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Two Cell Types in Familial Medullary Thyroid Carcinoma

A Histochemical Study

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Zelltypologie des Schilddrüsencarcinoms

Eine histochemische Untersuchung

Zusammenfassung. Medulläre Schilddrüsencarcinome und eine Lymphknotenmetastase von 4 Patienten — alle Mitglieder einer einzigen Familie mit vermehrtem Auftreten von medullärem Schilddrüsencarcinom und Phäochromocytom — wurden mit histologischen Methoden (argentaffine, chromaffine, Jodat-, Diazo-Kupplungs- und Ninhydrin-Reaktion) auf Monoamine untersucht.

Ungleichmäßig verteilte Haufen von argentaffinen und chromaffinen Zellen wurden in sämtlichen Schilddrüsentumoren ebenso in der Lymphknotenmetastase nachgewiesen. Jodat-positive Zellen wurden auch in den beiden Fällen gefunden, die mit dieser Methode untersucht wurden. Die argentaffinen und chromaffinen Zellen wiesen eine bemerkenswerte spindelige Form mit langen cytoplasmatischen Ausläufern auf, die nichtreagierende, runde Tumorzellen mit klarem Cytoplasma umschlossen. Sie standen oft in enger Beziehung zu den Blutgefäßen und unterschieden sich dadurch von den nicht reagierenden Zellen. Es wurde daher die Möglichkeit in Betracht gezogen, daß die hier untersuchten Schilddrüsencarcinome zwei funktionell verschiedener Zellpopulationen aufgebaut sein könnten. Dabei könnte die eine Population mit der Polypeptid-(Calcitonin-)Synthese verknüpft sein, die andere monoaminergische Funktionen besitzen. Es wird betont, daß der Nachweis von monoaminhaltigen Zellen dafür spricht, daß diese Tumorform zumindest teilweise neuroepithelialen Ursprungs ist. Dies deutet auf eine nahe Verwandtschaft zu Phäochromocytom und anderen Crista neuralis-derivierten Tumoren hin, die beim familiär auftretenden medullären Schilddrüsencarcinom-Phäochromocytom gehäuft vorkommen.

Summary. Medullary thyroid carcinomas and one lymph node metastasis from four patients belonging to a family with a high incidence of medullary thyroid carcinoma and phaeochromocytoma were studied histochemically for moncamines (argentaffin, chromaffin, iodate, diazo-coupling and ninhydrin reactions). Unevenly distributed clusters of argentaffin and chromaffin cells were demonstrated in all the thyroid tumours as well as in a lymph node metastasis. Iodate positive cells were found in the thyroid tumours of two patients. Positive cells showed a remarkable, spider-like shape with long cytoplasmic processes "embracing" non-reacting, more rounded tumour cells with clear cytoplasm, and were often located close to blood vessels. Since they differed from the non-reacting cells it seemed possible that the thyroid tumours were composed of two different cell populations derived from two functionally different types of nonfollicular epithelial cells of the thyroid gland, viz., one possibly concerned with polypeptide (calcitonin) synthesis, the other having monoaminergic functions. It was emphasized that the presence of cells containing monoamine in the medullary thyroid tumours studied suggested at least a partial neuroepithelial origin of these tumours, and pointed to a close relationship to the phaeochromocytoma and other neural crest tumours, that are frequently encountered in the familial "medullary thyroid carcinoma-phaeochromocytoma"-syndrome.

Medullary thyroid carcinoma is a tumour with several curious and interesting features, distinguishing it from other types of thyroid neoplasia. One of its characteristics is the occurrence of amyloid substances in the stroma and possibly also in certain cells of the tumour and its metastases (Hazard *et al.*, 1959; Albores-Saavedra *et al.*, 1964; Ibanez *et al.*, 1966); another is the common familial occurrence, and then often associated with phaeochromocytoma (Cushman, 1962; Finegold *et al.*, 1962; Grundstein *et al.*, 1962; Manning *et al.*, 1963; Nourok, 1964; Schimke *et al.*, 1965; Williams, 1965; Block *et al.*, 1967; Ljungberg *et al.*, 1967; Huang *et al.*, 1968; Sarosi *et al.*, 1968; Steiner *et al.*, 1968) and sometimes also with other neural tumours such as neuroma and ganglioneuroma (Mielke *et al.*, 1965; Ljungberg, 1966; Williams *et al.*, 1966; Gorlin *et al.*, 1968; Schimke *et al.*, 1968).

