

## Medullary Carcinoma and Thyrocalcitonin\*

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**S**ummary: Seven patients with medullary carcinoma of the thyroid were investigated, and the thyrocalcitonin activity of the primary tumours, metastatic deposits, and the serum was estimated.

Medullary carcinomas of the thyroid with amyloid stroma contain 100 to 600 times more thyrocalcitonin than normal thyroid tissue, and lymph nodes with metastatic deposits are equally rich in the hormone. High values of thyrocalcitonin are also found in the blood of these patients. Medullary carcinoma of the thyroid with amyloid stroma is the first disease to be recognized in which there is hypersecretion of thyrocalcitonin.

### Introduction

Medullary carcinoma with amyloid stroma is now a well-defined entity among thyroid tumours from a pathological point of view (Hazard *et al.*, 1959; Gérard-Marchant, 1965; Williams, 1966). The undifferentiated histological pattern of this slow-growing tumour presents a paradox which is still the subject of much speculation (Williams, 1966; Williams *et al.*, 1968).

We have recently reported (Milhaud *et al.*, 1968) the finding of significant amounts of thyrocalcitonin in two cases of thyroid medullary carcinoma, and in the metastatic lymph nodes and blood of these patients. Previously a high level of thyrocalcitonin activity had been reported in two patients with pseudohypoparathyroidism (Aliapoulos *et al.*, 1966; Tashjian *et al.*, 1966). Later, Meyer and Abdel-Bari (1968) published the study of a case of medullary carcinoma in which thyrocalcitonin was found in the gland. Since our preliminary communication we have studied four additional cases of medullary carcinoma. It is the aim of this paper to report the results of these investigations and to discuss their meaning.

### Case Reports

*Case 1.*—This patient was a 40-year-old man. In 1959 he presented with an extremely hard tumour of the right lobe of the thyroid gland and multiple cervical lymph node enlargements. Surgical extirpation was not possible, and he was given cobalt, 6,000 rads. Since 1960 there has been practically no increase in size of the irradiated masses. In 1964 he had a transient functional diarrhoea, which was resistant to all methods of treatment. A new hard cervical mass appeared in 1967 in a region which had not been irradiated in 1959, and this was excised. Study of the specimens from the 1959 and 1967 operations showed a medullary carcinoma with amyloid stroma.

*Case 2.*—This patient, a 37-year-old man, presented with a left testicular tumour in December 1966. Orchidectomy was carried out in January 1967 followed by telecobalt therapy to the para-aortic lymph nodes, which had been shown by lymphography to be invaded, and also to the left supraclavicular nodal region. Enlargement of the left cervical lymph nodes appeared in October

1967, and he was referred to the Institut Gustave-Roussy for chemotherapy. The left lobe of the thyroid was hard to palpation, and scintigraphy confirmed a lack of uptake in the left lobe. The presence of several confluent circular areas of pigmented skin over the face and shoulders was also noted. In November 1967 a left thyroid lobectomy was carried out with removal of the isthmus, and a lymphadenectomy on the left side of the neck. Histological section of the testicular tumour showed amyloid substance in the tumour stroma. The final diagnosis was "thyroid carcinoma with an amyloid stroma, metastasis to the testicle."

*Case 3.*—A 28-year-old Guadeloupean woman had been suffering since the age of 12 from a poorly defined disease of bone which was thought to be tuberculous in origin. Skeletal survey films taken in 1964 showed osteolytic lesions of the entire skeleton, with zones of condensation. In addition, bilateral cervical lymphadenopathy was present. Bone and lymph node biopsy revealed an epithelioma, the primary site of which could not be established. The patient was studied in the Institut Gustave-Roussy in June 1967, and as a result a thyroidectomy was performed. Histologically the specimen showed a medullary carcinoma with amyloid stroma. Bone biopsy confirmed the metastatic character of these lesions, but no amyloid substance was seen. A biopsy of the liver, however, showed the presence of metastatic tumour with amyloid substance. After a year diarrhoea occurred, and this responded favourably to symptomatic treatment.

