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The embryonal carcinoma of the parotid gland

A rare example of an embryonal tumor*, **

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Summary. Embryonal tumors are a neoplastic proliferation of cells of organ rudiments. Morphologically, these tumors are similar to the developmental stages of these organ rudiments. Embryonal tumors of the salivary glands have not been previously described. In the salivary gland register, reviewing the years 1965–1982 ($n = 8043$), we diagnosed 2,878 tumors of the salivary glands, of these 73 were tumors in children. One case was a malignant epithelial tumor in a 12-year-old boy, which showed the criteria of an embryonal carcinoma in light- and electron microscopy. The tumor revealed solid undifferentiated areas, epidermoid structures with keratinization and acinic structures. Immunhistochemically, the better differentiated epidermoid cells reacted positively with anti-CEA and anti-keratin, the acinic cells were positive with anti-amylase. The ultrastructure was characterized by primitive ductular epithelial cells and acinic cells with their typical morphological features. The embryonal carcinoma has to be distinguished from undifferentiated carcinomas of the salivary glands, which consist of primitive ductular structures only. The failure to detect other tumor markers (lactoferrin, tissue polypeptid antigen) indicates that poorly and well differentiated areas can exist simultaneously in embryonal carcinomas.

Key words: Embryonal carcinoma – Parotid gland – Differential diagnosis to undifferentiated carcinoma – embryonal tumors

Embryonal tumors (Willis 1962; Meister 1978) represent a neoplastic proliferation of cells of organ rudiments. Morphologically they are similar to the developmental stages of the rudiment and can be poorly or well differentiated. In the head-neck region embryonal tumors have been described in the nasopharynx, in the orbit and in the inner ear. Embryonal carcinomas

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of the salivary glands have not been reported. During the years 1965–1982, we diagnosed 2,878 salivary gland tumors out of 8,043 collected cases in the salivary gland register. 73 tumors occurred in childhood and adolescence. One case was a malignant epithelial tumor in a 12-year-old boy, which showed the criteria of an embryonal carcinoma in light- and electron microscopy. This study analyses this case and discusses the differential diagnosis to undifferentiated carcinomas of the salivary glands (Donath et al. 1982).

Materials and methods

The present case was compared with 73 salivary gland tumors of young patients (age 0–20). The pathohistological classification of these tumors shows Table 1, the localization is given in Table 2. The pleomorphic adenomas made up 65% of all tumors, the mucoepidermoid tumors about 15%. 70% of all tumors were found in the parotid gland, about 15% in the

Table 1. Salivary gland tumors in children and adolescents ($n=73$). Salivary Gland Register 1965–1982 ($n=8043$)

Tumor type	Age (Years)		Total number
	0–10	11–20	
Pleomorphic adenomas	2	44	46
Monomorphic adenomas	1	1	2
Acinic cell tumors	–	4	4
Mucoepidermoid tumors	1	9	10
Adenoid cystic carcinomas	–	3	3
Adenocarcinomas	2	–	2
Epidermoid carcinomas	1	1	2
Carcinomas in pleomorphic adenomas	–	2	2
Undifferentiated carcinomas	–	1	1
Embryonal carcinomas	–	1	1
Total number	8	66	73

Table 2. Localization of salivary gland tumors in children and adolescents ($n=73$)

Tumor type	Pa	Sm	Sl	Pg[a]	Cheek	Lip
Pleomorphic adenomas	35	2	–	6	1	2
Monomorphic adenomas	–	–	–	–	2	–
Acinic cell tumors	4	–	–	–	–	–
Mucoepidermoid tumors	4	1	–	4	–	1
Adenoid cystic carcinomas	3	–	–	–	–	–
Adenocarcinomas	2	–	–	–	–	–
Epidermoid carcinomas	2	–	–	–	–	–
Carcinomas in pleomorphic adenomas	2	–	–	–	–	–
Undifferentiated carcinomas	–	1	–	–	–	–
Embryonal carcinomas	1	–	–	–	–	–
Total number	53	4	–	10	3	3

[a] Pa = Parotid gland; Sm = Submandibular gland; Sl = Sublingual gland; Pg = Palatinal glands

palatine glands. The present case was examined with light- and electron microscopy and immuncytochemistry.

For lightmicroscopy paraffin-embedded material was stained by the following methods: haematoxylin-eosin, PAS-reaction, astra-blue-staining, Masson-Goldner-staining, silvering technique (Gomori) and karmin-staining (Best).