The problems bearing on the histogenesis of the tumour have received extensive attention in recent years. Its derivation from follicular thyroid epithelial cells seems improbable, since it has not a papillary or follicular growth pattern; nor has it been possible to induce the tumour in animals by long term thyrotropin stimulation.

It has recently been propounded that the tumour is a neuroepithelial tumour, or at least contains neuroepithelial cell elements, originating from cells other than follicular eptihelial cells (Ljungberg *et al.*, 1967; Schimke *et al.*, 1968). This assumption is based on the multifocal nature of the tumour (multiple growths in one and the same thyroid gland) and its tendency to occur in association with phaeochromocytoma (found to be bilateral in more than two thirds of the cases on record, and then sometimes also as multiple intra- and extra-adrenal phaeochromocytoma) (Ljungberg, 1966; Huang *et al.*, 1968) and other neural tumours, often with a familial occurrence as well as its morphologic similarity to phaeochromocytoma and carotic body tumour.

Whether the thyroid tumour contains monoamines of the same types as those in the phaeochromocytoma and other closely related tumours is an important question from a histogenetic point of view. The existence of biochemical functions similar to those of phaeochromocytoma tumour cells and their normal analogues may lend support to — though not prove — the view that the medullary thyroid tumour and the phaeochromcytoma of this syndrome are derived from a common cell system. A histochemical study of familial medullary thyroid carcinoma for the demonstration of monoamines, is described below.

Material and Methods

The material consisted of four medullary thyroid carcinomas and one lymph node metastasis. The patients belong to a family, which has been preliminary reported previously (Ljungberg *et al.*, 1967) and in which medullary thyroid cancer and/or phaeochromocytoma have been found to be common. Data about the patients are given in Table 1. The patient in case 1 is a cousin of those in cases 2, 3 and 4, who are siblings. Specimens of the thyroid tumours and, in case 1, also of a supraclavicular lymph node metastasis, were taken immediately after the operation and placed in the following solutions: in 10 per cent buffered formalin (pH 7.5—8.0), in 1 per cent potassium dichromate with 2 per cent formalin (pH 5.0) and in 10 per cent potassium iodate in aqueous solution, respectively. After 48 hours' fixation, the formalin treated specimens were embedded in paraffin, sectioned and treated according to Masson-Hamperl (Romeis, 1848a) for demonstrating argentaffin sub-

stances. After 24 hours' treatment the dichromate-formalin fixed specimens were transferred to 5 per cent potassium dichromate for another 24 hours, rinsed in running water for 24 hours, embedded in paraffin and sectioned. The sections were stained with the Giemsa method, as modified by Sevki (Pearse, 1960a). After the Giemsa stained sections had been examined, some were retreated according to the Masson-Hamperl method to find out whether chromaffin material, if any in the sections, also showed argentaffinity. The iodate reaction was carried out according to Hillarp-Hökfelt (Pearse, 1960a). The sections were stained with a weak solution of hematoxylin. Sections of formalin fixed tissue were also treated according to the diazo-coupling (Pearse, 1960b) and ninhydrin methods (Barka *et al.*, 1963) for 5-hydroxytryptamine. Specimens of all four thyroid tumours as well as of the lymph node metastasis from the patient in case 1 were examined with the methods for demonstrating argentaffin and chromaffin substances and with the diazo-coupling and ninhydrin methods.

The iodate method was used in cases 1 and 2 only. Furthermore, sections from formalin and dichromate-formalin fixed material from all patients were stained with hematoxylin-erythrosin and control-stained with Prussian Blue and Turnbull Blue (for iron pigments) (Romeis, 1948b) and according to the long Ziehl-Neelsen method (for acid fast lipo pigments) (Pearse, 1960c).

