CALCITONINOMA SYNDROME

INTERNAL MEDICINE GRAND ROUNDS
PARKLAND MEMORIAL HOSPITAL
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Figure 1 (Cover Page):

Ultrastructural immunocytochemistry of tumor cells from liver metastases (peritoneoscopic biopsies) in patient L.J. Regions containing immunoreactive cells were first identified on semithin sections of glutaraldehyde fixed material by using anti-calcitonin antiserum. Once these areas were identified the sections were further cut for immunoelectron microscopy. The protein A-gold method (46) was used for ultrastructural immunocytochemistry. Thin sections were incubated for 2 hours at room temperature with anti-calcitonin serum, washed, and then exposed to a solution of protein A-gold complex for 1 hour. Electron microscopic examination revealed a population of endocrine type cells containing round, small electron dense granules. Gold particles, which appear as black dots in the picture are concentrated over these secretory granules indicating that the granules contain calcitonin. (Drs. Lelio Orci and Mariella Ravazzola, Institut d'Histologie et d'Embryologie, Ecole de Médecine, Genève, Suisse)

INTRODUCTION

In 1962 Copp and coworkers (1) observed hypocalcemia in dogs whose thyroid-parathyroid complex was perfused with hypercalcemic blood. This observation led to the discovery of the calcium-lowering and phosphate-lowering hormone calcitonin. Although it was soon viewed as an important calcium-regulating factor, the exact role of calcitonin in human physiology has remained open to question, despite almost two decades of active investigation of the polypeptide (2). One reason why the role of calcitonin has remained controversial is the lack of clearly definable syndromes of calcitonin excess or deficiency.

In this Grand Rounds I will present a patient with calcitonin excess and I will discuss clinical, pathophysiological and morphological features of this syndrome. The workup and management of this complicated case required the help of a great number of my colleagues. Their contributions are acknowledged in Table 1.

TABLE 1. LIST OF PHYSICIANS AND SCIENTISTS COLLABORATING IN THE DIAGNOSTIC AND THERAPEUTIC MANAGEMENT OF PATIENT L.J.

WILLIAM MUNSON, M.D.	COLORADO SPRINGS, CO	ENDOCRINOLOGIST WHO DIAGNOSED HYPERCALCITONINEMIA
JAMES McMANUS, M.D.	TEMPLE, TEXAS	REFERRING GASTROENTEROLOGIST
JAMES LAMME, M.D.	WALSENBURG,	PRIVATE PHYSICIANS
LEONCE EVANS, M.D.	COLORADO	IN WALSENBURG, COLORADO
NEIL BRESLAU, M.D.	DALLAS, TEXAS	ENDOCRINOLOGICAL INVESTIGATION
LELIO ORCI, M.D.	GENEVA,	IMMUNOCYTOCHEMICAL
MARIELLA RAVAZZOLA, Ph.D.	SWITZERLAND	STUDIES
CHARLES PAK, M.D.	DALLAS, TEXAS	MINERAL METABOLISM GCRC PROGRAM DIRECTOR
ANDREW GAFFNEY, M.D.	DALLAS, TEXAS	CARDIOLOGY STUDIES
JOY ROBERTSON, M.D.	DALLAS, TEXAS	PULMONARY CONSULTANT
EUGENE FRENKEL, M.D.	DALLAS, TEXAS	CHEMOTHERAPY
EDWIN EIGENBRODT, M.D.	DALLAS, TEXAS	HISTOLOGICAL STUDIES
MAX BUJA, M.D.	DALLAS, TEXAS	HISTOLOGICAL STUDIES
PHILIP RASKIN, M.D.	DALLAS, TEXAS	SOMATOSTATIN THERAPY
THOMAS DICKINSON, M.D.	DALLAS, TEXAS	GI-FELLOW
BERNIE ROOS, M.D.	CLEVELAND, OH	CALCITONIN MEASUREMENT
ROBERT GAGEL, M.D.	BOSTON, MA	CALCITONIN, NEUROTENSIN
STEPHEN BAYLIN, M.D.	BALTIMORE, MARYLAND	DOPA-DECARBOXYLASE, HISTAMINASE
IRVIN MODLIN, M.D.	NEW YORK, NEW YORK	PROSTAGLANDIN, PANCREATIC POLYPEPTIDE RIA
CRAIG BRATER, M.D.	DALLAS, TEXAS	INDOMETHACIN MEASUREMENTS
SAMI SAID, M.D.	DALLAS, TEXAS	VIP RIA
ROGER UNGER, M.D.	DALLAS, TEXAS	GLUCAGON, SOMATOSTATIN AND INSULIN RIA
BRYAN ELLMAN, M.D.	DALLAS, TEXAS	SPECIAL RADIOLOGY
WILLIAM SNYDER, M.D.	DALLAS, TEXAS	SURGERY CONSULTANT

CASE REPORT

L.J. is a 55 year old female who along with her husband runs a radio station in Walsenburg, Colorado. She was in good health prior to 1965 (15 years ago) when she noted a lump in the right side of her neck which had become progressively larger. This eventually caused difficulty in swallowing and the patient sought medical advice. She underwent a thyroidectomy with a total lobectomy on the right and a subtotal lobectomy on the left side (Presbyterian Hospital, Denver).

The histological interpretation of the resected thyroid was somewhat difficult. Frozen sections were read as undifferentiated thyroid carcinoma. On definite sections, the differential diganosis lay between poorly differentiated follicular carcinoma and medullary carcinoma. Amyloid stains failed to show the amyloid deposition classically associated with medullary carcinoma. A team of pathologists decided on the diagnosis of medullary carcinoma of the thyroid. The patient and her family were informed about the poor prognosis of thyroid carcinoma. Following surgery radioiodine (2 x 25 mC after TSH stimulation) was given and radiation to the neck was carried out to a total of 1200r. Subsequent repeated thyroid scans showed no activity in the neck. Thyroid replacement therapy was commenced.

