

The Early Diagnosis of Medullary Carcinoma of the Thyroid Gland in Patients with Multiple Endocrine Neoplasia Type II

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Pentagastrin is a potent stimulator of thyrocalcitonin secretion from "C" cells. Since medullary carcinoma of the thyroid gland (MCT) is a tumor composed of "C" cells, pentagastrin was used to screen a large kindred with multiple endocrine neoplasia type II (MCT, pheochromocytoma (s), and parathyroid hyperplasia). Four children with no thyroid abnormalities evident on physical examination, with negative thyroid scans, and with normal levels of plasma thyrocalcitonin both before and after calcium infusion, were found to have elevated peripheral levels of this hormone following pentagastrin injection. All four children were found to have very small foci of MCT, in both thyroid lobes at the time of total thyroidectomy. Pentagastrin stimulation used conjointly with selective catheterization of the inferior thyroid vein provided even greater diagnostic accuracy in detecting elevations in thyrocalcitonin secretion. This test has great diagnostic utility, especially in screening patients with multiple endocrine neoplasia type II.

IN 1959, Hazard, Hawk, and Crile⁶ described medullary carcinoma of the thyroid gland (MCT), a tumor which has subsequently been shown to account for 5-10% of all thyroid malignancies. Clinically, it presents either sporadically or in a familial pattern inherited as a Mendelian dominant trait and associated with parathyroid hyperplasia and pheochromocytoma (s). This syndrome has been referred to as multiple endocrine neoplasia, type II

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(MEN-II).¹⁷ Although genetically there seems to be complete penetrance, there is variable expressivity and affected patients usually do not develop all three components of the disease. Almost all, however, develop MCT, and it is this lesion that develops earliest⁹ and is most life threatening. It has been shown that medullary carcinomas of the thyroid are composed of parafollicular or "C" cells which apparently arise from the neural crest. These tumor cells are capable of great biosynthetic activity, and have been shown to secrete ACTH,¹² prostaglandins,²¹ histaminase,¹ serotonin,¹³ and thyrocalcitonin (TCT).¹⁸ It is the TCT which has proved to be of greatest clinical importance because it has served as a tumor marker for MCT. Measurement of TCT in kindreds with MEN-II has been especially helpful because an elevated serum concentration of this hormone often provides the diagnosis of MCT before the lesion is palpable clinically or is demonstrable by thyroid scanning.^{9,18} Calcium,¹¹ glucagon,¹¹ and pentagastrin⁷ have all been shown to stimulate the secretion of TCT from medullary carcinoma cells, and have thereby increased the clinician's ability to diagnose this malignancy. This report demonstrates the usefulness of pentagastrin, a potent and reliable

stimulator of TCT release in screening asymptomatic family members with MEN-II for medullary carcinoma of the thyroid gland. Of special utility has been the combination of selective venous catheterization and pentagastrin stimulation in diagnosing MCT early, and in localizing metastatic disease in patients with recurrent MCT.

Materials and Methods

Patients: The patients studied were from a large kindred with multiple endocrine neoplasia, type II. All diagnostic tests were performed on the Clinical Research Units of the Duke University Medical Center or the North Carolina Memorial Hospital. The calcium and pentagastrin provocative tests were performed on separate days within a 3 to 5 day hospitalization, while the patients were receiving a diet constant in calcium (800 mg elemental calcium a day). The order in which the tests were administered was varied so that some of the patients received the calcium infusion test initially, (followed by the pentagastrin test), while others received the pentagastrin test initially, (followed by the calcium infusion test). The calcium infusion test was begun at 8:00 a.m. after an overnight fast. A total dose of 15 mg/kg of calcium ion was administered by intravenous infusion of calcium gluconate in 500 ml of 0.15 M NaCl over a 4-hr period. Blood samples were collected through an indwelling needle in the antecubital vein at 0, 15, 30, 60, 120, 180, and 240 minutes for subsequent determinations of calcium, phosphate, and TCT. Samples for TCT analysis were collected in heparinized tubes, chilled, and the plasma promptly separated and frozen at -20°C until assayed. Serum calcium³ and phosphate⁴ concentrations were determined by the automated methods of Gitelman.