Table 1. *Four familial cases of medullary thyroid carcinoma, three of which associated with phaeochromocytoma*

Case No.	Sex	Age at thyroid-ectomy (Years)	Associated phaeochromocytoma
1	♀	44	Left adrenal (operation 7 months after thyroidectomy)
2	♀	52	Right adrenal (operation 8 months after thyroidectomy)
3	♀	53[a]	Multiple microscopic tumours of the right adrenal medulla (autopsy)
4	♂	49	Not found (no symptoms, normal urinary catecholamines and 3-methoxy-4-hydroxymandelic acid, normal angiography)

[a] (Died 3 years later from metastases of thyroid cancer).

Table 2. *Reaction pattern with three known monoamines, as found under conditions used in the present study (see text)*

Reaction	Argentaffin	Chromaffin	Iodate	Diazo	Ninhydrin
Noradrenaline	+/−	+	+	−	−
Adrenaline	−	+/−	−	−	−
5-Hydroxytryptamine	+	(+)	−	+	+

The histochemical methods used and the various monoamines, which they demonstrate are summarized in Table 2. The alkaline silver (argentaffin reaction), dichromate (chromaffin reaction) and iodate methods are based on oxidation reactions, where silver ions, dichromate and iodate salts act as oxidants.

The argentaffin reaction indicates reducing substances of various kinds; not only monoamines but also certain iron and lipo pigments, for instance, give a positive argentaffin reaction. But some of these false positive reactions can

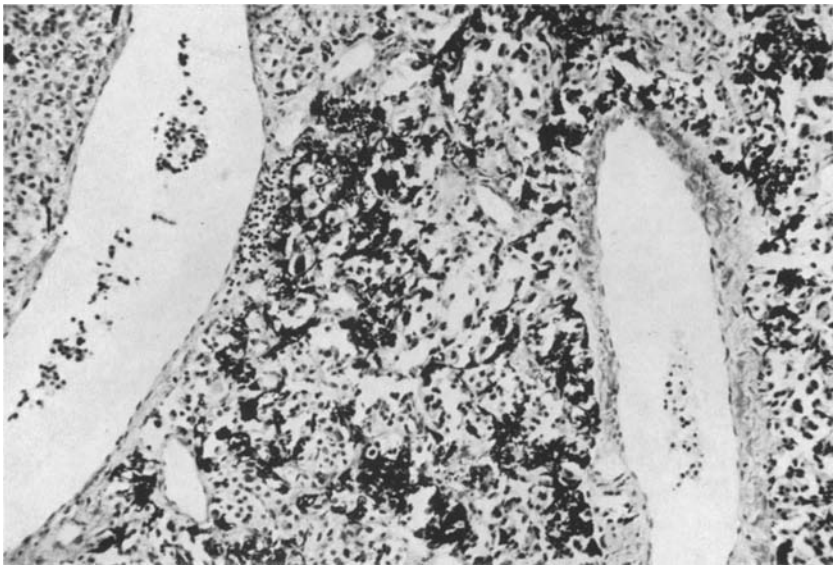
be ruled out by other methods, more specific for these substances. When used for demonstrating monoamines, the argentaffin reaction requires previous fixation in formaldehyde in weakly alkaline solution (pH 7.6), during which the water soluble monoamines react with formaldehyde to form condensation products, some of which are bound in the tissue; the latter preserve their reducing capacity and can, therefore, be demonstrated by black silver precipitates (Ratzenhofer *et al.*, 1959). According to *in vitro* model experiments, the rate of the reaction between formaldehyde and monoamine in alkaline milieu, is highest for 5-hydroxytryptamine, somewhat lower for noradrenaline and lowest for adrenaline. Accordingly, under the conditions used for the fixation, embedding and staining procedures, the method can only be expected to demonstrate 5-hydroxytryptamine and, to some extent noradrenaline. Adrenaline will usually escape detection, because it will diffuse into the fixing solution before the condensation reaction has taken place (Ratzenhofer *et al.*, 1959).