She was doing well and leading a normal life. She was followed for 15 years by yearly physical examination which was negative for recurrence and adenopathy on the neck and by chest x-ray which also remained negative for metastatic disease.

In September 1979 she noted the onset of diarrhea. Stool frequency progressively increased to 18 watery bowel movements per day (1-3 during the night). There was no mucus or blood in the stool and no associated abdominal pain but she noticed increased passage of gas. The diarrhea did not respond to dietary permutations such as a lactose-free diet.

A GI workup at that time included the following negative tests: X-ray studies of the stomach, small bowel and colon, proctoscopy with biopsy and colonoscopy, stool: enteric pathogens, ova and parasites.

The diarrhea did not respond to Metamucil, Lomotil, Imodium, Donnatal, Librax, Tetracycline and Flagyl. Thyroid overreplacement was suspected by her endocrinologist in Colorado Springs. When the thyroid replacement Rx was cut by half (0.3 mg to 0.15 mg Synthroid) there was no change in her diarrhea. Further GI workup was likewise unrevealing (normal: D-Xylose test, serum magnesium, urinary catecholamines, metanephrines and VMA).

Her endocrinologist in Colorado Springs started to consider a possible relation between her previous removal of a medullary carcinoma of the thyroid and her diarrhea. (Prostaglandins and calcitonin are considered the mediators of the diarrhea in this syndrome). She was put on Motrin, the rationale being inhibition of prostaglandin synthetase but this was of no avail. Blood was then drawn for calcitonin measurements but the results were pending when the patient decided to come to Texas.

She was admitted to Scott & White in Temple (June 1980). Normal studies included: Proctoscopy, small bowel biopsy and stool analysis for ova and parasites. She was found to have an elevated serum alkaline phosphatase. Subsequently a liver-spleen scan revealed multiple filling defects suggesting metastatic implants. The patient was then referred for further workup to our institution (General Clinical Research Center). Shortly after her arrival in Dallas the calcitonin plasma levels were reported (blood had been drawn by her endocrinologist in Colorado Springs).

TABLE 2 PLASMA CALCITONIN LEVELS IN L.J.* (June 1980)

			NORMAL
BASAL		85 ng/ml	< 0.105
AFTER CALCIUM INJECTION (200 mg Ca^{+} in 1 minute i.v.)	After 1 min	312 ng/ml	< 0.120
	After 2 min	312 ng/ml	< 0.120

(Serum Ca rose from 10.5 to 13.9 and 13.6 mg/dl at 1 and 2 min, resp.)
*(MAYO MEDICAL LABORATORY)

Thus, extreme calcitonin excess was demonstrated. Basal calcitonin concentration in plasma was 800 times higher than normal. Provocation (see below) with calcium injection resulted in massive release of tumor calcitonin (fourfold increase in plasma calcitonin).

AIM OF INVESTIGATIONS IN PATIENT L.J.

Our subsequent investigations in this patient were designed to answer two major questions:

1. Are the liver metastases secondary to the medullary carcinoma of the thyroid (removed 15 years ago, no local recurrence)? Or may the liver metastases be secondary to a pancreatic calcitoninoma? The importance of this differentiation lies in the availability of good chemotherapy (streptozotocin) in the case of a primary tumor in the pancreas and the lack of effective chemotherapy in the case of the medullary carcinoma of the thyroid being the primary.

2. What are the mechanisms causing the severe watery diarrhea? Can diarrhea be controlled by drug therapy?

CALCITONIN

Structure and Location

Calcitonin is a 32-amino acid polypeptide. Like with many other polypeptides calcitonin synthesis involves a large molecular precursor (MW 15,000). Clearance of a "leader sequence" and subsequent removal of several peptide sequences result in the calcitonin monomer which has a molecular weight of 3500 daltons (3,4).

The amino acid sequence of human, rat, cow, pig, sheep, eel and salmon calcitonin are all different (5-7). They have in common the 32-amino acid length, a 1 to 7 disulfide bridge that forms a ring at the amino terminus of the molecule, and a carboxyl-terminal proline amide. Salmon calcitonin is the only form available in the United States for therapeutic purposes, and it differs from human calcitonin in 16 amino acids. This structural difference makes salmon calcitonin immunogenic in man - a feature that occasionally limits its medical use.

The parafollicular cells of the thyroid, also called C-cells were identified by Foster in 1963 as the source of calcitonin (8). The histological description of these C-cells goes back to 1876 when Baber distinguished them from the follicular (thyroxine-secreting) cells (9). Many investigators have now confirmed that the C-cells are the major source of calcitonin in man (10,11).

The C-cells of the thyroid belong to the APUD (amine precursor uptake and decarboxylation) series (12,13) and their origin from the neural crest can be considered proven (14,15).

PHARMACOLOGICAL ACTIONS

In pharmacological doses calcitonin lowers plasma calcium and inorganic phosphorus concentration by acting on bone metabolism and kidney function. The pharmacological actions of calcitonin are summarized in Table 3.

TABLE 3 PHARMACOLOGICAL ACTIONS OF CALCITONIN

BONE:	INHIBITION OF OSTEOCLASTIC BONE RESORPTION
KIDNEY:	INCREASED RENAL CLEARANCE OF CALCIUM AND PHOSPHATE
GI-TRACT:	DECREASED GASTRIN AND GASTRIC ACID SECRETION
	INCREASED SMALL BOWEL SECRETION

PHYSIOLOGY

Several physiological functions have been proposed for calcitonin, but none are universally accepted.

