

Formaldehyde-Ozone-Induced Fluorescence and ACTH Immunoreactivity in Basophil Tumours of the Pituitary

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Summary. The ACTH cells of human foetal pituitary displayed intense formaldehyde-ozone-induced fluorescence characteristic of peptides or proteins with NH_2 -terminal tryptophan. Fourteen pituitary tumours were examined histochemically. Of these, two displayed formaldehyde-ozone-induced fluorescence. These two tumours were the only ones to exhibit ACTH immunoreactivity.

Introduction

The intermediate lobe and a cell population in the anterior lobe of the mammalian pituitary display intense fluorescence upon formaldehyde-ozone treatment (cf. Håkanson, Sundler, Nobin, Sjöberg, Edvinsson, and Larsson, 1974). Chemical and histochemical analysis indicates that this fluorescence reflects the presence of peptides or proteins having NH_2 -terminal tryptophan (Håkanson, Larsson, Nobin, and Sundler, 1972; Håkanson *et al.*, 1974). By immunohistochemistry, the fluorescent cells of the anterior lobe of hamster, cat and pig pituitaries have been shown to be the ACTH cells (Håkanson, Sundler, Larsson, Ekman and Sjöberg, 1975). From studies on pig pituitary the postulated tryptophyl-peptide seems to occur in the cytoplasmic granules of the ACTH and the MSH cells (Håkanson *et al.*, 1975).

This paper is concerned with the fluorescence histochemical features of human pituitary tumours.

Materials and Methods

Pituitary specimens were collected from one human foetus (induced abortion; gestational age 20 weeks). The specimens were frozen to the temperature of liquid nitrogen in a propane-propylene mixture, freeze-dried and embedded in paraffin. Pituitary tumours were obtained at surgery or at autopsy. The tumour specimens were fixed in 10% neutral formalin and embedded in paraffin. Sections were cut at $5\ \mu$, deparaffinized, mounted in xylene and examined by fluorescence microscopy. The sections were then treated in one of the following ways: a) They were stained according to Adams and Swettenham (1958). b) They were exposed to formaldehyde vapour in the presence of ozone (*formaldehyde-ozone-treatment*; for details see Håkanson and Sundler, 1971a and b). As a specificity control, sections thus treated were reacted with a fresh 0.1% solution of sodium borohydride in 90% isopropanol for 1–5 min (Corrodi, Hillarp and Jonsson, 1964). They were then reexamined in the fluorescence microscope. c) They were carried down to water and subjected to an indirect immunohistochemical method for the demonstration of *ACTH immunoreactivity* as described in detail elsewhere (Håkanson *et al.*, 1975). The ACTH antiserum was generously supplied by Dr. U. Lundkvist,

Pharmacia, Sweden. Controls used were those recommended by Goldman (1968) and included the application of ACTH-inactivated antiserum (ACTH antiserum containing 100 μ g ACTH per ml undiluted serum).

The sections were examined in a Leitz Orthoplan fluorescence microscope equipped with an epi-illumination system (standard filter setting No. 1 for formaldehyde-ozone-induced fluorescence; peak excitation at 365 nm, and No. 3 for immunofluorescence; peak excitation at 490 nm). An HBO 200 mercury lamp served as light source.

The spectral properties of the formaldehyde-ozone-induced fluorescence were analysed in a Leitz microspectrograph as described in detail elsewhere (Björklund, Falck and Owman, 1972). For recording of excitation spectra, the sections were placed on quartz slides and the optical system consisted of quartz components. The background fluorescence of the sections was used to obtain blank spectra. The spectra were corrected for blanks and instrumental errors. The values for excitation and emission maxima are calculated from at least 4 separate recordings.

Case Histories

For reasons that will be obvious from the results, case histories are given of two patients only:

Case No. 1. An 88 year old woman was admitted to hospital because of sudden precordial pain and died of cardiac insufficiency due to left coronary thrombosis and stenosis of the mitral valves. At necropsy a basophil adenoma of the pituitary was found incidentally. The left adrenal gland showed nodular cortical hyperplasia whereas the right gland was grossly and histologically normal.

Case No. 2. This 75 year old woman was the only patient in the series that displayed the clinical features of Cushing's syndrome. In addition, she suffered from diabetes mellitus (treated by diet alone) and had a three year history of thyrotoxicosis. Two years prior to death, sellar enlargement was diagnosed and the pituitary tumour was unsuccessfully treated by irradiation. The cause of death was bronchopneumonia and cardiac failure. At necropsy a basophil tumour was found to invade the surrounding bone.