Pentagastrin stimulation tests were begun at 8:00 a.m. after an overnight fast. After collection of baseline blood samples, patients were given pentagastrin (Peptavlon): a gift from Ayerst Laboratory, in a dose of 0.5 mcg/kg by rapid infusion injection (over 5-10 seconds) in 2-4 ml of 0.15 M NaCl. Blood samples were taken at 0, 1, 2, 3, 5, 10, 15, 30, and 60 minutes. Approximately 30 seconds after the injection, a majority of patients noticed mild epigastric pain which usually persisted for 45-60 seconds. No serious side effects were encountered.

Other studies performed during hospitalization included a complete blood count, blood chemistry analysis, urinalysis, and 24-hour urinary catecholamine determination. All patients had a chest X-ray, a metabolic bone survey, and an ¹³¹I thyroid scan and 24-hour uptake.

Selective venous catheterization: In two patients, an inferior thyroid vein was catheterized using the Muller guided catheter system as described by Doppman.² Blood was sampled from an inferior thyroid vein and simultaneously from a peripheral antecubital vein before and at 1, 2, 3, 5, and 10 minutes following the intravenous injection

of pentagastrin. The blood samples were placed in heparinized tubes, immediately chilled, and the plasma separated for subsequent TCT determination.

In one patient who had previously undergone total thyroidectomy for medullary carcinoma and who had one year later demonstrated an elevated level of TCT following pentagastrin injection, four catheters were placed simultaneously into the left inferior thyroid vein, the hepatic vein, the basilic vein, and the root of the aorta. Before and at 1, 2, 3, 5, and 10 minutes following the injection of pentagastrin, blood samples were collected in heparinized tubes from each of these veins. Subsequently the plasma was separated from the chilled tubes and frozen for TCT determination.

Detection of thyrocalcitonin in plasma: The radioimmunoassay used to detect thyrocalcitonin in the plasma of patients studied has been previously described.⁸ The lower limit of sensitivity of the assay in human plasma was 240 pg/ml. Using the assay as described, we have been unable to detect TCT in any plasma samples from normal subjects, even after injection of pentagastrin.

Radioimmunoassay of thyroid glands for thyrocalcitonin content: Fresh tissue slices removed from the upper, middle, and lower portions of each thyroid lobe were taken at the time of surgery and homogenized in 0.1 N HCl in a volume of 10 ml/gm. The material was then centrifuged at 15,000 rpm for 10 minutes and the supernatant was radioimmunoassayed for thyrocalcitonin content.⁸

Histologic studies: At the time of total thyroidectomy, additional tissue slices from the upper, middle, and lower parts of each lobe were taken. Half of these slices were fixed in Zenker's formol solution for routine pathologic evaluation. This fixed material was subsequently embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The adjacent portions of tissue were fixed in buffered formalin and washed three times in phosphate buffered saline. This material was stained with the immunoperoxidase bridge technic by Dr. T. C. Peng to localize thyrocalcitonin in "C" cells. This technic which depends upon the binding of a specific anti-TCT antibody to the surface of cells containing TCT has been previously described.¹⁵

Surgical Procedure

Once a patient had demonstrated an elevated level of thyrocalcitonin following pentagastrin stimulation, they were advised to undergo a total thyroidectomy. Under general anesthesia, a standard anterior cervical incision was made and a total thyroidectomy was performed. Lymph nodes as far as the carotid sheaths bilaterally were removed with the thyroid mass. Great care was taken not to compromise the vascularity or the substance of the parathyroid glands.