Semithin sections (1 μm) were obtained from material embedded in (EPON 812). These sections were stained with toluidine-blue, and they were used to select thin sections.

In immuncytochemistry, the immunperoxidase-method was applied to demonstrate CEA (carcino-embryonal antigen) (Caselitz et al. 1981a), lactoferrin (Caselitz et al. 1981b), tissue polypeptide antigen (Caselitz et al. 1983a) and amylase (Caselitz et al. 1983b). As intermediate-sized filament protein keratin was analysed (Caselitz et al. 1981c). Further details about methods to demonstrate tumor markers in salivary gland tumors can be found in Caselitz et al. (1982) and Seifert and Caselitz (1983).

For electron microscopy small tissue blocks were fixed in 3% glutaraldehyde-cacodylate buffer (ph 7.2–7.4; 300 mol/sm) at 4° C over 2 h. After washing, 1.33% s-colloidin buffered osmium tetroxide was used as another fixative. Then the material was embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate, then examined and photographed on a Philips EM 300 electron microscope (beam voltage 80 kV).

Alternative. For electron microscopy small tissue blocks were fixed in 1% buffered osmium tetroxide and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate, then examined and photographed on a Philips EM 300 electron microscope.

Results

Case report

A 12-year-old boy presented to his general practitioner with pain and swelling over the right cheek and the right submandibular region. These painful swellings had developed over two months. The patient was referred to hospital for further investigation.

On clinical examination, two tumor masses, each measuring 3 cm in diameter, were found. They were localized around the parotid duct and in the right sub mandibular region. Both tumors were totally excised and sent for histology. The pathologist could not make a definite diagnosis. In his report he suggested a malignant lymphoma or an undifferentiated carcinoma. During the following weeks, the patient was treated with urbason, vincristin, asparaginase and immunoglobulins on a paediatric ward. But the treatment was not successful and the tumor recurred and continued to grow.

The patient, therefore, was referred to the Department of Oral Surgery (University of Hamburg) for further surgical treatment. A subtotal parotidectomy, a neck dissection and a partial resection of the cheek was performed. The defect was reconstructed by Collin's lock. There were no postoperative complications. After a four-week-stay in hospital, the patient was discharged home, but he had to be readmitted two months later.

A third operation was performed which included the removal of the right zygoma, the lateral orbital lamellae and the right ramus of the mandible. After bone reconstruction and free dermatoplasty the patient under went radiotherapy, but the tumor recurred two months later. On the chest X-ray some well defined rounded shadows appeared and tumour spread

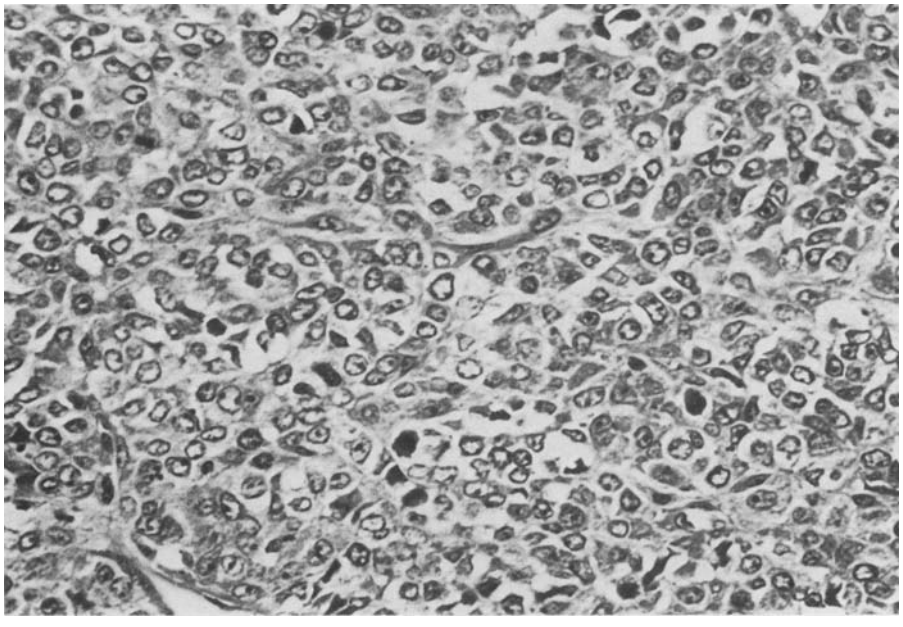

Fig. 1. Cells with different-sized, partly clear nuclei in a solid and medullary arrangement. Haematoxylin-eosin. ×400