The indicators formed in the dichromate and iodate reactions are yellow-brown respectively red-brown monoamine oxidation products, which precipitate in or near the *in situ* location of the monoamines. Noradrenaline is usually demonstrable by both methods, because it is oxidized fast enough to be bound in the tissues. Adrenaline, on the other hand, does not react quickly enough and diffuses into the aqueous solvents and probably escapes demonstration (Ratzenhofer *et al.*, 1959). 5-hydroxytryptamine can give a chromaffin reaction, provided the tissue is pretreated in weakly alkaline formalin, during which it is bound in the form of condensation products, which can still be oxidized by the subsequent dichromate treatment with the formation of coloured, insoluble products (Ratzenhofer *et al.*, 1959).

Results

Argentaffin Reaction

Clusters of argentaffin cells were observed in all thyroid tumours studied. They were unevenly distributed and most abundant in those parts of the tumour exhibiting a carcinoid-like and spindle-cell picture with scanty or no amyloid deposits (Fig. 1 a). Positive cells contained densely packed, fairly coarse, black, intracytoplasmic granules, while no signs of any reaction were seen in their nuclei. A large number of these cells had a remarkable spider-like shape with often very long, slender, cytoplasmic processes, sometimes "embracing" adjacent, more rounded tumour cells with light, non-argentaffin cytoplasm (Fig. 1 b). They were situated centrally as well as peripherally in the tumour alveoli and seemed to be most abundant near vascular spaces, from which they were sometimes separated only by the endothelial lining cells (Fig. 1 c). The lymph node metastasis from case 1, which showed a trabecular growth pattern with fairly large amyloid deposits, contained also a few argentaffin cells of the type described above. In hematoxylin-erythrosin stained sections a few clusters of tumour cells with rather coarse, red-brown intracytoplasmic granules were seen, but it could not be determined with certainty whether these corresponded to the argentaffin cells. Control staining for iron and lipo pigments did not reveal such pigments within the argentaffin cells.



Figs. 1a—c. Medullary thyroid carcinoma. Case 2. Treatment according to Masson-Hamperl. a Clusters of argentaffin cells in tumour area devoid of amyloid deposits. × 152

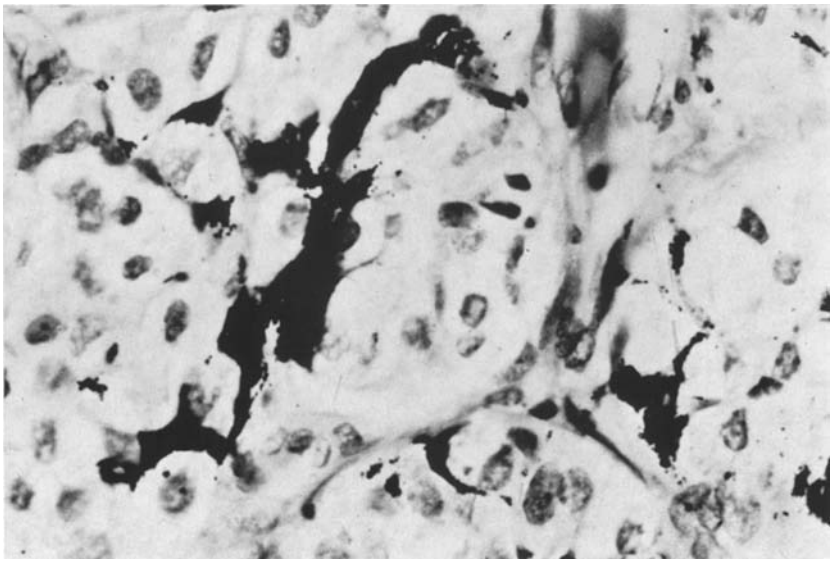


Fig. 1b. Spider-like, argentaffin cells with long cytoplasmic processes, often seen "embracing" adjacent, more rounded tumour cells with clear cytoplasm. × 680