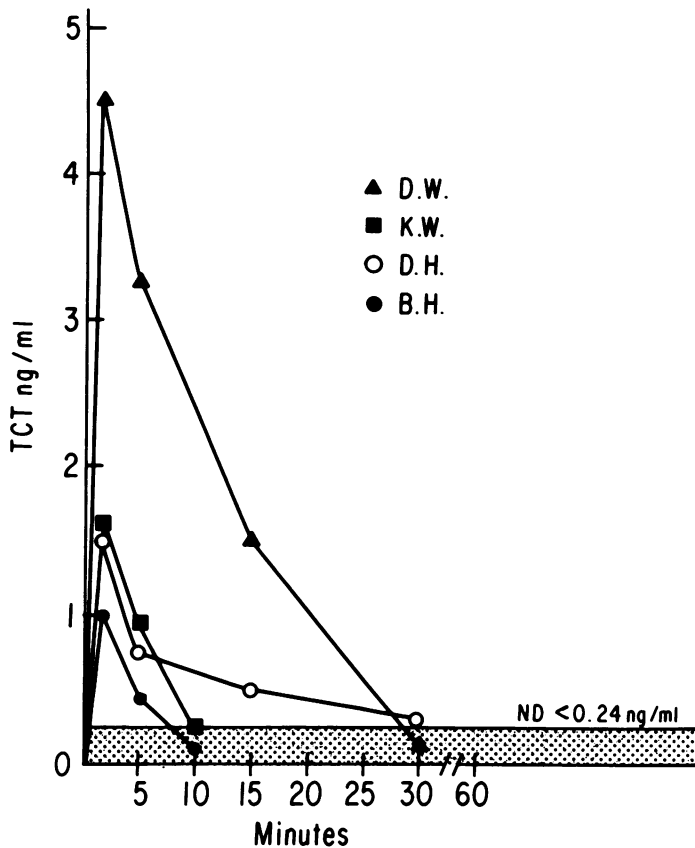


FIG. 1. Thyrocalcitonin response to pentagastrin stimulation in four children (D.W., K.W., D.H., and B.H.) with a family history of multiple endocrine neoplasia type II.

Results

Over the last two years a total of 40 members from a large kindred with multiple endocrine neoplasia type II

have been screened. During the first year of screening, three children, D.W., B.H., and D.H., ages 13 to 17, were diagnosed as having medullary carcinoma of the thyroid gland. None of these children had detectable TCT in the peripheral blood, even in response to a calcium infusion. However, all had elevated levels of thyrocalcitonin (greater than 1.0 ng/ml) within 1 to 3 minutes following pentagastrin injection (Table 1 and Fig. 1). During the second year of study, the fourth patient (K.W.) had an elevated level of TCT following pentagastrin injection (Table 1 and Fig. 1). He had shown a negative response to both calcium infusion and pentagastrin injection the previous year and remained unresponsive to calcium stimulation the second year. None of these four patients had palpable thyroid nodules or evidence of thyroid lesions on ^{131}I scanning.

The operative procedure used in these children was a total thyroidectomy combined with removal of lymphatic tissue laterally as far as the carotid sheaths. At the time of surgery no mass was palpable in either thyroid lobe in 2 of the 4 patients, however lesions were visible when the thyroid parenchyma was sectioned following removal of the gland from the neck (Fig. 5). In the remaining two patients small thyroid nodules were noted at surgery in the upper regions in each thyroid lobe. Only one patient (D.W.) had tumor extending to the thyroid capsule. In no patient was tumor detected in the lymph nodes adjacent to the thyroid gland.

In addition to the tumor foci which were grossly visible in the thyroid lobes of each patient, there were smaller microscopic foci of "C" cell clusters. The content of thyrocalcitonin in the thyroid gland of each patient was determined by radioimmunoassay. None of the sections

TABLE 1. Patients Diagnosed as Having Medullary Carcinoma of the Thyroid Gland by Pentagastrin (Pg) Stimulation

Patient	Baseline TCT Level* With Ca^{++} Infusion	TCT Peak* With Pg Stimulation (Peripheral Vein)	TCT Peak* With Pg Stimulation (Thyroid Vein)	Clinical Findings	Findings at Surgery
D.W.	N.D.	N.D.	4.5 ± 1.3	N.T.	Macroscopic Bilateral MCT
K.W.	N.D.	N.D.	1.4 ± 0.5	18	Macroscopic Bilateral MCT
D.H.	N.D.	N.D.	1.5 ± 0.6	N.T.	Macroscopic Bilateral MCT
B.H.	N.D.	N.D.	1.0 ± 0.1	N.T.	Macroscopic Bilateral MCT
C.P.	N.D.	N.D.	N.D.	9.6 ± 2.1	No. Macroscopic Disease. "C" Cell Hyperplasia Microscopically.

N.D. = not detectable (<240 pg/ml)

*Values of TCT expressed in ng/ml \pm S.E.M.

assayed contained grossly visible tumor. As can be seen in Table 2, the highest levels of TCT were detected in grossly normal tissue from each patient, mainly in the middle and upper regions of the thyroid lobes. The values ranged from 10 to 7200 ng per gram of tissue. This is consistent with the findings of Wolfe, and associates.²⁰

In all 4 patients there was no detectable thyrocalcitonin following pentagastrin stimulation in the immediate post-operative period.