TABLE 4 PUTATIVE PHYSIOLOGIC FUNCTIONS OF CALCITONIN

REGULATION OF PLASMA CALCITONIN CONCENTRATION
MODULATION OF BONE GROWTH AND REMODELING
CONTROLLING CALCIUM BALANCE
NEUROTRANSMITTER OR PARACINE REGULATOR

CALCITONIN DEFICIENCY

Calcitonin plasma levels are reduced or unmeasurable in thyroid agenesis or following thyroidectomy (16,17). It has been difficult, however, to show that persons with deficiency of calcitonin have disturbed calcium homeostasis. Thus, the body must be able to easily adapt to the absence of calcitonin.

Women have lower plasma calcitonin levels than men (18) and since calcitonin inhibits osteoclastic bone resorption (19) the question arose whether relative calcitonin deficiency may be responsible for osteoporosis which is more frequent in women than men. The role of calcitonin in osteoporosis is, however, controversial (20,21) and treatment with calcitonin has not been very successful (22,23).

CALCITONIN EXCESS

Elevated plasma concentrations of calcitonin are found in a number of malignancies, but primarily in medullary carcinoma of the thyroid (see below). A wide variety of other tumors cause hypercalcitoninemia (24-27). These include bronchogenic and oat cell carcinomas, pheochromocytomas and islet cell tumors of the pancreas.

Besides ectopic secretion, other mechanisms may account for hypercalcitoninemia, such as stimulation of calcitonin release by hypergastrinemia in Zollinger-Ellison Syndrome (28) or decreased clearance in renal failure (29). High plasma immunoreactive calcitonin is also found in pancreatitis, pernicious anemia, pregnancy, and nonmalignant pulmonary disease (26,30).

CLINICAL INVESTIGATIONS IN PATIENT L.J.

Question #1: Are the liver metastases secondary to the medullary carcinoma of the thyroid (MCT) resected 15 years ago?

In answering this question consideration must be given to 1) the natural history of MCT and 2) to the recognition of pancreatic calcitoninomas.

NATURAL HISTORY OF MCT

A striking variability has been noted in the clinical behavior of MCT. The spectrum of aggressiveness ranges from years of well controlled local disease in some patients to rapid and wide spread metastatic disease in others (31). Looking at the course of the disease following initial surgery (as is relevant in our patient) Hill, reporting 72 MCT patients seen at M.D. Anderson, described three different groups (32): A group that is cured, a second group that exhibits progressive increase in extent of tumor following surgery (average survival 6 years) and a third group (11 out of 72 patients) with a long latent period (often greater than 10 years - mean survival 9 years). Patients would be completely free of symptoms, return to their usual occupation, and live essentially normal lives. A recurrence and/or metastases developed later which produced symptoms and progression inexorably to cause death.

In a series from the Mayo Clinic, Chong (33) described 128 patients with MCT operated upon for cure. Forty four (34%) developed recurrence between 2 months and 19 years after operation (90% had their recurrence within 8 years after surgical treatment). Sites of recurrence were cervical alone (50%), cervical and mediastinal (9%), cervical, mediastinal, and distant (7%), distant alone (18%), cervical and distant (13%), and mediastinal and distant (2%). Distant metastases involved the liver in 7 patients, the bone in 7, the lungs in 6, and the kidney, pericardium and pancreas in 1 each.

The course observed in our patient (15 years between thyroidectomy and detection of liver metastases) would certainly fit the clinical picture described in a few patients in both the M. D. Anderson and Mayo series.

PANCREATIC PRIMARY ?

Based on negative immunocytochemical studies, calcitonin is not present in cells of the normal pancreas. The observation that calcitonin can be extracted from pancreatic tissue (34) is probably based on the binding of circulating peptide to calcitonin receptors. Calcitonin has, however, been found in pancreatic endocrine tumors. We have ourselves reported two such cases: one patient with pancreatic cholera syndrome and high levels of both calcitonin and VIP (35) and one patient with somatostatinoma (plasma somatostatin and calcitonin elevated) (36).

In the latter case, somatostatin and calcitonin immunoreactive material co-existed in the secretory granules of pancreatic tumor cells. Subsequently, Galmiche et al (37) have described another case of calcitonin-producing somatostatinoma in which individual tumor cells contained both somatostatin and calcitonin. These observations suggest a possible link between the pathways for somatostatin and calcitonin in C- and D-cells. Calcitonin production in pancreatic tumor cells may be the result of gene derepression due to carcinomatous changes of the endocrine cells.

There are other reports of pancreatic tumors containing calcitonin in association with such polypeptides as VIP, neurotensin, motilin, GIP, enkephalins, gastrin and pancreatic polypeptide (38-41).

Thus, an extensive effort was made to search for a pancreatic primary tumor in patient L.J. The various tests which were employed and their results are listed in Table 5.

TABLE 5. INVESTIGATIONS IN SEARCH OF A PRIMARY ENDOCRINE TUMOR IN THE PANCREAS OF L.J.