Chromaffin Reaction

Dichromate treated sections from all thyroid tumours as well as from the lymph node metastasis revealed cells with a greenish to yellow-greenish granular, intracytoplasmic material (Fig. 2a). Distribution and number of positive cells

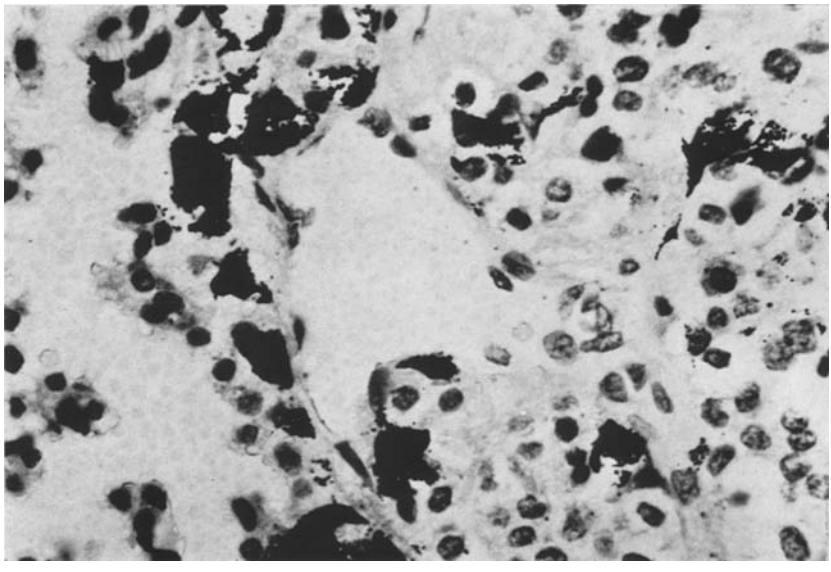


Fig. 1 c. Argentaffin cells in close relationship to blood vessels. × 520

seemed to be similar to that of the argentaffin cells. Many of them were rather polyhedric, sometimes with distinct cytoplasmic processes of the type seen in the argentaffin cells. On subsequent treatment with silver salt of one and the same section, chromaffin intracytoplasmic, granular material was found to give an argentaffin reaction as well, though not so strong as in the formalin fixed sections (Fig. 2 b).

Iodate Reaction

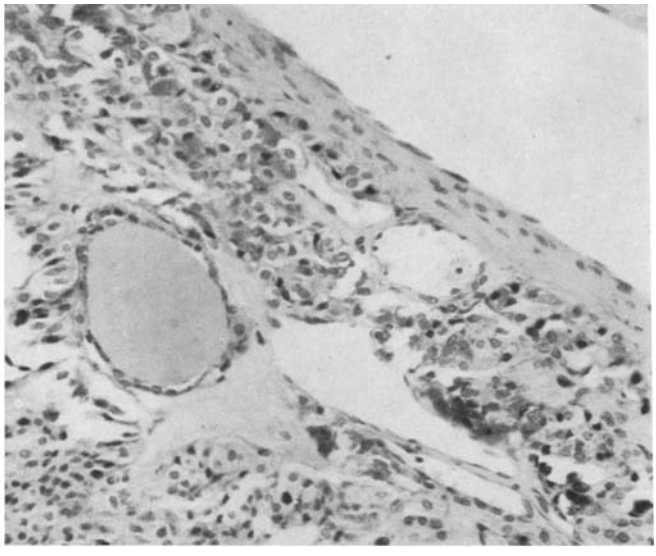
The thyroid tumours in cases 1 and 2, contained unevenly distributed clusters of cells, whose cytoplasm was red-brown and diffuse or faintly granular. The distribution of such cells within the tumour tissue seemed to be similar to that of the argentaffin and chromaffin cells, in case 1, however they were very sparse. No distinct cytoplasmic processes could be demonstrated, possibly because of derangement of cell boundaries during freezing and/or treatment with iodate salt.