One patient (B.H.) was hypercalcemic preoperatively, and at surgery was found to have two grossly enlarged and microscopically hyperplastic parathyroid glands. Another patient (D.W.) had microscopic evidence of parathyroid hyperplasia, although she had no elevation in her serum calcium concentration preoperatively. Two other patients, (K.W. and D.H.), had no hypercalcemia preoperatively and their parathyroid glands appeared normal both to gross and microscopic examination.

All 4 patients are now on thyroid replacement therapy but none requires Vitamin D or calcium supplement.

Selective venous catheterization: In patient K.W., who was known to have an elevated peripheral level of TCT following pentagastrin injection, a selective venous catheterization study was performed just prior to his surgery. As demonstrated in Fig. 2, a markedly higher

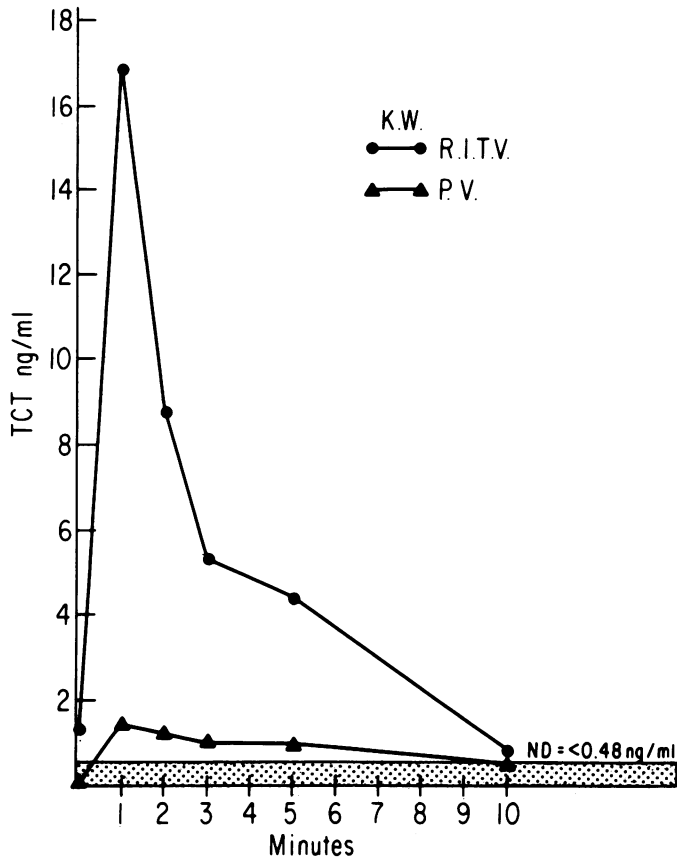


FIG. 2. Thyrocalcitonin response to pentagastrin stimulation in patient K.W. Note the higher level of TCT in the right inferior thyroid vein compared to the level in a peripheral vein.