	TEST	RESULT
1.	Pancreatic Sonogram	Normal pancreas, texture of head somewhat difficult to evaluate due to shadowing from falciform ligament.
2.	CAT Scan	Diffuse enlargement of the pancreas. Mass in the region of the head of the pancreas which could be within the pancreas itself or could be within nodes adjacent to the pancreas.
3.	Angiogram	First read as positive for tumor blush in the head of the pancreas, on subtraction films 2 days later read as normal.
4.	ERCP (Endoscopic retrograde cholangio-pancreatography)	Normal pancreatic duct system
5.	i.v. Tolbutamide Test (Investigational test, a rise in calcitonin levels would be expected if calcitonin were of islet cell origin)	No significant rise in basal calcitonin levels for 60 min following i.v. Tolbutamide

CONT'D

TABLE 5 CONT'D

TEST	RESULT
6. Search for elevation of pancreas related polypeptide levels in plasma (using radioimmunoassays)	
Pancreatic Polypeptide	16 (normal 20-70 fmol/ml)
VIP	80 (normal 82-120 pg/ml)
Somatostatin*	285 (normal 50-150 pg/ml)
Gastrin**	78 (normal <200 pg/ml)
Glucagon	160 (normal 50-150 pg/ml)
Insulin	25 (normal 2-20 μU/ml)
	NT***
7. Histological comparison of liver metastases (peritoneoscopic biopsies) with resected thyroid tumor	Similar histologic appearance
8. Immunocytochemistry and immunoelectron microscopy (protein A-gold method) (peritoneoscopic biopsies) (46)	Homogenous population of endocrine type cells containing round, small electron dense granules. Immunofluorescence positive for alcitonin and negative for the following other peptides: somatostatin, glucagon, insulin, glicentin, pancreatic polypeptide, neurotensin, ACTH, gastrin and PTH (see Figure 1, cover page).

CONT'D

*Somatostatin was found in some C-cells (42-43) in human, dog and rat thyroids (calcitonin and somatostatin coexist in the same cells). Also, somatostatin has been found in tissue of medullary carcinoma of the thyroid (44,45). Thus, the slightly elevated somatostatin level in plasma does not help in the search for a pancreatic tumor. Immunofluoresence was negative for somatostatin in the liver metastases of our patient.

**Gastric acid secretion in patient L.J. was normal: Basal: 0.7 meq/h, peak acid output after pentagastrin 12.1 meq/h.

***Plasma neurotensin concentration was also normal (140 fmol/ml).

TABLE 5 CONT'D

TEST			RESULT
9. DOPA-Decarboxylase-Histaminase-Calcitonin distribution in liver metastases (frozen biopsy specimen obtained at peritoneoscopy) (47)			
Pattern expected in:	Islet Cell Tumor	MCT	MCT Pattern found:
DOPA-Decarboxylase	+++	++	274 nmol/g wet weight(mod. high)
Histaminase	+	+	None detected
Calcitonin	+	+++	15,000 ng/g wet weight (very high)

Based on the results presented in Table 5 we ruled out the possibility of a primary pancreatic tumor. Overwhelming evidence sugge ted that the metastases originated from the medullary carcinoma of the thyroid removed 15 years earlier. Unfortunately, these results precluded the use of Streptozotocin which has been found to be an effective chemo herapeutic agent in islet cell tumors (48).

DIARRHEA

As indicated in the CASE REPORT (see above) diarrhea was the presenting symptom that led to the discovery of metastatic liver disease.

Bernier (49) and Williams (50) were the first to recognize diarrhea as a prominent symptom in patients with MCT. Diarrhea is encountered in at least one third of patients with this syndrome (51-55).

Bernier's initial studies (51) showed decreased intestinal transit time and implied that hypermotility (intestinal hurry) was the major cause of diarrhea ("Diarrhee motrice par cancer medullaire thyroidien"). Later Fordtran and coworkers clearly showed that calcitonin was an intestinal secretagogue in man (56,57). Using small bowel perfusion techniques Fordtran and coworkers were able to abolish absorption and induce secretion of water and electrolytes when salmon calc tonin was infused in healthy volunteers (Fig. 2).

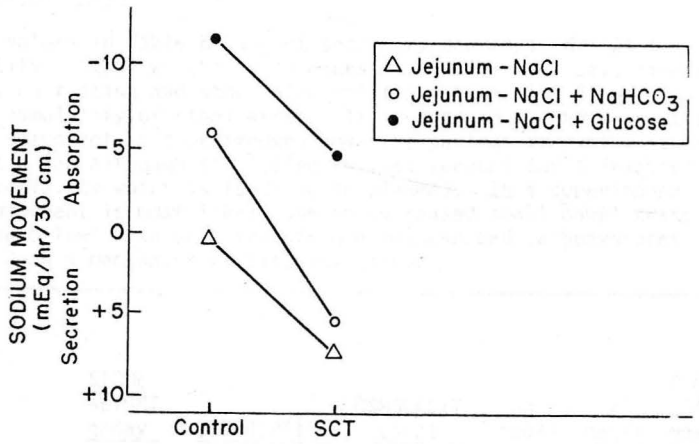


Figure 2: Effect of salmon calcitonin (SCT) on sodium movement in 30 cm jejunal segments of healthy volunteers. Calcitonin induced intestinal secretion when isotonic solutions of NaCl or NaCl plus $NaHCO_3$ were perfused. In the presence of glucose in the lumen calcitonin reduced sodium absorption but did not cause net secretion. [From Gray, Brannan, Juan, Morawski and Fordtran, 1976 (57)]. Similar transport changes can be demonstrated in the small intestine of experimental animals in vitro (58,59).

So far one MCT patient has been reported in whom perfusion studies revealed abnormal Na and Cl transport in the small bowel (52). In another patient increased ileo-cecal flow was measured by the slow-marker technique (60), however, the validity of this method is controversial (61). Thus, only very limited observations are so far available on intestinal transport changes in patients with MCT.