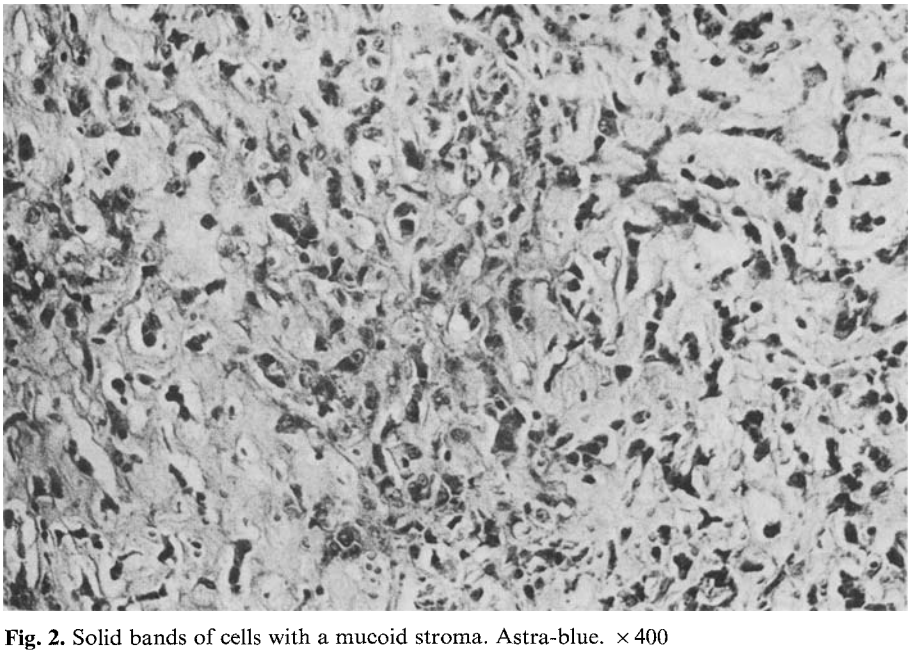

Fig. 2. Solid bands of cells with a mucoid stroma. Astra-blue. ×400

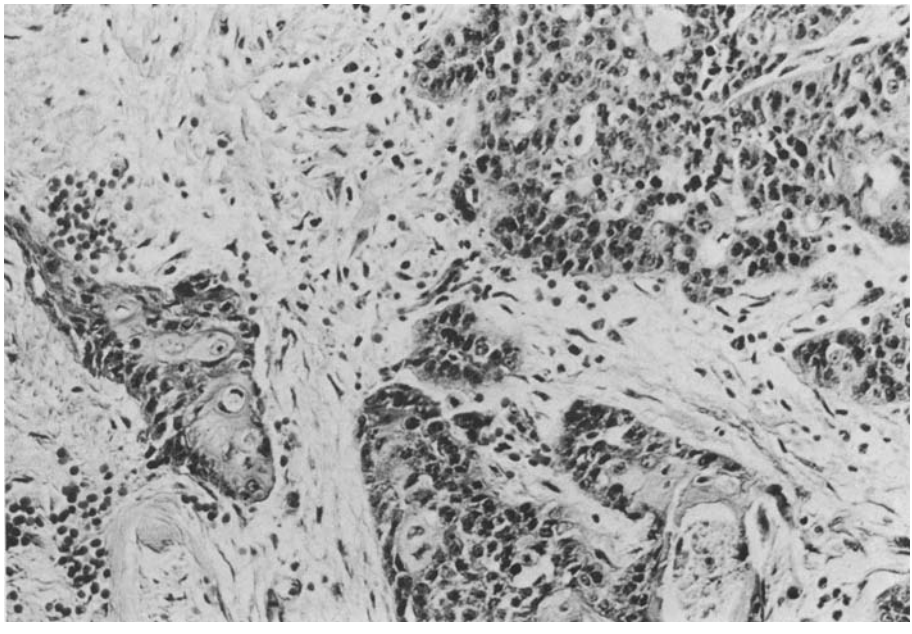

Fig. 3. Epidermoid differentiated cells with focal keratinization. Masson-Goldner. ×400