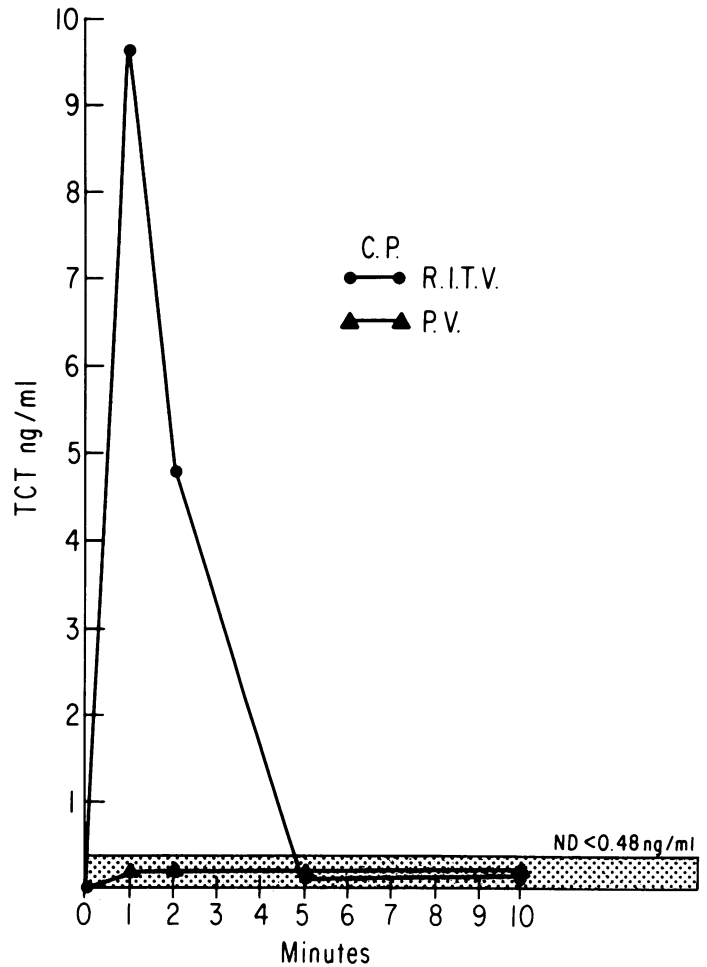


FIG. 3. Thyrocalcitonin response to pentagastrin stimulation in patient C.P. Note the absence of TCT elevation in the peripheral blood following pentagastrin stimulation. An elevated TCT level is only detected in the right inferior thyroid vein.

concentration of thyrocalcitonin was detected in the right inferior thyroid vein following pentagastrin injection than was measurable in the peripheral vein sampled concurrently.

Patient C.P., a 40-year-old man, had no measurable TCT level in the peripheral blood following pentagastrin stimulation. However, because of his positive family history he underwent selective venous catheterization and pentagastrin stimulation. As shown in Fig. 3, no TCT was detectable in the antecubital vein during the pentagastrin stimulation test, but there was a peak value of 9.8 ng/ml of TCT in the right inferior thyroid vein 1 minute following pentagastrin stimulation. A total thyroidectomy was performed and no macroscopic tumor was visualized in the sectioned thyroid gland at surgery or subsequently by pathological examination. On microscopic examination of H and E stained sections, however, foci of "C" cell hyperplasia were noted (Fig. 6). Furthermore, on immunoperoxidase staining, clusters of "C" cells were readily detected, due to their specific content of TCT. Radioimmunoassay measurement of

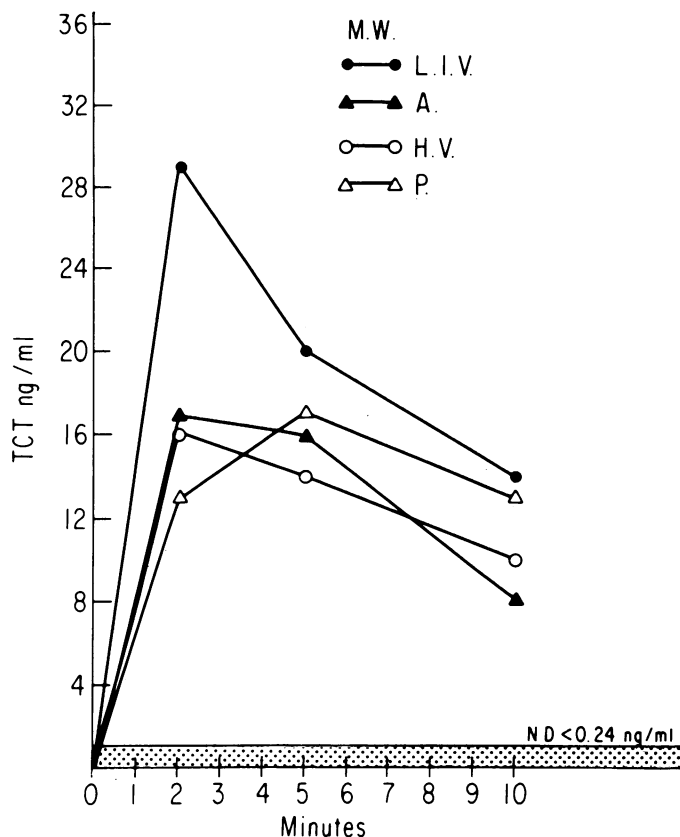


FIG. 4. Thyrocalcitonin response to pentagastrin stimulation in patient M.W. Note the highest level of TCT in blood sampled from the left innominate vein.

portions of the thyroid gland removed at surgery demonstrated abnormally high concentrations of TCT in the thyroid glands, especially in the middle regions of both lobes (Table 2). Two parathyroid glands were removed from patient C.P.; one was enlarged and one was of normal size grossly; however, both showed absence of fat cells and histological characteristics of parathyroid hyperplasia.

