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Esthesioneuroblastoma*

Histological, Histochemical and Electron Microscopic Studies of a Case

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Summary. A case of esthesioneuroblastoma was examined by histological, histochemical and electron microscopic techniques. The tumour cells showed an argyrophil reaction with the Grimelius technique and contained cytoplasmic secretory granules, but in contrast to previous reports were devoid of histochemically demonstrable biogenic amines. For routine diagnosis the argyrophil technique may be useful in differentiating this type of tumour from epidermoid carcinoma.

Key words: Esthesioneuroblastoma – Light microscopy – Electron microscopy – Argyrophil reaction – Formaldehyde-induced fluorescence.

Introduction

The olfactory esthesioneuroblastoma is an infrequent tumour, which was first described by Berger et al. in 1924. Up to 1966, 97 cases of the tumour had been reported in the literature (Skolnik et al., 1966), the classification being mainly based on its histological appearance and location. Recently this type of tumour has been examined by histochemical (Micheau et al., 1975; Judge et al., 1976) as well as ultrastructural methods (McGavran, 1970; Kudo et al., 1972; Kahn, 1974; Judge et al., 1976; Osamura and Fine, 1976). In the present report a case of esthesioneuroblastoma is described with particular reference to the ultrastructural appearance and the histochemical and histological properties of the tumour.

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Case Report

A 49-year-old man with cured pulmonary tuberculosis was examined because of left-sided rhinorrhea and nasal congestion. In the left nasal cavity a polyp-like tumour mass with a partly necrotic surface was seen both at anterior and posterior nasoscopy. Biopsies were taken for histopathological examination. X-ray examination revealed involvement of the left antrum, the left ethmoidal cells and the medial wall of the left orbit. Radiation therapy with 5500 rad was given before radical excision of the tumour, which appeared to arise from the ethmoidal region and infiltrated the surrounding bone tissue. After the operation the patient was followed up for one year and no relapse occurred. Preoperatively, determination of urinary 5-HIAA showed a normal value. No sign of any endocrine symptoms was seen.

Material and Methods

Light Microscopy. Tumour tissue was fixed in 10% formalin, dehydrated in graded ethanols, cleared in xylene and embedded in paraffin. Sections 4 μ thick were stained with van Gieson stain, haematoxylin-eosin, period acid Schiff (PAS), the argentaffin technique of Masson-Hamperl (1927), the argyrophil stains of Grimelius (1968) and Sevier-Munger (1965), and with Hellerström-Hellman's modification of the Davenport argyrophil technique (1960). The Bodian technique was used for visualizing nerve fibres.

Fluorescence Microscopy. Part of the material was frozen to the temperature of liquid nitrogen in a propane-propylene mixture, freeze-dried, and exposed to formaldehyde gas at 80° C for 1 h (Björklund et al., 1972). Control specimens were heated in the absence of formaldehyde. The specimens were embedded in paraffin in vacuo, cut in 4 μ thick sections, mounted in entellan (Merck) and examined in a Leitz Orthoplan fluorescence microscope equipped with an epi-illumination system (standard filter setting no. 2, peak excitation at 405 nm). The light source was an HBO 100 mercury lamp.

The presence of GH, ACHT, gastrin, insulin, glucagon, human pancreatic polypeptide (HPP), vasoactive intestinal polypeptide (VIP), gastric inhibitory peptide (GIP) and somatostatin was demonstrated by an indirect immunofluorescence technique (Coons et al., 1955). All anti-sera except insulin were raised in rabbits by repeated subcutaneous injections. The anti-insulin was raised in guinea-pigs. These anti-sera were used as the first layer. The second layer consisted of goat antiserum to rabbit IgG (except for insulin, for which rabbit anti-serum to guinea-pig IgG was used) labelled with fluorescein isothiocyanate (Hyland), in a dilution of 1:10–1:20. After being mounted in buffered glycerine the sections were examined in a fluorescence microscope (standard filter setting no. 3, peak excitation at 490 nm). Controls were those recommended by Goldman (1968).

Electron Microscopy. Tumour tissue was fixed in 2,5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 for 4 h, postfixed in 1% osmium tetroxide and embedded in Epon 812. Ultra-thin sections were prepared with an LKB ultrotome, and were mounted on Formvar-Coated copper grids, contrasted with uranyl acetate and lead citrate and viewed in a Zeiss electron microscope EM 9 at 60 kV.

