndrome have no apparent susceptibility to infections.

About 20 percent of patients with autoimmune polyglandular syndrome type I have fat malabsorption,<sup>2-4</sup> which is often associated with weight loss, growth retardation, and erratic absorption of medications. Malabsorption is considered to be a nonendocrine manifestation of the disorder,<sup>2-5</sup> but its cause is unknown.<sup>6,7</sup> We describe a patient with autoimmune polyglandular syndrome type I who had a severe malabsorption syndrome caused by a deficiency of cholecystokinin-producing enteroendocrine cells in the mucosa of his proximal small intestine.

### CASE REPORT

A 46-year-old man had had autoimmune polyglandular syndrome type I since the age of 9 years. His age at the onset of the various manifestations of the syndrome and his treatment (at the time of his referral to Baylor University Medical Center, Dallas, in March 1999) are shown in Table 1.

At the age of 34 years, diarrhea developed in association with recurrent episodes of hypocalcemia. Laboratory studies revealed a stool weight of 800 g per day (normal value, <200) and fecal fat

excretion of 97 g per day (normal value, <7). Upper gastrointestinal endoscopy, endoscopic retrograde cholangiopancreatography, and duodenal biopsies showed no abnormalities. The patient was treated with pancreatic enzymes, metronidazole, and famotidine (a histamine H<sub>2</sub>-receptor antagonist). His diarrhea improved after several months. The metronidazole and famotidine were subsequently discontinued, but he continued to take pancreatic enzymes for the next 12 years.

Despite treatment with pancreatic-enzyme replacement, the patient had a second episode of diarrhea and recurrent hypocalcemia at the age of 43 years. He was again treated with metronidazole and famotidine, and after several months the diarrhea improved.

In October 1998, while the patient continued to take pancreatic enzymes, diarrhea developed again, with weight loss and recurrent episodes of hypocalcemia requiring intravenous infusions of calcium. The doses of several of his oral medications had to be increased. Extensive studies showed no evidence that the diarrhea had an infectious cause. Trials of famotidine, metronidazole, tetracycline, a lactose-free diet, and a gluten-free diet had no effect.

In March 1999, the patient was evaluated at Baylor University Medical Center with the use of studies that have been described previously.<sup>8-11</sup> The studies were approved by the center's institutional review board, and the patient gave written informed consent.

### RESULTS

The patient had malabsorption of all major nutrients (Table 2). Fat malabsorption was especially severe. Upper gastrointestinal endoscopy and colonoscopy showed a normal appearance of the mucosa, and no abnormalities were detected in biopsy specimens of the duodenum, ileum, and colon. Examination of the biopsy specimens and fecal cultures showed no infectious agents, and tests for giardia and parasites were negative. A quantitative culture of duodenal fluid was negative for the bacterial overgrowth syndrome. Urinary excretion of 5-hydroxyindoleacetic acid was normal, and a small-bowel barium study and computed tomographic studies of the abdomen and pelvis showed no abnormalities. Measurement of serum antigliadin antibodies, gastrin, and seven gastrointestinal peptides<sup>16</sup> (with assays designed to detect high concentrations produced by neuroendocrine tumors) revealed no abnormalities. Intestinal absorption of water and electrolytes and of bile acids was normal.<sup>8,9</sup>

These diagnostic tests revealed no known cause or mechanism of malabsorption. We therefore suspected a deficiency of an enteric hormone. To evaluate this possibility, we performed hepatobiliary scintigraphy, which revealed a gallbladder of normal size but no contraction of the gallbladder in response to a meal rich in protein and fat. This result suggested that the malabsorption was due to a deficiency of cholecystokinin. We then obtained additional duodenal-biopsy specimens for staining of enteroendocrine cells, a sample of the duodenal contents after the patient had consumed a liquid test meal, and serum samples before and after he had consumed a meal rich in protein and fat.

Pending analysis of these samples, the patient returned to his home in Montana. His therapy was not changed except for the substitution of medium-chain triglycerides for long-chain triglycerides in his diet.