CLINICAL AND PATHOPHYSIOLOGICAL INVESTIGATIONS OF THE LARGE VOLUME DIARRHEA IN PATIENT L.J.

1. Stool Analysis

The values in Table 6 suggest secretory diarrhea (62) as the major abnormality: Stool weight is in excess of 1 liter per day, diarrhea persists on fasting and stool electrolytes [(Na + K) x 2] account for all the osmolality of stool water. It is noteworthy, however, that an osmotic component is superimposed when the patient is eating (there is an osmotic gap although stool electrolytes account for a fraction of stool osmolality which is isotonic to plasma). This superimposed osmotic component is most likely due to decreased small bowel transit time (see below) with delivery of some malabsorbed carbohydrates to the colon. Such a mechanism is also indicated

TABLE 6

DIET	STOOL WEIGHT g/day	DESCRIPTION	OSMOLALITY mosm/l	Na meq/l	K meq/l	OSMOT. GAP mosm/l	pH
REGULAR	1850	Viscous	421	101	41	137	5.9
FASTING	1100	Watery	308	126	19	18	7.9

by the low stool pH while on a regular diet (formation of short-chain fatty acids from carbohydrates by bacterial metabolism). Mild steatorrhea (ranging from 9 to 29 g of fecal fat per day) is also consistent with decreased small bowel transit time.

2. Intestinal Perfusion Studies

In order to assess intestinal water and electrolyte transport, the triple-lumen perfusion technique was used (Figure 3). Absorption or secretion is estimated in either 30 cm segments of small intestine (jejunum or ileum) or in the entire colon. Measurements are made under steady state conditions. Polyethylene glycol serves as a nonabsorbable marker (63,64).

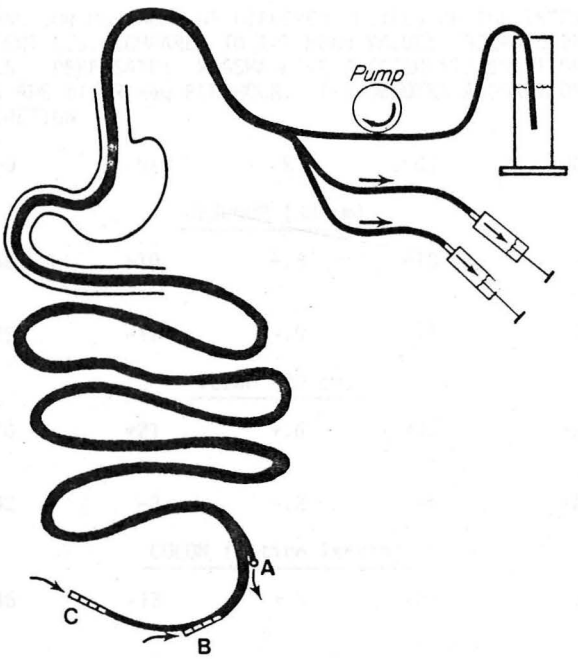


Figure 3: Perfusion technique of the ileum. The perfusate enters the lumen at Point A (200 cm from teeth, infusion rate 11 ml/min. The proximal sampling site (B) (aspiration 1.5 ml/min) is located 10 cm distal to A. The test segment extends from B to C (30 cm). Sampling at Point C (1.5 ml/min) is staggered by 10 min. Absorption (or secretion) in the test segment is calculated from the perfusion rate (660 ml/h), the change in PEG concentration and the change in concentration of the respective solute (65).

Table 7 gives the results of intestinal perfusion studies. Major transport abnormalities were found in the jejunum and ileum: Water

TABLE 7: WATER AND ION MOVEMENT AT DIFFERENT LEVELS OF THE INTESTINE IN PATIENT L.J. COMPARED TO THE MEAN VALUES OBSERVED IN HEALTHY CONTROLS. PERFUSATE: PLASMA-LIKE ELECTROLYTE SOLUTION. NUMBERS ARE ml OR meq PER HOUR. (-) DENOTES ABSORPTION, (+) SECRETION

	H_2O	Na	K	Cl	HCO_3
			JEJUNUM (30 cm)		
L.J.	+68	+10	+.3	+10	-.5
HEALTHY CONTROLS	-129	-17	-.9	-11	-5.5
			ILEUM (30 cm)		
L.J.	+170	+21	+.6	+22	-2
HEALTHY CONTROLS	-42	-8	-.2	-6	-2
			COLON (entire length)		
L.J.	-46	-13	+.5	-21	·7
HEALTHY CONTROLS	-181	-31	+1	-32	·1

and NaCl were secreted instead of absorbed. Secretion was higher in the ileum than in the jejunum. Absorption in the colon (water and NaCl) was low (lower limit of normal).

From the endogenous flow in the ileum (fluid from above the test segment that mixes with perfusate) we estimated the daily ileocecal flow to be 4 liters. While the reserve capacity of the normal human colon provides for absorption of 4 liters of an electrolyte solution per day (66), the low normal colonic absorption in our patient (see colon perfusion results in Table 7) allows only absorption of part of the fluid volume entering the cecum per 24 hrs. Moreover, our assessment of ileocecal flow applies to the fasting state and not to times with normal dietary intake when food and digestive secretions add to the luminal contents.

In summary, these perfusion studies traced the cause of the secretory diarrhea to pathological small bowel secretion in the jejunum and ileum.

3. Small Bowel Transit Time

Using dye dilution curves (injection of 1 ml bolus of ^{3}H-Mannitol, ^{14}C-PEG and bromsulphalen at site A (Fig. 3) we estimated small bowel transit time in a 30-cm segment of perfused intestine (67). Mean transit time in the jejunum of L.J. was 1.7 min/30 cm as compared to 9.0 min/30 cm in healthy controls. A similar reduction in transit time was observed in the ileum.