*Case 4.*—The patient, a 68-year-old man, was seen in October 1965 suffering from diarrhoea and weight loss. At that time several cervical and supraclavicular lymph nodes were enlarged. The aetiology was not established, and he was treated with telecobalt, 6,000 rads, to the left cervical lymph nodes. In January 1968 a mass appeared in the left axilla. A biopsy specimen of this mass showed a medullary thyroid cancer with numerous areas containing amyloid substance. The diagnosis was further confirmed by a scan which showed poor uptake of <sup>131</sup>I in the left lobe of the thyroid and by the presence of thyrocalcitonin in the blood. An attempt at definitive surgery was complicated by the marked degree of post-radiation fibrosis, and consisted of the removal of a block of tissue containing the left posterior carotid lymph nodes, and of a subtotal left thyroidectomy.

*Case 5.*—A 13-year-old girl had undergone seven major abdominal surgical procedures for a colonic disease considered to be Hirschsprung's disease. Finally a total colectomy was performed in June 1967, but a rectovaginal fistula persisted and severe diarrhoea developed. In December 1967 she was seen at the Institut Gustave-Roussy because of paralysis of the right recurrent laryngeal nerve. The right lobe of the thyroid was greatly enlarged and extremely hard. Bilateral cervical lymphadenopathy was present. The thyroid scan demonstrated a lack of <sup>131</sup>I uptake in the right lobe. Surgery was performed in February 1968. Only three-quarters of the right lobe could be excised because of the fixation of the tumour.

*Case 6.*—This patient, a 46-year-old woman, had been aware of a nodular goitre on the right side since 1959. In May 1967 right supraclavicular lymphadenopathy appeared, for which she was operated on in June 1967. A right thyroid lobectomy and isthmectomy were performed. She was treated with telecobalt, 5,500 rads, to the superior mediastinum and to the right supraclavicular region.

*Case 7.*—A 44-year-old woman was operated on in March 1966 for removal of a left nodular goitre. Histology showed a medullary carcinoma, without amyloid stroma by specific colouration, with poor vesicular differentiation. After a hemilobectomy she was irradiated by telecobalt, 5,000 rads. In January 1968 several bone metastases were observed. Her general condition was poor, and she died at home two months later.

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## Bioassay of Thyrocalcitonin

## Methods

The assay of thyrocalcitonin activity is based on its hypocalcaemic (Kumar *et al.*, 1965) and hypophosphataemic action. It is carried out in 28-day-old Wistar C.F. female rats, of the same strain, raised under the same conditions and fasted for 24 hours before the test.

The thyrocalcitonin activity of serum to be tested is assayed by comparing in the same animal the serum calcium and phosphorus levels before injecting the serum with the levels one hour later. Several control groups of three rats are used for each assay: animals injected with normal serum in various dilutions, animals injected with a known quantity of thyrocalcitonin (5 and 20 mU. M.R.C.) diluted in an acetate buffer, and animals injected with the acetate buffer alone.

The thyrocalcitonin activity of an organ is assayed according to the same principles after extraction of the acetone-dried powder by HCl following the technique of Baghdiantz *et al.* (1964), dialysis in a solution of acetate buffer, and salt fractionation.

## Results

No thyrocalcitonin activity was found in two human papillary carcinomas, neither in the tumour nor in a metastatic cervical lymph node (Table I). In contrast a marked thyrocalcitonin activity was found in all the five cases of medullary carcinoma with amyloid stroma which were assayed (three specimens were thyroid tumours and three metastatic cervical lymph nodes).

TABLE I.—*Hypocalcaemic and Hypophosphataemic Activity of Thyrocalcitonin in Specimens*

Case No.	Diagnosis	Specimen	Hypocalcaemic Activity (mU. MRC/mg.*)	Hypophosphataemic Activity (mU. MRC/mg.)
1	Medullary carcinoma with amyloid stroma	Cervical lymph node	4.3 (2.6-8.1)	4.6 (3.8-6.1)
2		Thyroid gland	4.0 (2.7-5.9)†	4.1 (3.2-5.4)
3	" "	Cervical lymph node	5.4 (4.7-6.3)	4.4 (3.0-6.4)
4		Thyroid gland	8.2 (5.5-12)	8.0 (6.0-10.7)
5	" "	Cervical lymph node	7.5 (5.2-10.9)	8.0 (6.4-10.0)
8		Thyroid gland	2.5 (1.7-3.4)	10.8 (6.5-17)
9	Papillary carcinoma of thyroid gland	Cervical lymph node	No activity	No activity
	" "	Thyroid gland	" "	" "

\* Milliunit of the Medical Research Council per milligram of dry tissue.

