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Calcitonin- and ACTH-Producing Cells in a Case of Medullary Carcinoma of the Thyroid

Immunofluorescence Investigations

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Summary. The presence and distribution of calcitonin- and ACTH-producing cells have been investigated, by means of specific immunofluorescence in a case of medullary-type thyroid carcinoma associated with hyper-calcitoninaemia and Cushing's syndrome. An ultrastructural study of the same tumour already reported (Bordi, Anversa and Vitali-Mazza, 1972) revealed the presence of two different types of endocrine cell. Two main endocrine cell populations have been demonstrated by immunofluorescence: one, arranged in clusters, producing calcitonin, the other, less numerous and mostly arranged in duct-like structures, containing ACTH.

The implications of these findings for the theory of the neural crest origin of cells of the APUD series are also discussed.

An electron microscopical investigation has recently been reported, on a medullary-type thyroid carcinoma associated with hyper-calcitoninaemia and Cushing's syndrome (Bordi, Anversa and Vitali-Mazza, 1972). The latter is considered to be due to ectopic ACTH production, since Crooke cells were found in the pituitary gland (Bordi, unpublished observation). With the electron microscope two distinctly different types of endocrine cell were observed: one, more numerous, was characterized by cytoplasmic granules of variable electron density, closely resembling those of the calcitonin-producing thyroid C cells; the other, more scantily represented, contained large granules resembling those of the pituitary ACTH cells. Immunofluorescence investigations have now been undertaken on the same tumour tissue, and these have confirmed the presence in it of two different endocrine cell populations.

Material and Methods

Lymph node and hepatic metastases of the tumour were examined: the former were collected during the surgical operation, the latter at the autopsy (which was performed several months after the operation, and a short time after death). Two types of processing were carried out.

a) The tissues were fixed in Bouin's fluid or in buffered formalin and embedded in paraffin. The sections were used for immunofluorescence tests or stained with the Grimelius (1968) and the lead haematoxylin (Solcia *et al.*, 1969) methods.

b) Tissue was frozen in liquid nitrogen and subsequently freeze-dried at —45° C overnight with a thermo-electric tissue dryer. After fixation in formaldehyde vapour at 80° C for 1 h the tissue was embedded in paraffin under vacuum. Sections were examined by ultra violet microscopy for the detection of 5-hydroxytryptamine by formaldehyde-induced fluorescence.

Immunofluorescence. The rabbit antiserum to synthetic human calcitonin (calcitonin M) was supplied by Dr. Mary Clark, Royal Postgraduate Medical School. The rabbit antiserum to ACTH was supplied by Dr. B. A. L. Hurn, The Wellcome Research Laboratories. Both antisera were used in an indirect immunofluorescence test, employing rhodamine- or fluorescein-conjugated antibodies (goat anti-rabbit gamma globulin, Hyland) as the second step. Control staining was done on normal human pituitary and rat thyroid, and by using the fluorescent serum alone or the anti-hormone antibodies absorbed with excess hormone.

A double staining procedure was carried out in order to show the presence of ACTH- and calcitonin-producing cells in the same tumour section. The procedure is outlined below:

Step 1. Rabbit anti-calcitonin or anti-ACTH.
Step 2. Fluorescein-labelled goat anti-rabbit serum.
Step 3. Rabbit anti-ACTH or anti-calcitonin.
Step 4. Rhodamine-labelled goat anti-rabbit serum.

Results

The general morphology of the tumour has already been reported (Bordi *et al.*, 1972) and will be described only briefly here. The tumour cells were usually arranged in clusters, but small duct-like or tubular structures were also observed. Most of the cells were argyrophilic with the Grimelius method, while with the lead haematoxylin method only a few cells appeared intensely reactive.

In freeze-dried, formaldehyde vapour-fixed tissue no 5-hydroxytryptamine-containing cells were detected. By immunofluorescence, numerous roundish or elongated calcitonin-containing cells were observed, both in the lymph node and in the hepatic metastatic tumoral tissue. The cells were mainly arranged in clusters or, sometimes, in small groups.

ACTH-containing cells were detected only in the liver metastasis, where they were rather scarce but distributed all over the sections. These cells were roundish or square, and mainly grouped in small cords or duct-like structures.