Results

Light Microscopy. In routinely stained sections the tumour consisted of fairly uniform cells with a round to ovoid, hyperchromatic nucleus with a small nucleolus and scanty cytoplasm. The tumour cells were arranged in anastomosing cords and were surrounded by a loose fibrovascular stroma (Fig. 1). No degenerative changes were seen before or after radiation therapy. No PAS positive material

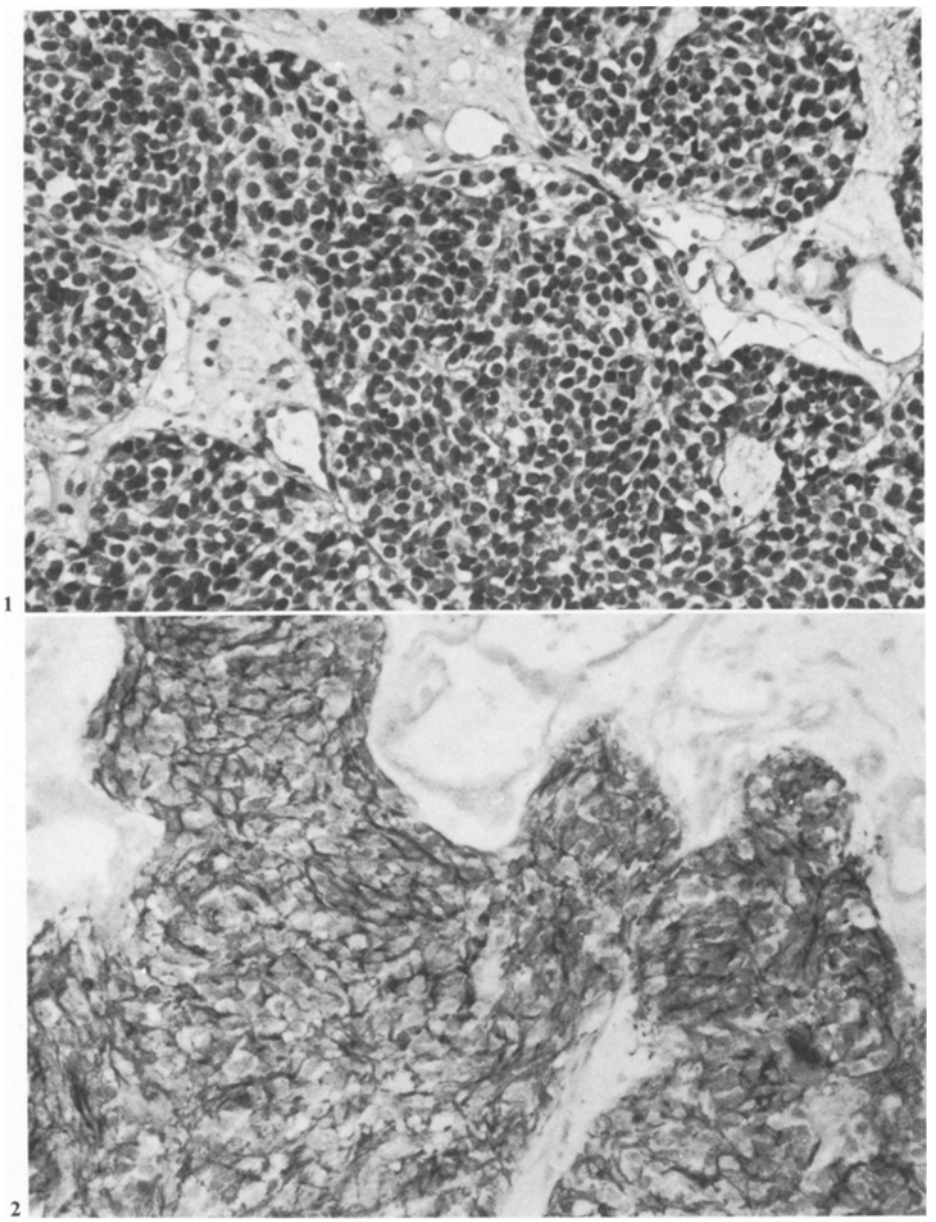

Fig. 1. The esthesioneuroblastoma, showing anastomosing cords of uniform cells surrounded by a loose fibro-vascular stroma. Haematoxylin-eosin. ×250

Fig. 2. The tumour stained with the Grimelius silver nitrate technique. The argyrophil reaction appears in most, but not all, tumour cells. ×250