Diazo-Coupling and Ninhydrin Reactions

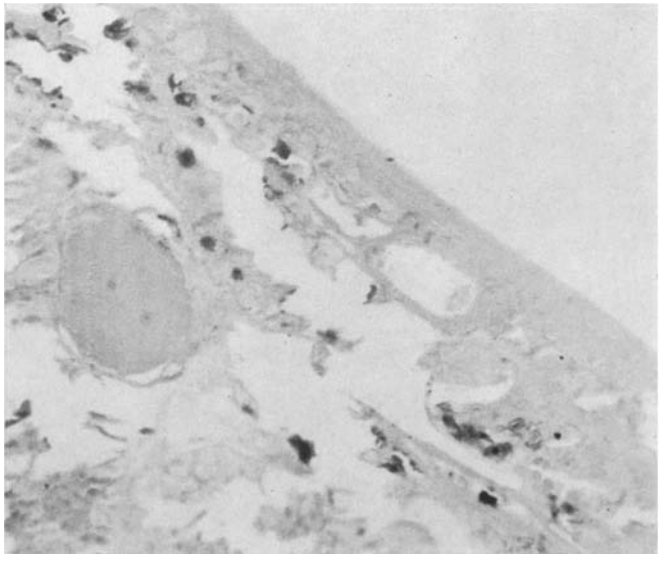
No 5-hydroxytryptamine was demonstrated by the methods used here.

Discussion

All the four familial medullary thyroid tumours as well as a lymph node metastasis contained unevenly distributed argentaffin and chromaffin cells. Iodate positive cells were also demonstrated in two medullary thyroid tumours analyzed with this method. Furthermore, on subsequent silver salt treatment of one and the same section, the chromaffin cells proved to be also argentaffin. These findings argue strongly for the presence of monoamines in certain cells in the



a



b

Figs. 2 a and b. Medullary thyroid carcinoma. Case 2. a Chromaffin cells (with dark cytoplasm). Dichromate-formalin. Modified Giemsa stain. × 240. b Same section as in Fig. a, subsequently treated according to Masson-Hamperl. The chromaffin cells in Fig. a are seen to be argentaffin as well. × 240

medullary thyroid tumours and in the lymph node metastasis investigated here. Noradrenaline is the only one of the above mentioned amines that gives positive reactions in all three methods, although substances closely related to noradrenaline, such as precursors of that amine (DOPA, dopamine) may have a similar reaction pattern.

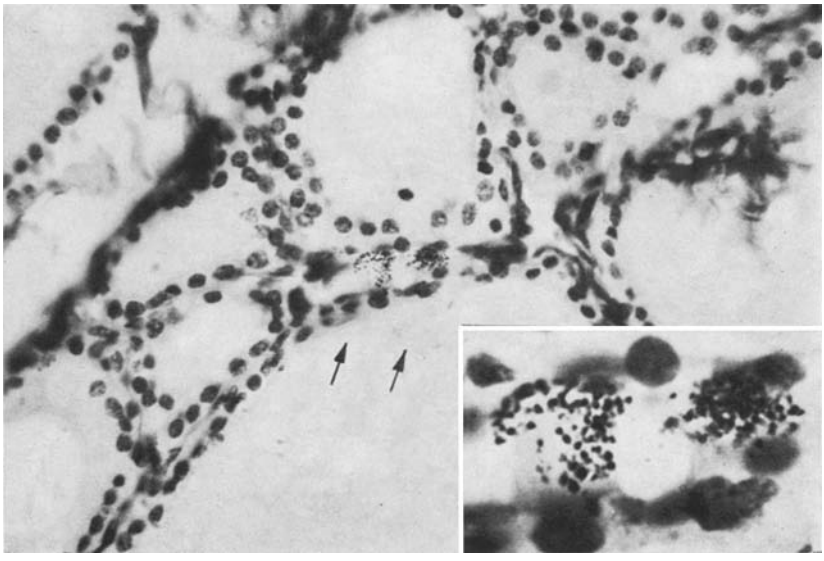


Fig. 3. Normal human thyroid gland (boy, $2^1/_2$ year old, autopsy specimen). Arrows indicate argentaffin cells, apparently parafollicular in position, with closely packed, argentaffin granules. Masson-Hamperl. × 460. The inset shows the same cells at higher magnification (× 1,500)