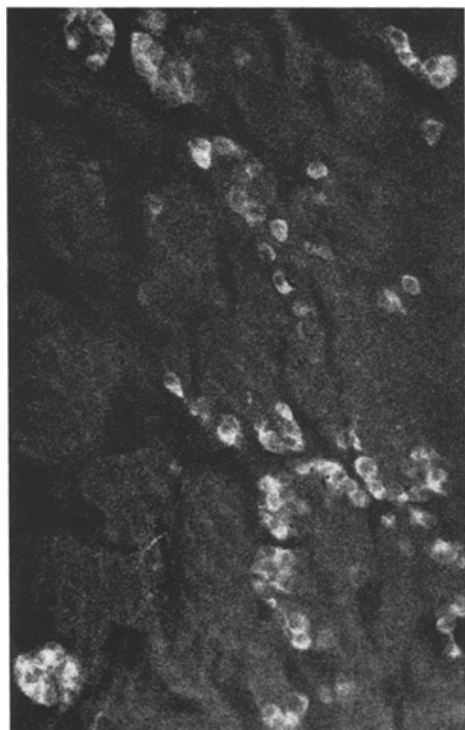
Results

Numerous cells in the anterior lobe of the human foetal pituitary displayed formaldehyde-ozone-induced fluorescence (375/500 nm) characteristic of peptides with NH_2 -terminal tryptophan. By immunohistochemistry, these cells could be shown to be identical with the ACTH cells (Fig. 1).

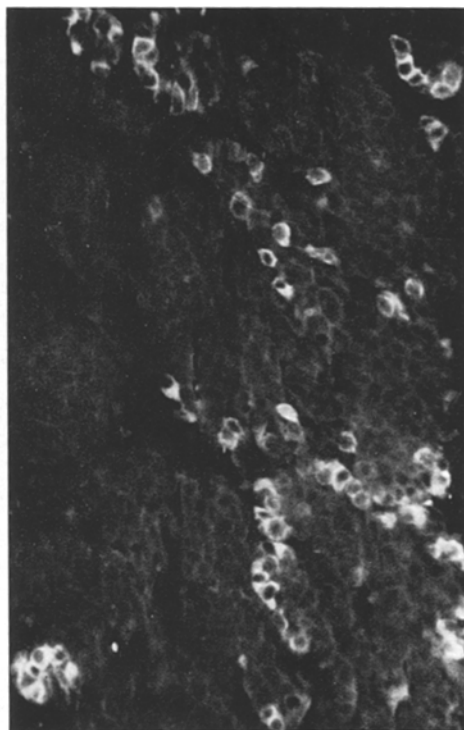
Of fourteen pituitary tumours examined, eight were chromophobe, two acidophil and four basophil as defined by staining according to Adams and Swettenham (1958). Two of the basophil tumours (cases No. 1 and 2) displayed formaldehyde-ozone-induced fluorescence (380/500 nm), the spectral properties of which were similar to those of the ACTH cells of the human foetus. The fluorescence disappeared following treatment with the reducing agent sodium

Fig. 1a and b. Human foetal pituitary (gestational age 20 weeks) (a) Numerous cells display formaldehyde-ozone-induced fluorescence. (b) Same section after staining with ACTH antiserum. The cells showing fluorescence following formaldehyde-ozone treatment are ACTH cells. Note that the formaldehyde-ozone-induced fluorescence is quenched by the processing in aqueous media necessary for immunofluorescent staining (cf. Håkanson *et al.*, 1975). $\times 175$

Fig. 2a and b. Pituitary tumour, case No. 1. Formaldehyde-ozone-induced fluorescence (a) and ACTH immunofluorescence (b). $\times 300$