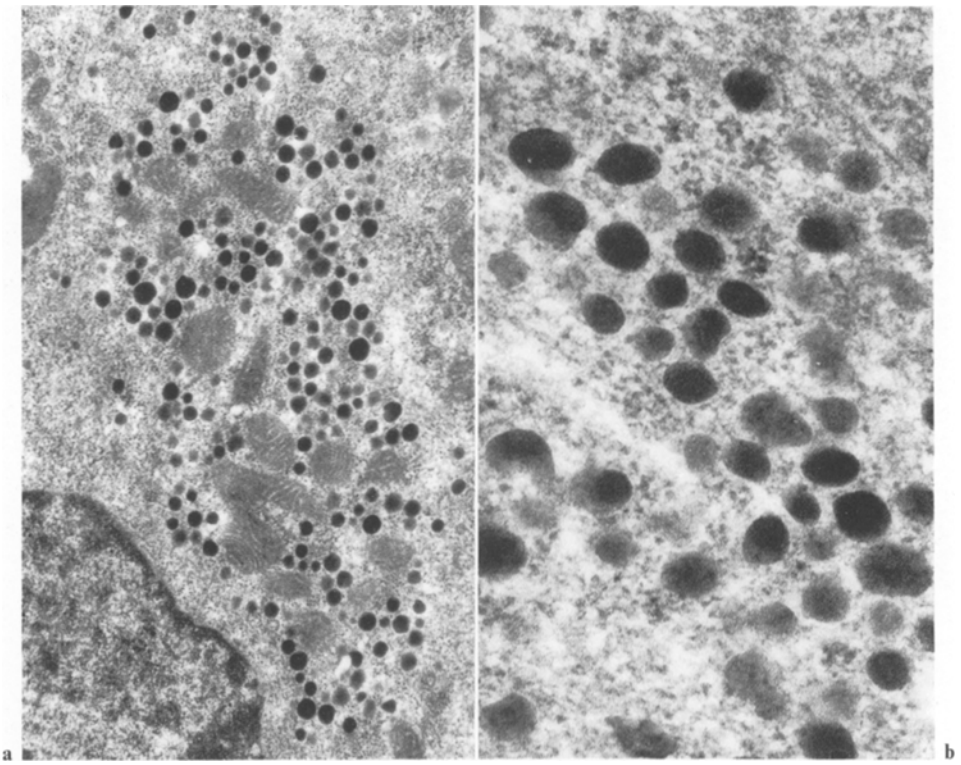

Fig. 3a and b. Ultrastructural picture of the esthesioneuroblastoma, showing endocrine granules in the cytoplasm of a tumour cell (**a**). ×14,800. The granules have an electron dense eccentric core and a paler halo (**b**). ×64,000

was found in the tumour, nerve fibres were not seen using the Bodian technique, nor did the tumour show any argentaffin reaction. Most tumour cells were argyrophil in the Grimelius stain, in which the brown to black silver grains were spread throughout the cytoplasm (Fig. 2). The reaction with the two other argyrophil methods was negative.

Fluorescence Microscopy. No formaldehyde-induced fluorescence was seen and no polypeptide hormones were demonstrated with immuno-fluorescence techniques.

Electron Microscopy. Most tumour cells contained ovoid to round membrane-limited, cytoplasmic granules (Mean diameter 100 nm) with a dense sometimes eccentrically located core and a paler halo (Fig. 3a and b).

Discussion

The olfactory esthesioneuroblastoma can be difficult to differentiate histo-pathologically from nasal epidermoid carcinoma (Gerhard-Marchant and Micheau,

1965; Lewis et al., 1965; Skolnik et al., 1966; McGavran, 1970; Schenk and Ogura, 1972; Judge et al., 1976). Electron microscopy has revealed the occurrence of cytoplasmic secretory granules (McGavran, 1970; Kahn, 1974; Judge et al., 1976; Osamura and Fine, 1976), and histochemical studies have shown the presence of biogenic amines (Micheau et al., 1975; Judge et al., 1976) and dopamine-β-hydroxylase (Micheau et al., 1975). The tumour cells also exhibit argyrophilia (Judge et al., 1976).

The tumour cells of the present case contained characteristic cytoplasmic granules but were devoid of formaldehyde-induced fluorescence. Formaldehyde-induced fluorescence has been suggested as a method for classifying this type of tumour, but negative results should thus be interpreted with care. Electron microscopy may also be helpful in showing the occurrence of secretory granules (McGavran, 1970; Kudo et al., 1972; Kahn, 1974; Judge et al., 1976; Osamura and Fine, 1976). In our experience argyrophil staining represents the easiest means of diagnosing this type of tumour. The chemical or physiochemical background of the argyrophil reaction is unknown, but ultrastructural studies have shown that the silver reaction is mainly localized at cytoplasmic granules (Grimelius, 1969; Vassallo et al., 1971; Grimelius et al., 1971; Wilander and Westermark, 1976).

Before classifying these tumours as esthesioneuroblastoma, metastases of other endocrine tumours must be excluded. In our case no endocrine symptoms were evident, nor did the sella turcica show any changes suggestive of neoplasia. Further, no polypeptide hormones were found in the tumour cells.

The few esthesioneuroblastomas which have been examined with argyrophil and histochemical and ultrastructural techniques have displayed somewhat differing properties. In routine pathological material argyrophil techniques may be of great value, especially in differentiating this type of tumour from epidermoid carcinoma. No silver positive reaction has been observed in epidermoid carcinoma (unpublished observations). It should be noted, however, that out of the three tested argyrophil techniques only the Grimelius method gave a positive reaction.

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