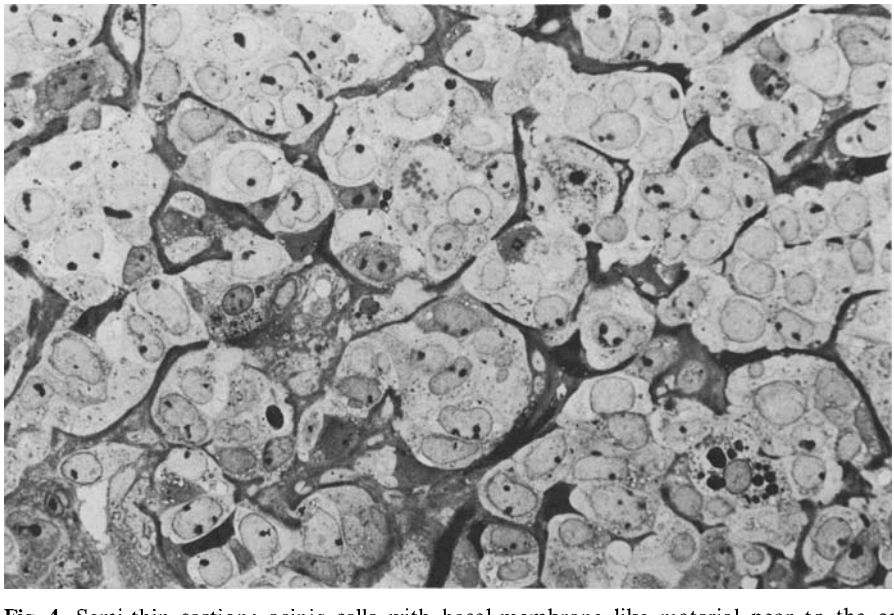

Fig. 4. Semi-thin section: acinic cells with basal-membrane like material near to the outer surface. Toluidine-blue. ×800

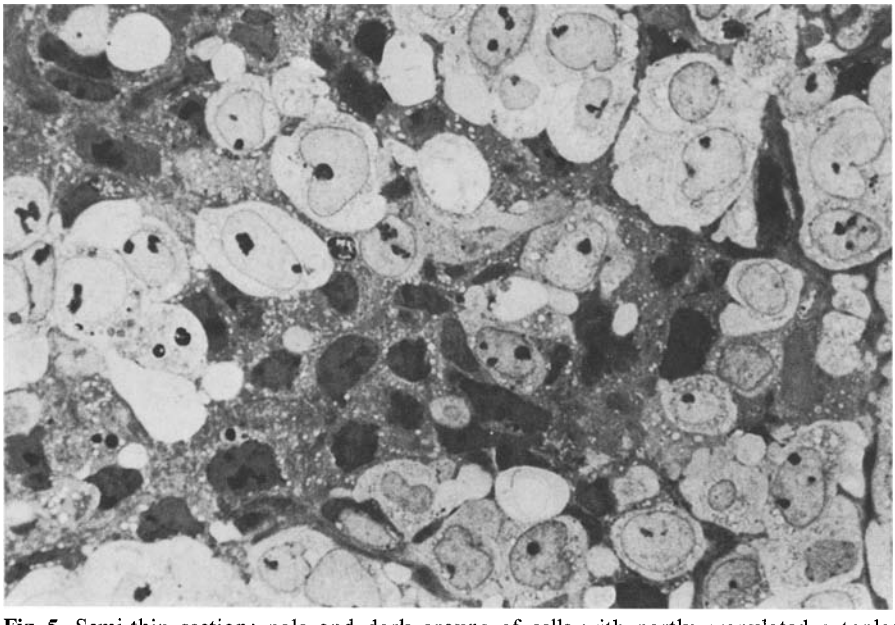

Fig. 5. Semi-thin section: pale and dark groups of cells with partly granulated cytoplasm. Toluidine-blue. × 800