Patient M.W. had a total thyroidectomy in July 1973 for medullary carcinoma of the thyroid gland. Preoperatively, he had a basal plasma level of thyrocalcitonin of 51 ng/ml which increased to 352 ng/ml at 2 minutes following pentagastrin stimulation. Postoperatively, he

TABLE 2. TCT Concentration (ng/gm)* in the Thyroid Glands of Patients With MCT Diagnosed by Pentagastrin Stimulation

Thyroid lobe	D.W.	K.W.	D.H.	B.H.	C.P.
R Upper	190	64	7200	2500	85
R Middle	70	483	870	71	5030
R Lower	70	10	70	85	135
L Upper	1300	10	75	2300	77
L Middle	70	566	1200	260	5400
L Lower	70	420	70	470	650

*TCT concentration in the thyroid tissue of normal individuals = 10 to 300 ng/gm.

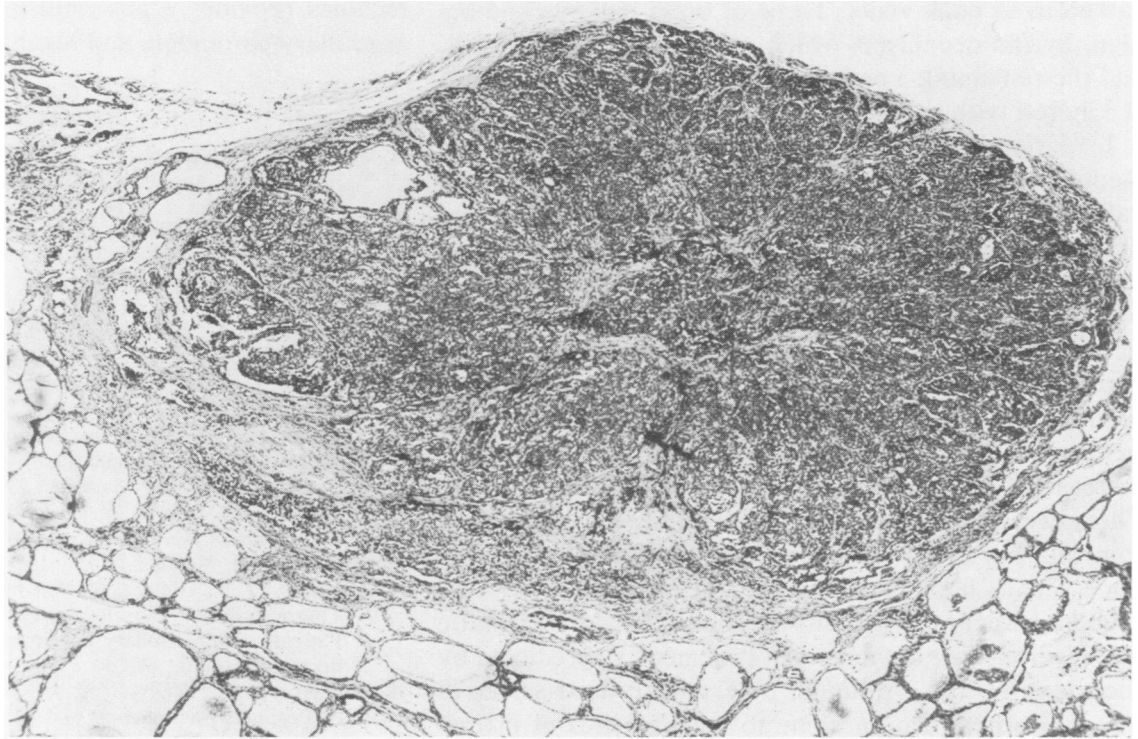
had no detectable TCT in the peripheral blood following pentagastrin stimulation. One year following surgery he was re-tested, and although the basal level of TCT was still undetectable, there was an elevated level of 17 ng/ml in peripheral venous plasma following pentagastrin stimulation. In an attempt to localize the recurrent tumor, catheters were selectively placed in the left innominate vein, the hepatic vein, a peripheral vein, and the root of the aorta. As can be seen in Fig. 4, the highest concentration of TCT following pentagastrin injection was detected in the left innominate vein. Accordingly, the patient's neck was re-explored and he was found to have lymph nodes containing tumor in the left side of the neck. A left radical neck dissection was performed. There was no disease by palpation in the right neck and frozen section examination of random nodes revealed no evidence of metastatic disease there.