Thus, in addition to small bowel secretion these studies identified decreased intestinal transit time as a second underlying mechanism leading to the large volume diarrhea as described above under "Stool Analysis."

4. Other Tests for the Workup of Diarrhea in L.J.

Since other secretagogues may be circulating in patients with medullary carcinoma of the thyroid, the following analyses were performed:

Prostaglandin RIA revealed normal levels of prostaglandin E_1 in plasma (730 pg/ml, normal 150-1000; levels seen in prostaglandin-mediated diarrhea are in the 10,000 range). Additional proof that prostaglandins did not play a role came from the observation that secretion was unaltered when indomethacin was given during the last hour of intestinal perfusion on the day of the jejunal study and on the day of the ileal study. Indomethacin blood levels were well within the range of inhibition of prostaglandin synthetase. Also, indomethacin therapy had no effect on daily stool weight (see below).

Blood serotonin concentration was normal (90 ng/ml, normal 50-200), plasma cortisol was normal and so were metanephrines in urine.

5. Therapeutic Trials to Control Diarrhea

As listed in Table 8, several drug regimens were used in an attempt to control the large volume diarrhea.

TABLE 8: THERAPEUTIC TRIALS

DIET	DRUG	STOOL WEIGHT (g/day)	RATIONALE
Regular	None	1850	(Baseline)
Fasting	None	1100	(Secretory Diarrhea?)

CONT'D

TABLE 8 CONT'D

DIET	DRUG*	STOOL WEIGHT (g/day)	RATIONALE
Regular	Indomethacin (50 mg q.i.d.)	1400	Inhibition of prostaglandin synthetase (68,69).
Regular	Codeine Phosphate (60 mg q.i.d.)	170	Increased absorption and/or decreased motility (70-73).
Regular	Lithium Carbonate (300 mg q.i.d.)	1820	Lithium carbonate can control diarrhea in pancreatic cholera by unknown mechanism (74).
Regular	L-DOPA (500 mg q.i.d.)	850	Inhibition of calcitonin release from MCT in vitro and in vivo (75).
Regular	Prednisone (100 mg q.d.)	1050	Increased activity of Na/K ATPase in basolateral membrane of enterocytes (76,77) beneficial effect in pancreatic cholera (35,78,79).
Regular	Somatostatin (4 µg/kg/h i.v. or s.c.)	1565	Somatostatin inhibits secretion in experimental animals (80), and in patients with severe watery diarrhea (81,82).
Regular	Trifluoperazine (Stelazine)	1510	Chlorpromazine and trifluoperazine decrease stool output in enterotoxic cholera (83,84) and pancreatic cholera (85).

Out of the numerous drug regimens that were tried, only Codeine proved to be effective. During the subsequent course the diarrhea was well controlled on an even much lower dose (30 mg t.i.d.).

Continuous somatostatin infusion at a dose of 4 µg/kg/h was of no benefit. Although we had previously found that this dose can control secretory diarrhea in carcinoid syndrome (81), it was probably too low in patient L.J. When double the dose (8 µg/kg/h) was given during perfusion studies, intestinal secretion was not only abolished but normal absorptive rates were restored in both the jejunum and ileum (Table 9).

*Plasma calcitonin concentration remained unchanged during all seven therapeutic trials.

TABLE 9: WATER MOVEMENT (ml PER HOUR) IN THE JEJUNUM AND ILEUM OF PATIENT L.J. (-) ABSORPTION, (+) SECRETION.

i.v.	JEJUNUM (30 cm)	ILEUM (30 cm)	COLON (entire length)
SALINE	+68	+170	-46
SOMATOSTATIN i.v. 8 µg/kg/h	-124	-45	-68

For reasons of time, we were, however, unable to test the clinical effectiveness of this high somatostatin dose (No 24 hour infusion and stool collection available).

MINERAL METABOLISM

With a plasma calcitonin level of about 1000 times higher than normal in patient L.J., we wished to answer the question whether the known pharmacological effects of calcitonin on calcium and phosphorus metabolism had caused any obvious derangements in the patient's mineral metabolism.

The patient was placed on a 400 mg calcium metabolic diet and the studies listed in Table 10 were performed.

TABLE 10: MINERAL METABOLISM INVESTIGATIONS IN PATIENT L.J.

Test or Measurement	Expected Result (based on pharmacological action of calcitonin)	Result Observed in Patient L.J. (hypercalcitoninemia, MCT)	Comments
Serum Calcium	Low	Normal: 9.4 mg/dl	-
Serum Phosphorus	Low	Normal: 3.8 mg/dl	-
Urinary Calcium	High (decreased renal tubular reabsorption Ca) (86)	High 260 mg/day (normal <200)	Negative calcium balance. Calculated Ca loss: 150 mg/day