† Confidence limit:  $P < 0.05$ .

The hypocalcaemic activity per mg. of dry tissue is 100 to 600 times higher than in normal thyroid gland (0.04 mU./mg.). Furthermore, the hypocalcaemic and hypophosphataemic activities were approximately the same.

TABLE II.—*Hypocalcaemic Activity of Serum*

Case No.	Diagnosis	Serum Ca (mg./l.) (N=90-110)	Serum P (mg./l.) (N=30-40)	Hypocalcaemic Activity of Serum (mU. MRC/ml.)
1 (before surgery)	Medullary carcinoma with amyloid stroma	117	35	54 (32-92)
1 (after surgery)		110	25	No activity
2 (after surgery)	" "	107	27	" "
3 (after surgery)	" "	91	30	46 (26-122)
4 (before surgery)	" "	118	30	32 (25-42)
5 (after surgery)	" "	98	Not assayed	No activity
6 (after surgery)	" "	121	36	" "
7	Medullary carcinoma without amyloid	98	35	" "
8	Papillary carcinoma of thyroid	101	32	" "
9	" "			" "

A high hypocalcaemic activity was found in the serum in three of these five patients (Table II). In one of them (Case 1), a hypocalcaemic activity was found in the serum before surgery, but was not found after in spite of the fact that surgery was not complete and that diarrhoea persisted. The serum of another (Case 2) was assayed only after surgery, and no activity was found. In this case removal of the tumour had been macroscopically satisfactory. No activity was present in the serum in Case 6, but there was at that time no evidence of a cancerous recurrence.

Hypocalcaemia was never observed; in three cases, on the contrary, hypercalcaemia was present.

Conversely, the serum of a patient with a medullary cancer without amyloid stroma (Case 7) presented no hypocalcaemic activity at the time of the assay, though there was evidence of a recurrence of cancer. In this case hypercalcaemia could be explained by the bone metastases.

No activity was found in the serum of two patients with papillary cancer of the thyroid or in the serum of normal subjects.

## Discussion

*Clinically* our patients conformed to the characteristics of medullary cancers already reported (Williams *et al.*, 1966). The age of our patients ranged widely, from 13 to 72 years. In none of these cases was there any family history of a similar tumour or evidence suggesting a pheochromocytoma—vanillyl mandelic acid catecholamines, and urinary 5-hydroxyindole acetic acid being within normal range.

In all of our patients the general condition remained good for a long time. In two of them radiotherapy appears to have been effective, inducing regression of the tumour mass or arresting its growth for at least eight years in one case (Case 1) and for three years in another (Case 4). In one case an association with pigmented spots was observed, and in three cases functional diarrhoea was present. We have been particularly impressed by the extreme hardness of the tumours and the involved nodes, as well as by their pronounced tenderness to palpation.

The diagnosis was often difficult; in three cases the primary thyroid tumour was very small despite the existence of metastases. The location of these metastases is most peculiar. In addition to the involvement of the cervical lymph nodes observed in all six patients, axillary lymph node metastasis was seen in one (Case 4) and para-aortic nodes in another (Case 2). The metastases differ in nature from other thyroid cancers: case 3 with diffuse bone metastases presented a peculiar radiographic picture demonstrating diffuse osteolytic lesions of the entire skeleton, case 2 had a testicular metastasis, and case 3 had hepatic metastases. Liver metastases are exceptional in thyroid cancer, even though we have observed them in another patient, not considered in this paper, who also had a medullary cancer with amyloid stroma. Furthermore, Williams *et al.* (1966) reported five cases of liver metastases in 20 necropsies of patients who died from medullary thyroid carcinoma.

*Biologically*, the association between this histological type of tumour and the presence of a thyrocalcitonin activity in the tumour tissue is highly significant. A high level of thyrocalcitonin activity was found in tumour tissue in all cases which were assayed, whether in the thyroid or in the metastatic cervical lymph nodes.

Moreover, thyrocalcitonin activity was present in the blood in three patients. This shows that the tumours not only synthesize thyrocalcitonin but also secrete it. Of the four cases in which we did not find any thyrocalcitonin in the blood three had undergone surgery, but it is not known whether this surgery was complete or not. The fourth lacked amyloid in the stroma.