Discussion

A total of 40 patients were evaluated in the initial phase of this study. A comparison of the TCT response both to calcium infusion and pentagastrin injection was assessed and has been previously reported.⁸ Briefly, it was found that pentagastrin was a much more potent stimulator of thyrocalcitonin secretion from MCT than calcium infusion. Following the injection of pentagastrin (0.5 mcg/kg) the peak TCT response occurred at approximately 2 minutes, compared to a peak response at 30 minutes following initiation of the calcium infusion test (15 mg/kg/4 hours). Furthermore, the peak TCT response following pentagastrin was two to four fold higher than that following calcium infusion. These two obvious advantages combined with the rapidity and simplicity of pentagastrin administration, currently make it the agent of choice in screening for medullary carcinoma of the thyroid gland. Our interest in the current study was to determine if pentagastrin was effective in detecting MCT in patients with preclinical disease. That pentagastrin was effective in this regard was readily demonstrated by four patients (D.W., K.W., D.H., and B.H.) in this study. Medullary carcinoma of the thyroid gland was diagnosed in each by demonstrating an elevated concentration of TCT in the peripheral blood following pentagastrin stimulation. The sensitivity of this diagnostic test is impressive when one considers the absence of thyroid nodules on physical examination, the failure to demonstrate lesions by thyroid scan, the actual small size of the malignant primary tumors and the absence of metastatic disease in regional lymph nodes.

As demonstrated previously in selective venous catheterization studies in patients with parathyroid adenomas, higher concentrations of parathyroid hormone are detectable in the venous effluent closest to the source of its production.^{16,19} Similar observations have been made in patients with medullary carcinoma of the thyroid gland.^{5,14} A combination of pentagastrin stimu-

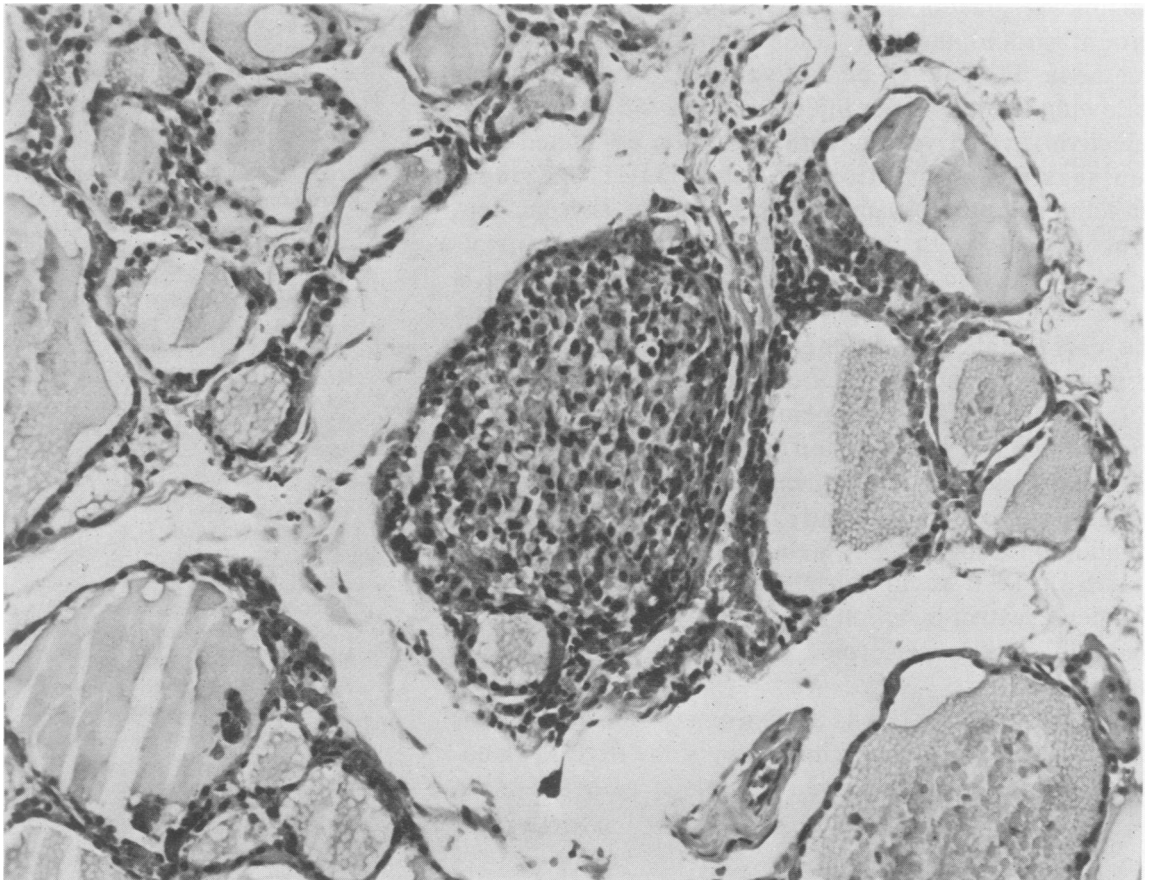
FIG. 5. Grossly visible medullary carcinoma nodule in the right thyroid lobe of patient K.W. Hematoxylin and eosin, original magnification $\times 25$.