From the Departments of Internal Medicine (C.H., K.H.L., C.A.S.A., J.L.P., J.S.E.) and Pathology (R.L.M., G.J.N., D.B.), Baylor University Medical Center, Dallas; and the Deaconess Billings Clinic, Billings, Mont. (L.F.). Address reprint requests to Dr. Fordtran at Baylor University Medical Center, 3500 Gaston Ave., Dallas, TX 75246.

**TABLE 1.** DISEASE MANIFESTATIONS, AGE AT ONSET, AND TREATMENT IN A PATIENT WITH AUTOIMMUNE POLYGLANDULAR SYNDROME TYPE I AND MALABSORPTION.

MANIFESTATION	AGE AT ONSET	TREATMENT AS OF MARCH 1999
	yr	
Hypothyroidism	9	Levothyroxine, 0.3 mg/day orally
Hypoparathyroidism	10	Calcium carbonate, 6000 mg/day orally; and calcitriol, 3 µg/day orally
Adrenal insufficiency	10	Hydrocortisone, 30 mg/day orally; and fludrocortisone, 0.1 mg/day orally
Keratopathy	10	Corneal transplantation (at 23 and 26 years of age), topical glucocorticoids, and lubricants
Malabsorption (diarrhea and recurrent hypocalcemia)	34	Pancreatic enzymes, 64,000 lipase units/meal, initially with loperamide, 2 mg/day orally, and famotidine, 40 mg/day orally
Diabetes insipidus	44	Desmopressin, 20 µg/day intranasally
Mucocutaneous candidiasis	45	Intermittent treatment with fluconazole
Hypogonadism	45	Testosterone enanthate, 200 mg intramuscularly every 2 wk

**TABLE 2.** FECAL AND DUODENAL OUTPUT WHEN THE PATIENT HAD SEVERE FAT MALABSORPTION (IN MARCH 1999) AND AFTER SPONTANEOUS IMPROVEMENT (IN SEPTEMBER 1999).

VARIABLE	MARCH 1999	SEPTEMBER 1999
Fecal output*		
Fat — g/day (% of dietary intake)	98.3 (79)	10.1 (8)
Protein — g/day (% of dietary intake)	25.9 (29)	17.4 (19)
Calories — kcal/day (% of dietary intake)	1546 (54)	373 (13)
Carbohydrates — g/day (% of dietary intake)	139 (36)	53 (14)
Duodenal output†		
Trypsin — U/hr	327,000	480,000
Lipase — U/hr	73,300	127,000
Colipase — U/hr	39,900	72,300
Bile acids — mg/hr	84.3	407.8
Bilirubin — mg/hr	16.3	61.6

\*The dietary intake was exactly the same in March and September. The intakes of fat, protein, and carbohydrates were 124, 90, and 383 g per day, respectively. Caloric intake was 2854 kcal per day. During the study, the patient took his routine medicines (shown in Table 1), except for pancreatic enzymes and loperamide. Stool samples were analyzed for fat, protein, and calories by methods described previously.<sup>11</sup> The carbohydrate content of stool was calculated from nonfat and nonprotein calories (carbohydrate [in grams] = nonfat and nonprotein kcal ÷ 4).

†After the patient had fasted overnight, a tube was placed in the duodenum with its opening midway between the duodenal papilla and the ligament of Treitz. The patient then drank a liquid test meal (237 ml of Ensure Plus containing 13.5 g of protein, 12.6 g of fat, and 46.8 g of carbohydrates), and the duodenal contents were aspirated continuously for one hour. Samples were frozen and stored at -70°C for subsequent analysis.<sup>12-15</sup> To convert the values for bile acids to micromoles per hour, multiply by 2.45. To convert the values for bilirubin to micromoles per hour, multiply by 1.71.

The diarrhea did not change initially, but after three months, it improved substantially, according to the patient's subjective assessment. He returned to Dallas in September 1999 for further evaluation. Malabsorption was still present, but it was greatly improved (Table 2). Hepatobiliary scintigraphy revealed normal contraction of the gallbladder, with a postprandial ejection fraction of 94 percent.

In March 1999, when the patient had severe malabsorption, serum cholecystokinin was undetectable, and serum gastric inhibitory polypeptide and peptide YY concentrations did not rise in response to the protein- and fat-rich meal (Fig. 1). In September, when the malabsorption had abated, serum cholecystokinin concentrations were normal, but serum gastric inhibitory polypeptide and peptide YY concentrations remained low.