This high concentration of thyrocalcitonin seems to be specific of medullary cancers in which the presence of an

amyloid stroma is demonstrated by specific staining techniques (Gérard-Marchant, 1965). We have not observed it in two cases of papillary cancer.

The question is now raised whether this activity is due to the presence of thyrocalcitonin or to another substance with a similar biological activity. Two arguments favour thyrocalcitonin hypothesis: the purification procedure used is that which serves to demonstrate the presence of thyrocalcitonin in normal human thyroid gland, and which currently is used to prepare pork thyrocalcitonin. Further, the ratios of hypocalcaemic and hypophosphataemic activity are very close to those for thyrocalcitonin. Our results, in particular the presence of thyrocalcitonin in involved nodes, suggest that medullary cancer with amyloid stroma is a secreting tumour whose cells are developmentally different from those that stem from the follicular cells. The fact that these tumours contain no iodine (Ljungberg, 1966) supports that hypothesis.

Williams (1966) has supposed that these cancers develop from the parafollicular cells and may produce thyrocalcitonin, but has not presented experimental evidence to support this. Our observations therefore are the first to show evidence of the part played in human pathology by these thyrocalcitonin-producing cells.

We have not observed hypocalcaemia in these cases in spite of the presence of thyrocalcitonin. Moreover, hypercalcaemia was present in three patients. These paradoxical findings have no good explanation at the present time, but suggest over-reaction of a compensatory mechanism.

What is the role of amyloid substance in medullary cancer of the thyroid? We have not found thyrocalcitonin activity in our one case without amyloid stroma. It would be interesting to determine if the medullary cancers without amyloid stroma described by Williams (1966) in dogs and rats have any thyrocalcitonin activity. On the other hand, if it is confirmed

in man that the medullary cancer without amyloid stroma has no thyrocalcitonin activity, it might be possible by biological assay to distinguish the two types of cancer.

Finally the diarrhoea cannot be explained by the hyperproduction of thyrocalcitonin, as no diarrhoea was observed in two patients (Cases 2 and 4) in whom tumour contained a high amount of thyrocalcitonin. Furthermore, animals injected with very high doses of thyrocalcitonin (1,000 milliunits) presented no diarrhoea (Milhaud, personal communication). One can reconcile this with the fact that Williams *et al.* (1968) demonstrated the presence of prostaglandin substance having a motor action on smooth muscle in several patients. It would be interesting to study this problem in order to determine if there is a correlation between prostaglandin and thyrocalcitonin concentration, or if they are independent.

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## Rapid Diagnosis of Herpesvirus hominis Infections in Superficial Lesions by Immunofluorescent Antibody Techniques

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[WITH SPECIAL PLATE BETWEEN PAGES 94 AND 95]

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**S**ummary: Scrapings from lesions of the skin and mucous membranes of 14 patients suspected of suffering from suspected Herpesvirus hominis infections were examined by both fluorescent antibody and routine isolation techniques. There was complete correlation between results by both methods in all 14 cases, 12 being positive. No positive fluorescence was obtained from scrapings of seven control patients with a variety of skin diseases. Thirteen patients with corneal lesions were similarly investigated. Of the 10 scrapings which showed positive fluorescence, nine were confirmed by virus isolation. It is suggested that as more antiviral agents become available the application of a fluorescent antibody technique for testing virus sensitivity in tissue culture could become a practical method.

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### Introduction

*Herpesvirus hominis* is a ubiquitous virus causing a variety of illnesses, and two of the most common conditions with which it is associated are corneal infections and lesions of the skin and mucous membranes. In recent years claims have been made that the virus is sensitive to the antiviral agent 5-iodo-2'-deoxyuridine (idoxuridine) (Kaufman, 1962; Kaufman, Martola, and Dohlman, 1962).

It is therefore surprising that greater attempts have not been made to establish methods of rapid diagnosis for this virus, especially as material is readily accessible from the superficial lesions. Biegeleisen, Scott, and Lewis as long ago as 1959 correlated virus isolation with the fluorescent staining of skin lesions, but no further attempts were made to exploit this method. Kaufman (1960) and Pettit, Kimura, and Peters (1964) stained cell scrapings both from rabbits whose corneas had been experimentally infected with *Herpesvirus hominis* and from human cases of ocular herpetic infections and found a close correlation between their isolation and fluorescent anti-