lation and selective venous catheterization provides a very sensitive method for diagnosing primary medullary carcinoma of the thyroid gland. As demonstrated by patient C.P. in this study, thyrocalcitonin elevation occurred only in the catheterized thyroid vein and

not in the peripheral vein, and only following pentagastrin stimulation. We have studied 6 other persons, using the selective catheterization technic combined with pentagastrin stimulation, and have not detected elevated levels of TCT in either the peripheral veins or the

FIG. 6. A focus of "C" cell hyperplasia demonstrable in the thyroid gland of patient C.P. Hematoxylin and eosin, original magnification $\times 190$.



catheterized neck veins. Three of these patients had benign thyroid neoplasms which were resected at surgery, and the remaining 3 patients were members of the MEN II kindred with as yet no evidence of MCT.

In patients who have undergone thyroidectomy for medullary carcinoma and subsequently developed elevated levels of TCT with or without pentagastrin stimulation, selective venous catheterization is advantageous in localizing the site of recurrence. In one of the patients in our study (M.W.), there was no detectable level of TCT in the aortic root draining the pulmonary bed, in the hepatic vein draining the liver, in the innominate vein draining the neck, or in the antecubital vein. Following pentagastrin injection, however, there was a marked increase of TCT detectable in the left innominate vein. There were increased levels of TCT in the other sites sampled, but they were all in the same general range and none was as high as the innominate vein level. At subsequent surgery the patient was found to have metastatic disease involving the left side of the neck as predicted by the assay results. Although others have used selective venous catheterization alone to localize sites of medullary carcinoma,^{5,14} the combination of this technic with pentagastrin stimulation, especially when the effluent from several organs is simultaneously sampled appears especially effective in localizing metastatic disease.

Our current method of following patients with a family history of multiple endocrine neoplasia type II is to perform pentagastrin stimulation tests yearly in all children above the age of 10 years. Should the level of TCT in peripheral plasma be 1.0 ng/ml or greater then the patient should undergo total thyroidectomy. Should the peak TCT level be between 0.5 and 1.0 ng/ml following pentagastrin injection then we favor using selective thyroid venous catheterization combined with pentagastrin stimulation to detect MCT. A single catheter is used to sample either inferior thyroid vein concomitantly with the peripheral vein following pentagastrin injection. At the time of surgery an attempt is made to remove all lymph node tissue laterally as far as the carotid sheaths. If metastatic disease is present grossly in the neck, a neck dissection is performed.

In the immediate postoperative period, the pentagastrin stimulation test is again performed, samples being obtained only from a peripheral vein. If this is negative, patients are followed at yearly intervals with pentagastrin stimulation tests and peripheral sampling. Should an elevation in TCT occur during one of these tests, then the pentagastrin injection combined with sampling from multiple sites is performed to localize metastatic disease.

The pentagastrin test is an extremely sensitive means for diagnosing medullary carcinoma of the thyroid gland. Its combination with selective venous catheterization

technics provides a powerful tool both for diagnosis of medullary carcinoma and localization of metastatic foci.

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