The duodenal-biopsy specimens obtained in March did not react with antibodies against chromogranin A,<sup>17,18</sup> Leu 7,<sup>19</sup> or cholecystokinin. However, the duodenal-biopsy specimens obtained in September reacted with antibodies against all three of these substances (Fig. 2). Immunohistochemical and immunofluorescence studies of the blood samples obtained in March and in September showed no antibodies against enteroendocrine- or cholecystokinin-producing cells.

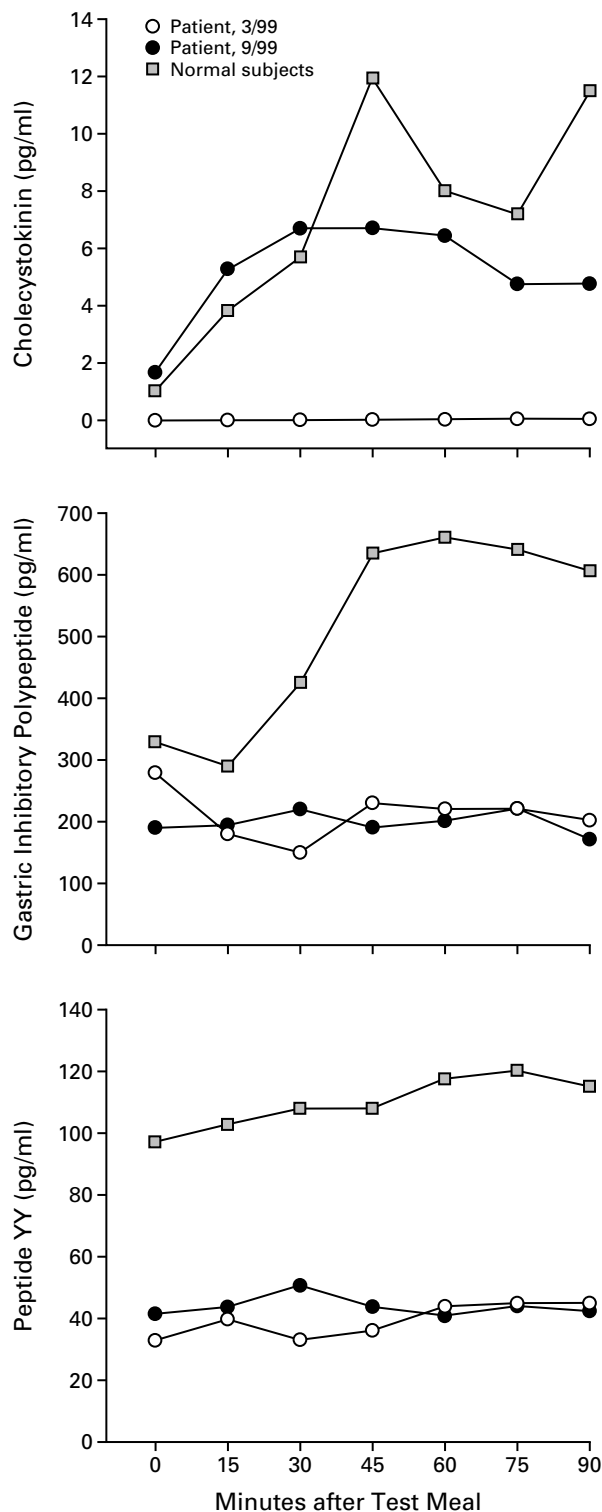
The output of duodenal-fluid bile acids, measured after the patient had consumed a liquid test meal, was almost five times as high in September as in March, and the output of bilirubin was almost four times as high in September (Table 2). The output of pancreatic enzymes was also higher in September than in March, but the increases were smaller.