CONT'D

TABLE 10 CONT'D

Test or Measurement	Expected Result (based on pharmacological action of calcitonin	Result Observed in Patient L.J. (hypercalcitoninemia, MCT)	Comments
PTH	High (secondary hyperparathyroidism in response to serum calcium - lowering effect of calcitonin and hypercalcuria)	Normal: 18 µl equiv./ml (normal 16-36)	Lack of PTH response to calcium loss may be due to destruction (removal) of parathyroid by surgery (and radiation?). Pt. also had a normal phosphate clearance: 92% tubular reabsorption (nl >85%, in hyperpara <85%)
Urinary cAMP			
Total	High	Normal: 4.5 nmol/100 ml GF	Confirms lack of PTH response
Nephrogenous	High (in response to ↑ PTH, no effect of calcitonin [87])	Normal 2.99 nmol/100 ml GF (normal <3.1)	
Calcium Absorption	No effect by calcitonin (88), high due to sec. hyperpara.		Possible reasons for lack of compensation for urinary losses: 1) Parathyroid missing or destroyed. 2) Inability of kidney to respond to PTH to synthesize 1,25-$(OH)_2$ Vit D_3. 3) Intestinal resistance to Vit D_3.
Fractional		Normal: 38% (normal 36-60)	
Perfused Jejunum		Normal: 219 µmol/30cm/h (normal 139-290)	
Plasma 1,25-$(OH)_2$ Vit D_3	High or normal	Pending	-
Bone Density	High (inhibition of osteoclastic bone resorption) (89,90)	Low .529 g/cm^2 (normal for 55 year old white female: .560 - .800)	Spine x-ray in L.J.: Osteopenia

Although the studies concerning the mineral metabolism of our patient with MCT are difficult to interpret, they do suggest that the excessive hypercalcitoninemia had no biological effects. Down-regulation of calcitonin receptors (decrease in number or affinity of receptors) could explain these observations. The only finding in patient L.J. that would be consistent with an effect of calcitonin is the hypercalcuria (91). However, several other conditions such as postmenopausal osteoposis with renal leak could explain the hypercalcuria (92). The relative high thyroid replacement therapy (T_4 10-13 µg/dl) could also play a role since bone demineralization, suppression of PTH and hypercalcuria are seen i. thyrotoxicosis (93).

GENETIC ASPECTS

Medullary carcinoma of the thyroid comprises some 5 to 10% of all thyroid malignancies (32,94). Approximately 80% of the cases of this tumor arise spontaneously with no apparent evidence for familial disease; the other 20% occur in the setting of familial multiple endocrine neoplasia syndromes (95-100). MCT occurs as part of MEN II (IIa or Sipple's Syndrome) and MEN III (IIb) (Table 11). All MEN syndromes are inherited as an autosomal dominant trait.

TABLE 11: MEDULLARY CARCINOMA OF THE THYROID IN MULTIPLE ENDOCRINE NEOPLASIA (MEN) SYNDROMES

MEN II (IIa or Sipple's Syndrome)

MEDULLARY CARCINOMA OF THE THYROID

PHEOCHROMOCYTOMA

PARATHYROID HYPERPLASIA AND/OR ADENOMAS

MEN III

MEDULLARY THYROID CARCINOMA

PHEOCHROMOCYTOMA

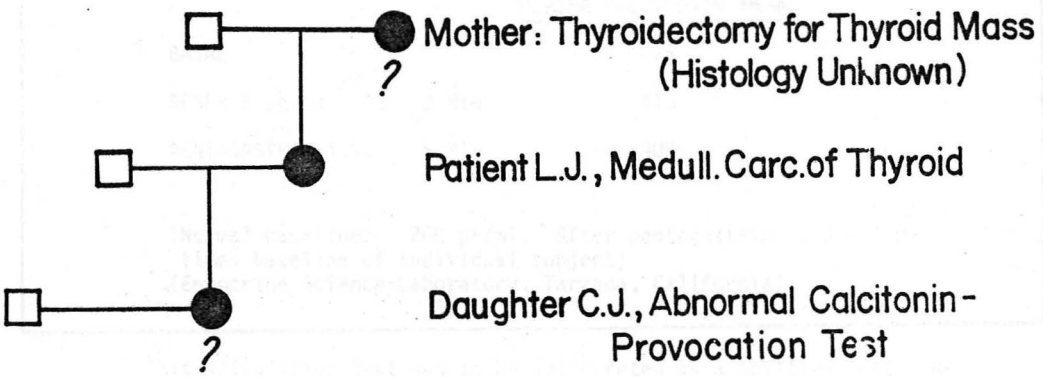
MUCOSAL NEUROMAS (LIPS, TONGUE, EYELIDS)

INTESTINAL GANGLIONEUROMATOSIS

MARFANOID HABITUS

In our patient L.J. no evidence for pheochromocytoma (normal urinary metanephrines on repeated analysis) and no evidence for hyperparathyroidism (see mineral metabolism results above) were found. Also, none of the features of MEN III (mucosal neuromas or intestinal ganglioneuromatosis) were present.

The patient's family history is depicted in Figure 4:



The patient's mother had a thyroidectomy in her 60's for a "benign" tumor. She lived for about 10 more years after the thyroidectomy during which time she was described as being very "slow." Our patient L.J. is the only child. She has one daughter (C.J.) who at the age of 33 is in good health (Ph.D. in Delaware) and has no children.

It is now recognized that the physician is obligated to screen all first-degree relatives once the diagnosis of MCT has been established in a patient (101). Screening of our patient's family for MEN II involved screening of only one person, namely the patient's daughter. Physical exam, routine laboratory tests and basal calcitonin concentration were normal in the daughter (C.J.).

Calcitonin is released abnormally by hyperplastic or malignant C-cells and thus constitutes a readily available hormone marker when elevations are encountered. "False-positive" results are possible since calcitonin is elevated in several other conditions (see CALCITONIN EXCESS, page 7). If basal calcitonin levels are normal MCT cannot be excluded and provocation tests need to be employed. Pentagastrin and calcium infusion have been used (101-104). Pentagastrin injection is now the preferred method. Table 12 gives the results of pentagastrin injection in C.J. (the patient's daughter).