In double-stained immunofluorescence preparations, two different cell populations could easily be distinguished, displaying respectively a green and a red fluorescence. Some degree of overlapping of the rhodamine-labelled antibodies (employed as step No. 4) with the fluorescein-stained areas was always observed: this was ascribed to the linkage of rabbit globulins (step No. 3) to free antibody sites of the fluorescein-conjugated antibodies (step No. 2). However when such preparations were examined with a Ploem system, it appeared that the fluorescein fluorescence was restricted to one type of hormone-containing cell.

Discussion

Production of ACTH, or ACTH-like substances, by tumours of other than pituitary or adrenal origin, has been reported by many authors. These substances have been shown to be similar to pituitary ACTH in chemical (Liddle *et al.*, 1963), immunological (Jarett *et al.*, 1964) and biological (Meador *et al.*, 1962; Jarett *et al.*, 1964) properties. Ectopic secretion of ACTH is often associated with production of other endocrine substances by the same tumour (O'Neal *et al.*, 1968). The medullary (C cell) carcinoma is the only thyroid tumour which is known to be

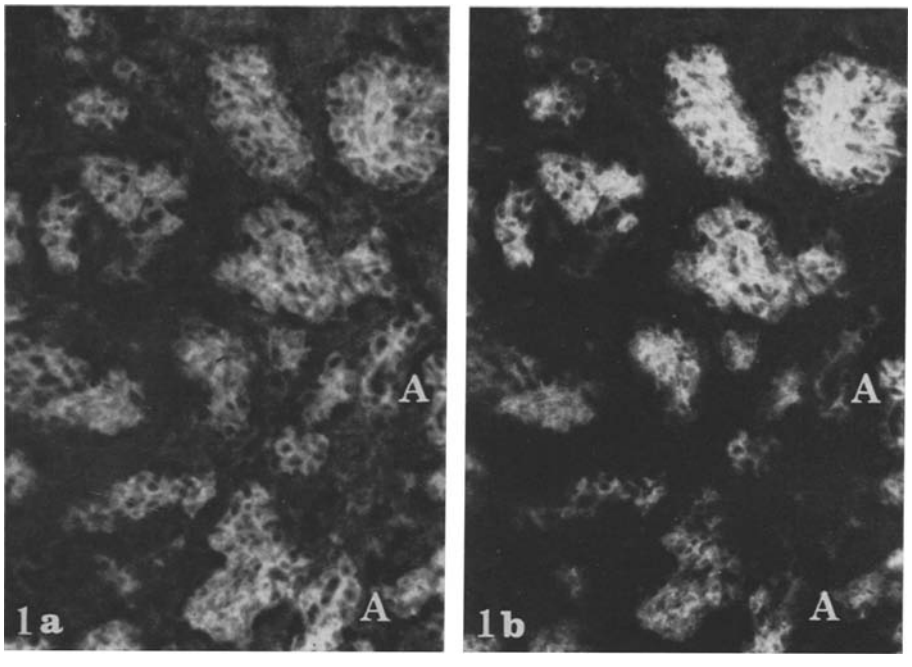

Fig. 1a and b (250 ×). a Double-staining preparation. Both calcitonin-(FITC, green fluorescence) and ACTH-(RITC, red fluorescence, *A*) containing cells are shown. b When the preparation is examined with a Ploem system (position 3, exc. filter KP 490, barrier filter S525) only the clusters of calcitonin-containing cells give specific FITC fluorescence