DISCUSSION

Gastrointestinal endocrine cells are derived from precursor cells in the proliferative zone of the crypts, from which they migrate to the rest of the mucosa.<sup>18</sup> Their secretory granules contain peptides that act as systemic or paracrine mediators for the regulation of digestion and motility.<sup>20</sup> After stimulation by fats and proteins in the intestinal lumen, the contents of the granules are secreted into adjacent blood vessels. The granules also contain chromogranin A and Leu 7, which can be used as immunohistochemical markers for enteroendocrine cells.<sup>17-19</sup>

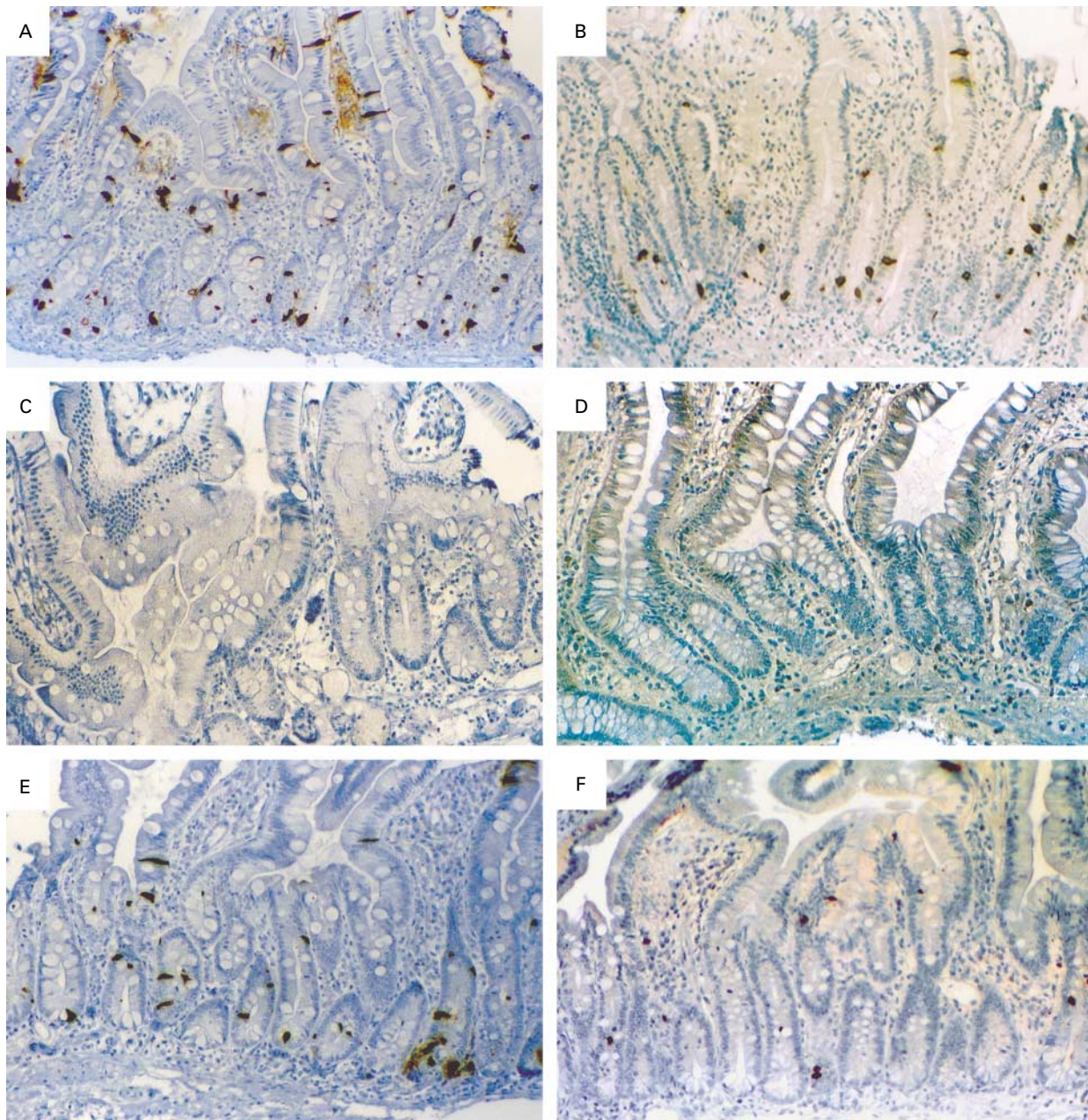
The findings presented here suggest that a deficiency of cholecystokinin-producing enteroendocrine cells in the proximal small bowel caused severe malabsorption in our patient with autoimmune polyglandular syndrome type I. When he had severe malabsorption, postprandial serum cholecystokinin concentrations were undetectable, and duodenal-biopsy specimens contained no enteroendocrine cells. When the malabsorption improved spontaneously, the serum cholecystokinin concentrations increased normally in response to food, and cholecystokinin-containing enteroendocrine cells were present in the duodenal mucosa. Bile acid secretion, which is stimulated by cholecystokinin, was reduced when malabsorption was severe. Decreased bile acid secretion impairs the formation of micelles within the intestinal lumen<sup>21</sup> and reduces the hydrolysis of dietary triglycerides by pancreatic lipase,<sup>22</sup> thereby reducing the absorption of fat. In our patient, the output of pancreatic enzymes was not as low as the output of bile acids, even though the secretion of both is regulated by cholecystokinin.<sup>23</sup> This finding suggests that other mechanisms of pancreatic stimulation, such as vagal activity, may have caused some degree of pancreatic-enzyme output, even when stimulation by cholecystokinin was absent.