TABLE 12: PENTAGASTRIN TEST IN C.J. (DAUGHTER OF L.J.)

		PLASMA CALCITONIN pg/ml
BASAL		<25
AFTER 5 µg/kg PENTAGASTRIN i.v.	2 Min	410
	5 Min	400

(Normal baseline: <200 pg/ml. After pentagastrin: 1.5 - 3.0 times baseline of individual subject)
(Endocrine Science Laboratory, Tarzana, California)

This stimulation test has to be interpreted as a positive test. We have repeated the test on February 10, 1981 (results pending; also pending are urinary metanephrines and PTH). Arrangements have been mad for an elective total thyroidectomy in the daughter. It is only through early detection of MCT and early surgery that the outcome can be improved. Sizemore, et al (105) found that persistance and recurrence was more than doubled for patients surgically treated in their 50's compared to patients in the second decade of life.

FOLLOW-UP IN PATIENT L.J.

The patient was referred for workup and therapy of the severe diarrhea (Admitted to General Clinical Research Center during August 1980). Codeine Phosphate successfully controlled the diarrhea (see above).

While many of the reported results (special histology, immunocytochemistry, plasma RIA's for polypeptide, etc.) were still pending she was dischaged and returned to Colorado.

When she was seen again in October 1980, her clinical status had deteriorated, she gave a history of hemoptysis, mild dyspnea and she was tachycardic. Extensive cardiopulmonary testing was commenced. There was no evidence for pulmonary embolism (lung scan, ABGA); spirometry was normal. However, right ventricular dilatation on echocardiography, increased interstitial lung markings, and a markedly impaired diffusion capacity (DL_{CO} = 6.9, about 30% of normal) suggested cor pulmonale. Cardiac output was increased (6 l/min) and with a narrow av-difference this was thought to be due to av-shunting. The patient's skin showed, indeed, multiple spiders which were probably due to the metastatic liver disease. The rapid development of cor pulmonale suggested lymphangitic spread of the tumor to the lungs.

Consideration was given to chemotherapy. Consulting with several centers around the country, it was learned that chemotherapy is usually not effective and is not used by most investigators who see relatively large numbers of patient with MCT. Since, however, Adriamycin (doxorubicin) had been found effective in one study (106) where it caused remission in 3 our of 7 patients, we decided to commence chemotherapy.

Adriamycin (40 mg) was started at monthly intervals and was tolerated without side effects. Plasma calcitonin, pulmonary diffusion capacity (DL_{CO}), echocardiography and chest x-ray were used as follow-up parameters (see Table 13).

TABLE 13: FOLLOW-UP DURING ADRIAMYCIN THERAPY, 40 mg WERE GIVEN i.v. ON OCTOBER 7, NOVEMBER 5, DECEMBER 3 AND JANUARY 2, 1981.

	PLASMA* CALCITONIN	DL_{CO} ml/min x mmHg	CHEST X-RAY	ECHO-CARDIOGRAPHY
October 7, 1980 (Before first dose of Adriamycin)	103,000	6.9 (30% of normal)	Increased Interstitial Markings	Enlarged right ventricle
November 5, 1980	121,000	6.1	Unchanged	Unchanged
December 3, 1980	117,000	6.0	Unchanged	Right ventricle slightly smaller
January 2, 1981	Pending	(Adriamycin administered in Walsenburg, Colorado and not in Dallas)		

*Assay of Dr. B. Roos, Cleveland, Ohio.

From the results listed in Table 13, it is impossible to prove or disprove any effect of the Adriamycin. Certainly, there was no obvious progression of the disease as judged by the listed parameters.

SUMMARY

In this Grand Rounds a patient with medullary carcinoma of the thyroid is presented. Subtotal thyroidectomy, postoperative radioiodine and neck radiation was the initial treatment. Subsequently, the patient was well for 15 years leading a normal life. A workup for large volume diarrhea led to the recognition of calcitonin-producing liver metastases. Using a wide variety of special techniques, every effort (except exploratory laparotomy) was made to search for a pancreatic primary. No evidence for pancreatic calcitoninoma was found, however.

The diarrhea (1850 g/day) was found to be mainly due to intestinal secretion (secretory diarrhea) although decreased intestinal transit time caused some carbohydrate and fat malabsorption. Abnormal secretion was localized by perfusion studies to the small bowel (ileum more abnormal than jejunum). Among many drug regimens used, Codeine Phosphate was the only drug which was clinically effective although somatostatin infusion abolished intestinal secretion during perfusion studies. We are uncertain whether chemotherapy had any effect on halting tumor progression in this patient.

Despite extreme hypercalcitoninemia there was no obvious effect on the patient's mineral metabolism as would have been expected from the pharmacological actions of calcitonin. An "escape phenomenon" (down-regulation of calcitonin receptors?) may protect the body from demineralization.

In about 20% of cases medullary carcinoma of the thyroid occurs as part of MEN II or MEN III. The patient has a positive family history for thyroid disease. All first-degree relatives of patients with medullary carcinoma of the thyroid must be tested by provocation test if basal calcitonin levels are normal. Early recognition of C-cell hyperplasia or tumor followed by early total thyroidectomy is the only proven measure which improves the prognosis of affected members of a family (autosomal dominant trait). In the patient's daughter we have found a normal basal calcitonin concentration but a pathological response to pentagastrin stimulation. An elective thyroidectomy is planned within the next few weeks.

After completion of this protocol, L.J., the patient reported in this Grand Rounds, died on February 3, 1981, in Walsenburg, Colorado. Increasing weakness and nausea preceded her demise. She died quietly at home. No autopsy was obtained.

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