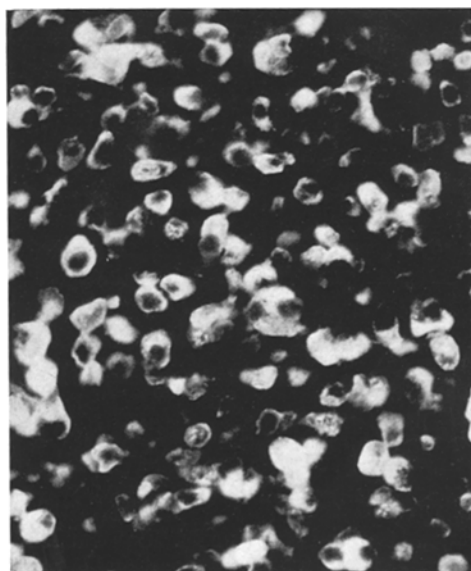


a

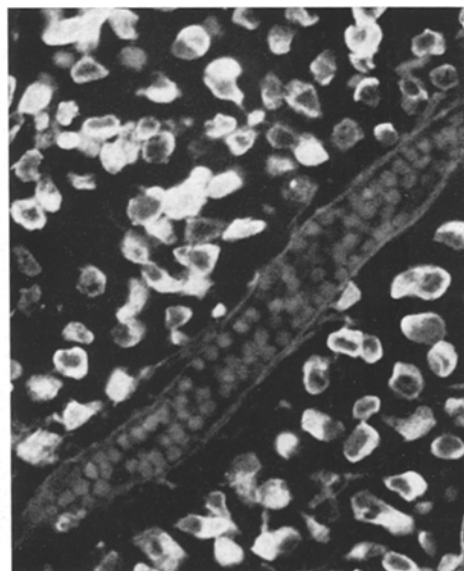


b

Fig. 1.



a



b

Fig. 2.

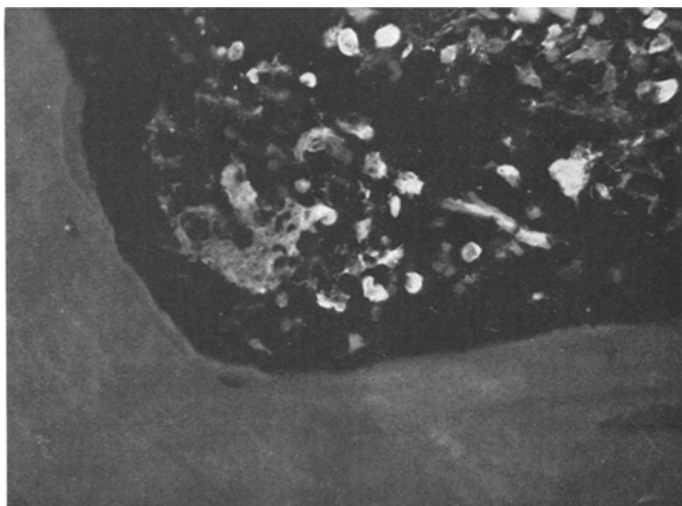


Fig. 3. Pituitary tumour, case No. 2. Formaldehyde-ozone-induced fluorescence is displayed by tumour cells in the immediate vicinity of bone structures (below). $\times 200$

borohydride but reappeared after renewed formaldehyde-ozone-treatment. These two tumours were the only ones that showed ACTH immunoreactivity. The cells displaying formaldehyde-ozone-induced fluorescence had the same distribution pattern as the ACTH cells, as was evident from examination of adjacent sections (Fig. 2). In the tumour of case No. 1 about half of the cell population displayed formaldehyde-ozone-induced fluorescence and ACTH immunofluorescence; the remainder being non-fluorescent with both techniques. In the tumour of case No. 2 (Fig. 3), all cells exhibited intense formaldehyde-ozone-induced fluorescence as well as ACTH immunoreactivity.

Outside the tumours there was quite often a small rim of compressed, seemingly normal, pituitary tissue. Here, cells showing formaldehyde-ozone-induced fluorescence and ACTH immunoreactivity were regularly seen.

Discussion

Our material consisted of 14 pituitary tumours of which four were basophil. Two of these showed formaldehyde-ozone-induced fluorescence and were the only ones that displayed ACTH immunoreactivity¹. Thus, neoplastic ACTH cells seem to exhibit the formaldehyde-ozone-induced fluorescence characteristic of normal ACTH cells. We have previously described similar fluorescence in the gastrin cells of man and some other species (Larsson, Sundler, Håkanson, Grmelius, Rehfeld and Stadil, 1974; Larsson, Håkanson, Sjöberg and Sundler, 1975) and in gastrinomas (Zollinger-Ellison tumours) of the pancreas and duo-

¹ We have frequently observed basophil pituitary adenomas in adult cats. The adenoma cells invariably displayed intense formaldehyde-ozone-induced fluorescence and ACTH immunofluorescence.

denum (Larsson, Sundler, Grimelius, Håkanson, Buffa and Solcia, 1975). On the basis of histochemical and chemical evidence we have suggested that in all these locations the fluorescence reflects the presence of peptides or proteins having NH_2 -terminal tryptophan, the functions of which are as yet unknown. From a practical point of view, it should be noted that formaldehyde-ozone-treatment is a simple procedure that has been shown to work satisfactorily on material fixed in aqueous formalin (Larsson *et al.*, 1975a and b). This technique may therefore find use in tumour diagnosis.

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