In contrast to the corrected cholecystokinin concentrations, the serum peptide YY and gastric inhib-



**Figure 1.** Serum Concentrations of Cholecystokinin, Gastric Inhibitory Polypeptide, and Peptide YY in a Patient with Autoimmune Polyglandular Syndrome Type I and Malabsorption and in Normal Subjects.

In March and September 1999, serum samples were obtained at six 15-minute intervals after the patient had fasted overnight and then eaten a meal of scrambled eggs, bacon, a biscuit, and coffee (440 kcal). The samples were immediately frozen and stored at  $-70^{\circ}\text{C}$  for later analysis by radioimmunoassay for cholecystokinin (American Laboratory Products, Windham, N.H.), gastric inhibitory polypeptide, and peptide YY (Peninsula Laboratories, San Carlos, Calif.). The threshold of detection for the cholecystokinin assay was 0.34 pg per milliliter, and the maximal cross-reactivity with human gastrin was 0.5 percent. The values in normal subjects are mean concentrations (in six subjects for cholecystokinin and in two subjects for gastric inhibitory polypeptide and peptide YY). To convert the values for cholecystokinin, gastric inhibitory polypeptide, and peptide YY to picomoles per liter, divide by 1.14, 4.98, and 4.31, respectively.



**Figure 2.** Results of Immunohistochemical Staining with Chromogranin A and Cholecystokinin Antibodies for the Detection of Enteroendocrine Cells in Duodenal-Biopsy Specimens from the Patient and from a Normal Subject ( $\times 100$ ).

Duodenal-biopsy specimens from the normal subject are positive for both chromogranin A<sup>17,18</sup> (Panel A, brown) and cholecystokinin (Panel B, brown). Duodenal-biopsy specimens obtained from the patient in March 1999 were negative for both chromogranin A (Panel C) and cholecystokinin (Panel D). The specimens obtained from the patient in September 1999 were positive for both chromogranin A (Panel E, brown) and cholecystokinin (Panel F, brown). The results of staining for Leu 7<sup>19</sup> were similar to those for chromogranin A. For the detection of cholecystokinin, 5- $\mu\text{m}$  sections of paraffin-embedded duodenal mucosa were immunostained with rabbit polyclonal antibody against cholecystokinin (Peninsula Laboratories), followed by swine biotinylated antirabbit IgG (Dako, Carpinteria, Calif.) and streptavidin-peroxidase (Vector Laboratories, Burlingame, Calif.). The peroxidase was combined with diaminobenzidine tetrahydrochloride for a brown stain (Vector Laboratories), and Mayer's hematoxylin (Sigma Chemical, St. Louis) was used as a counterstain.

itory polypeptide concentrations remained low despite spontaneous improvement in malabsorption and the presence of at least some enteroendocrine cells. A deficiency of these peptides hastens the transit of chyme through the gastrointestinal tract,<sup>20,24</sup> and a persistent deficiency might have contributed to the residual mild malabsorption in our patient.