The findings reported here are in accord with those obtained by the fluorescence method of Falck and Hillarp (also applied to thyroid tumour specimens of cases 1, 2 and 3) and published elsewhere (Falck *et al.*, 1968). Examination of the tumour tissue with this technique revealed a cell system with a green intracytoplasmic, granular fluorescence characteristic of primary catecholamines, such as noradrenaline or closely related substances.

Whether the unevenly distributed monoamine containing cells found in the thyroid tumours studied were neoplastic cells or merely residual elements of a monoamine containing cell system of the normal thyroid tissue is not clear. The demonstration of argentaffin as well as of chromaffin cells in a lymph node metastasis from the thyroid tumour in case 1 however, suggests that they constitute an integral part of the tumour tissue.

It should be pointed out that although monoamines can be demonstrated in certain cells in the thyroid tumours, it is not certain whether these amines are synthesized in these cells. The presence of such amines might be the result of an uptake of monoamines or of monoamine precursors that have been synthesized in, and secreted into the blood by, a co-existing phaeochromocytoma, removed some time after the operations on the thyroid. The so-called parafollicular cells (C-cells) of certain mammals have been shown to be able to take up and decarboxylate certain monoamine precursors, such as DOPA and 5-hydroxytryptophan (Falck *et al.*, 1964; Ritzén *et al.*, 1965; Larson *et al.*, 1966; Pearse, 1966). But no phaeochromocytoma was found in case 4. Yet monoamine containing cells were demonstrated in the thyroid tumour from this patient. Provided that monoamine synthesizing tissues normally occurring in the body

do not secrete amounts of monoamines large enough to result in such a storage, this finding suggests that — besides an uptake and storing mechanism — a capacity for synthesizing monoamines may really, also exist in certain medullary thyroid tumour cells.

Two recent findings, viz. the occurrence of the polypeptide hormone calcitonin in para- and epifollicular cell populations in the thyroid gland of the pig and the dog (Bussolati *et al.*, 1967) and of calcitonin-like activity in several human medullary thyroid tumours (Cunliffe *et al.*, 1968; Tubiana *et al.*, 1968) argue strongly for the assumption that human medullary cancer originates from nonfollicular thyroid epithelial cells.

It nevertheless remains to be established whether the so-called "parafollicular cell system" of the thyroid gland consists of a single cell system, in which one and the same cell possesses both characteristics *i.e.* synthesis of polypeptide hormone (calcitonin) as well as monoaminergic functions or of *two* "parafollicular cell systems", viz. one concerned with the synthesis of calcitonin; the other (topographically closely related to the former) having monoaminergic functions and possibly concerned with a neuro-humoral regulation of synthesis and/or release of the polypeptide hormone in calcitonin containing cells. The present findings in medullary thyroid carcinoma speak for the latter possibility. The unevenly and sparsely distributed monoamine containing cells with their characteristic shape and proximity to blood vessels may thus constitute the neuroepithelial part of the tumour, the predominating non-argentaffin, non-chromaffin and iodate-negative, more rounded cells with clear cytoplasm constituting the part synthesizing the polypeptide hormone. It is noteworthy that polyhedric, argentaffin and apparently parafollicular cells are occasionally found in the normal human thyroid gland of young individuals (Fig. 3). But the nature of these cells must abide further research.

It is possible that the ratio between the two types of cells of medullary thyroid carcinoma vary from one tumour to another and that the monoamine containing cells occasionally constitute the major part of the tumour, producing sufficient amounts of monoamines to affect the cardiovascular system and metabolic processes and cause symptoms mimicking phaeochromocytoma.

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