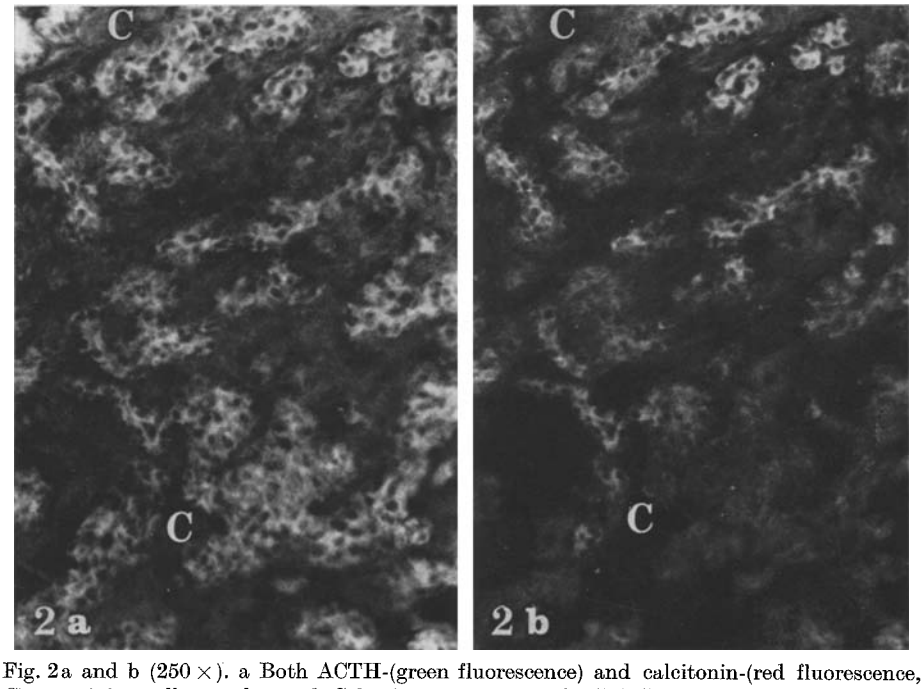

Fig. 2a and b (250 ×). a Both ACTH-(green fluorescence) and calcitonin-(red fluorescence, *C*) containing cells are shown. b Selective examination for FITC fluorescence with a Ploem system. The ACTH-containing cells are arranged in slender duct-like structures

able to produce ACTH (Donahower *et al.*, 1968; Williams *et al.*, 1968), and the simultaneous production of calcitonin and ACTH by the same tumour has repeatedly been demonstrated by means of radio-immunoassay (Melvin *et al.*, 1970; Vague *et al.*, 1971; Croughs *et al.*, 1972).

The present report provides morphological evidence of the cellular distribution of both hormones, for the first time to the best of our knowledge. Our results demonstrate the presence in the same tumoral tissue of two functionally distinct cell populations: one producing calcitonin and the other ACTH. The present immuno-histochemical findings are in agreement with the previously reported ultrastructural data; we are however unable, for technical reasons, to correlate the immunofluorescence and the electron microscopical studies on the same tissue and section. ACTH-producing cells have been detected only in the hepatic, and not in the lymph node metastatic tissue; the latter however was collected many months before death, when symptoms of Cushing's syndrome were not evident. We cannot exclude the possibility that the same cell could produce the two hormones, but our data seem at least to support the assumption that they are not synthesized simultaneously. The suggestion that two (genetically) distinct cell populations exist in this tumour is also supported by the different histological arrangement of the calcitonin- and the ACTH-producing cells. These findings could perhaps be explained by, and fit with, the theory of Lipsett *et al.* (1964) who attribute ectopic hormone production by tumoral tissue to DNA de-repression.

Both calcitonin and ACTH cells belong to the APUD cell series (Pearse, 1968, 1969), sharing common histochemical properties and, possibly a common embryological origin. It has recently been demonstrated that the calcitonin cells originate from the neural crest (Le Douarin and Le Lièvre, 1970; Pearse and Polak, 1971), and the origin of pituitary ACTH cells from the same source was suggested by Pearse (1966). In the tumour described here, cells able to produce calcitonin might possess the ability to switch to ACTH production. On the other hand, as suggested above, the two cells might well be phenotypically distinct.

Our findings nevertheless support the potential ability of cells of neural crest origin to differentiate into cells producing either calcitonin or ACTH.

Note Added in Proof. The metastatic liver tissue from this patient has now been examined by Dr. Lesley H. Rees, from the Department of Chemical Pathology of St. Bartholomews Hospital of London, for the presence of immuno-reactive ACTH.

The tumour ACTH levels (0.440 μg/g N-terminally immunoreactive ACTH; 0.120 μg/g C-terminally immunoreactive ACTH) are well in the range normally found in ectopic ACTH-secreting tumours.

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