Since autoimmunity is believed to cause failure of the endocrine glands in patients with autoimmune polyglandular syndrome type I, it seems logical to surmise that autoimmunity mediated the destruction of small-bowel enteroendocrine cells in our patient. Although we detected no serum autoantibodies against normal enteroendocrine cells, this does not rule out the role of autoimmunity, because autoantibodies are not found in all patients with classic endocrine-gland failure due to autoimmune polyglandular syndrome type I.<sup>2,3</sup> There was no inflammation in the intestinal mucosa in our patient, but since malabsorption had developed at least five months before the biopsy specimens were obtained, it is possible that an earlier, transient autoimmune attack had destroyed the enteroendocrine cells. Infection (e.g., candidiasis), medications, and failure of the differentiation of enteroendocrine cells from precursor cells are other possible causes of the enteroendocrine deficiency. Whatever the cause, the deficiency in this patient was at least partially reversible, in contrast to the classic endocrine manifestations of autoimmune polyglandular syndrome type I. This difference may be due to the unique regenerative potential of intestinal mucosal cells.

Three other points deserve mention. First, an unexplained association between idiopathic hypoparathyroidism and severe malabsorption has been recognized for many years.<sup>25-27</sup> We believe that the destruction of enteroendocrine cells may be the mechanism of malabsorption in some patients with these two disorders. Second, a deficiency of enteroendocrine cells should be considered in any patient in whom the usual tests fail to reveal an explanation for the malabsorption syndrome. Finally, oral bile acid-replacement therapy<sup>11</sup> may ameliorate fat malabsorption in patients with steatorrhea due to cholecystokinin deficiency.

Supported by grants from the National Institute of Diabetes and Digestive and Kidney Diseases (5-RO1-DK37172-14) and the Southwest Digestive Disease Foundation.

*We are indebted to Dr. Peter R. Durie for assistance in the analysis of pancreatic enzymes; to Drs. Alan F. Hofmann, Michael Emmett, Günther J. Krejs, and Jacques Bancheureau for their advice and critical review of the manuscript; to Drs. Ed Lee and Lindsey Inmen for advice and assistance with the photomicrographs; to Mr. Mau V. Tran for assistance with the immunohistochemical studies; and to Ms. Diana Santa Ana for assistance in the preparation of the manuscript.*

## REFERENCES

1. The Finnish-German APECED Consortium. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. *Nat Genet* 1997;17:399-403.
2. Betterle C, Greggio NA, Volpato M. Autoimmune polyglandular syndrome type I. *J Clin Endocrinol Metab* 1998;83:1049-55.
3. Brosnan P, Riley WJ. Autoimmune polyglandular syndrome. In: Sperling MA, ed. *Pediatric endocrinology*. Philadelphia: W.B. Saunders, 1996: 509-22.
4. Ahonen P, Myllärniemi S, Sipilä I, Perheentupa J. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. *N Engl J Med* 1990;322:1829-36.
5. Kronenberg HM. Polyglandular disorders. In: Bennett JC, Plum F, eds. *Cecil textbook of medicine*. 20th ed. Vol. 2. Philadelphia: W.B. Saunders, 1996:1345-8.
6. Eisenbarth GS, Verge CF. Immunoendocrinopathy syndromes. In: Wilson JD, Foster DW, Kronenberg HM, Larsen PR, eds. *Williams textbook of endocrinology*. 9th ed. Philadelphia: W.B. Saunders, 1998:1651-62.
7. Su AY, Bilhartz LE. Endocrine-related gut dysfunction. *Semin Gastrointest Dis* 1995;6:217-27.
8. Arrambide KA, Santa Ana CA, Schiller LR, Little KH, Santangelo WC, Fordtran JS. Loss of absorptive capacity for sodium chloride as a cause of diarrhea following partial ileal and right colon resection. *Dig Dis Sci* 1989; 34:193-201.
9. Schiller LR, Hogan RB, Morawski SG, et al. Studies of the prevalence and significance of radiolabeled bile acid malabsorption in a group of patients with idiopathic chronic diarrhea. *Gastroenterology* 1987;92:151-60.
10. Afzalpurkar RG, Schiller LR, Little KH, Santangelo WC, Fordtran JS. The self-limited nature of chronic idiopathic diarrhea. *N Engl J Med* 1992; 327:1849-52.
11. Gruy-Kapral C, Little KH, Fordtran JS, Meziere TL, Hagey LR, Hofmann AF. Conjugated bile acid replacement therapy for short-bowel syndrome. *Gastroenterology* 1999;116:15-21.
12. Schiller LR, Bilhartz LE, Santa Ana CA, Fordtran JS. Comparison of endogenous and radiolabeled bile acid excretion in patients with idiopathic chronic diarrhea. *Gastroenterology* 1990;98:1036-43.
13. Gaskin KJ, Durie PR, Hill RE, Lee LM, Forstner GG. Colipase and maximally activated pancreatic lipase in normal subjects and patients with steatorrhea. *J Clin Invest* 1982;69:427-34.
14. Hayakawa T, Kondo T, Yamazaki Y, Iinuma Y, Mizuno R. A simple and specific determination of trypsin in human duodenal juice. *Gastroenterol Jpn* 1980;15:135-9.
15. Stevenson GW, Jacobs SL, Henry RJ. Studies on the determination of bile pigments. IV. Spectrophotometric determination of free and total bilirubin in serum. *Clin Chem* 1964;10:95-102.
16. Feldman JM, O'Dorisio TM. Role of neuropeptides and serotonin in the diagnosis of carcinoid tumors. *Am J Med* 1986;81:Suppl 6B:41-8.
17. Facer P, Bishop AE, Cole GA, et al. Developmental profile of chromogranin, hormonal peptides, and 5-hydroxytryptamine in gastrointestinal endocrine cells. *Gastroenterology* 1989;97:48-57.
18. Polak JM. Endocrine cells of the gut. In: *Handbook of physiology*. Section 6, The gastrointestinal system. Vol. 2. Neural and endocrine biology. Bethesda, Md.: American Physiological Society, 1989:79-96.
19. Shioda Y, Nagura H, Tsutsumi Y, Shimamura K, Tamaoki N. Distribution of Leu 7 (HNK-1) antigen in human digestive organs: an immunohistochemical study with monoclonal antibody. *Histochem J* 1984;16:843-54.
20. Redfern J, O'Dorisio TM. Gastrointestinal hormones and carcinoid syndrome. In: Felig P, Baxter JD, Frohman LA, eds. *Endocrinology and metabolism*. 3rd ed. New York: McGraw-Hill, 1995:1675-702.
21. Hofmann AF. The enterohepatic circulation of bile acids in health and disease. In: Sleisenger MH, Fordtran JS, eds. *Gastrointestinal disease: pathophysiology, diagnosis, management*. 5th ed. Vol. 1. Philadelphia: W.B. Saunders, 1993:127-50.
22. Pandolfi SJ. Pancreatic physiology and secretory testing. In: Feldman M, Scharschmidt BF, Sleisenger MH, eds. *Sleisenger & Fordtran's gastrointestinal and liver disease: pathophysiology/diagnosis/management*. 6th ed. Philadelphia: W.B. Saunders, 1998:771-82.
23. Rehfeld JF. Cholecystokinin. In: *Handbook of physiology*. Section 6, The gastrointestinal system. Vol. 2. Neural and endocrine biology. Bethesda, Md.: American Physiological Society, 1989:337-58.
24. Taylor IL. Pancreatic polypeptide family: pancreatic polypeptide, neuropeptide Y, and peptide YY. In: *Handbook of physiology*. Section 6, The gastrointestinal system. Vol. 2. Neural and endocrine biology. Bethesda, Md.: American Physiological Society, 1989:475-543.
25. Jackson WPU. Steatorrhea and hypoparathyroidism. *Lancet* 1957; 272:1086-7.
26. Texter EC. Malabsorption syndromes in adults. In: Mellinkoff SM, ed. *The differential diagnosis of diarrhea*. New York: McGraw-Hill, 1964:206-61.
27. Williams E, Wood C. The syndrome of hypoparathyroidism and steatorrhea. *Arch Dis Child* 1959;34:302-6.

Copyright © 2001 Massachusetts Medical Society.