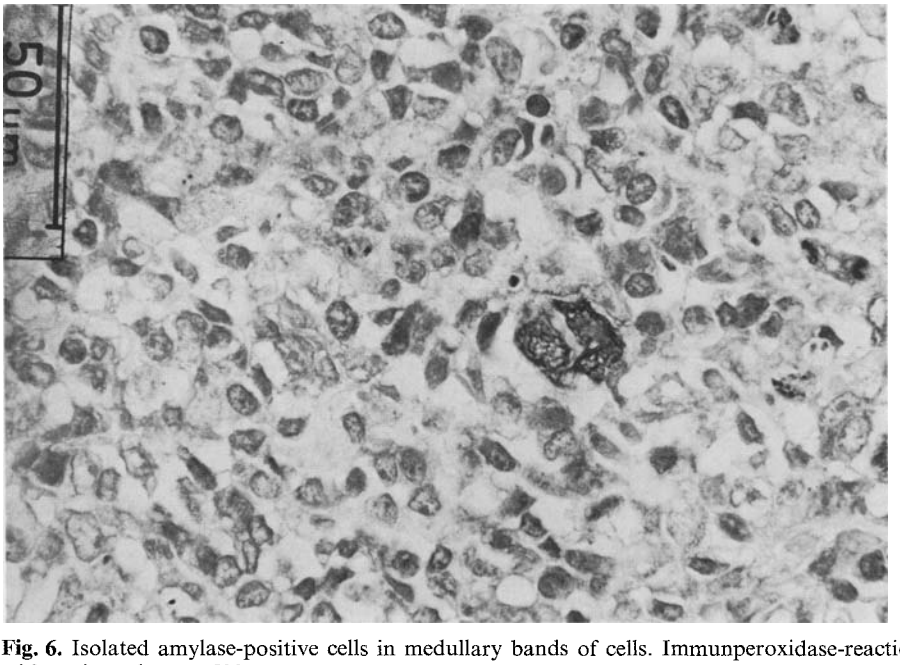

Fig. 6. Isolated amylase-positive cells in medullary bands of cells. Immunperoxidase-reaction with anti-amylase. × 500

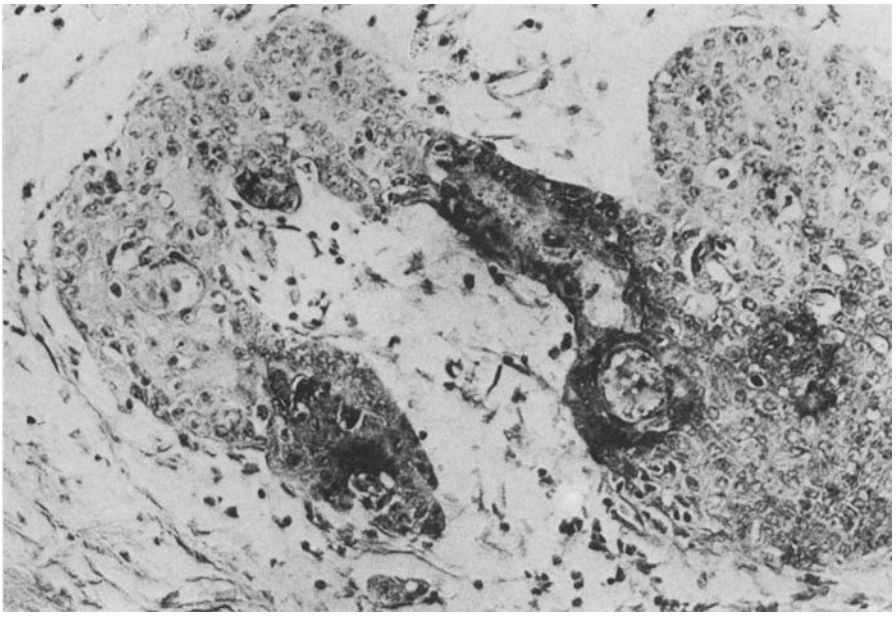

Fig. 7. Positive reaction with anti-CEA in keratinized epidermoid areas. Immunperoxidase-reaction with anti-CEA. ×400

to the lungs was considered to be likely. No further therapy wa given. The patient died a month later at home. An autopsy was not performed.

Macroscopy. The excision specimen of the cheek (J.-No. 31911/77) revealed a round tumor mass, measuring 2.5 cm in diameter. The tumor was not surrounded by a fibrous capsule. The cut surface was a grey-whitish colour.

Histology. The well preserved tumor tissue showed varying morphological features. There were some solid and medullary areas, which consisted of tumor cells with a pale cytoplasm. The nuclei contained heterochromatin in the periphery or a prominent nucleolus (Fig. 1). Other areas were characterized by bands of single tumor cells within a stroma filled with acid mucopolysaccharides (Fig. 2). Epidermoid differentiated tumor islands surrounded by fibrous tissue were also present (Fig. 3).

Semithin sections revealed groups of cells, bound by a basal membran like material (PAS-positive) and resembling acinar cells (Fig. 4). These cells had large oval or round nuclei, partly with two or more nucleoli. The pale cytoplasm contained small vesicles and varying dark granules. The cells with pale or dark granules around the nucleus looked like acinic cells. Solid areas consisted of polymorphic pale and dark tumor cells (Fig. 5). In the tumor and in the surrounding tissue, there was no stromal reaction. Some atypical mitotic figures were seen.

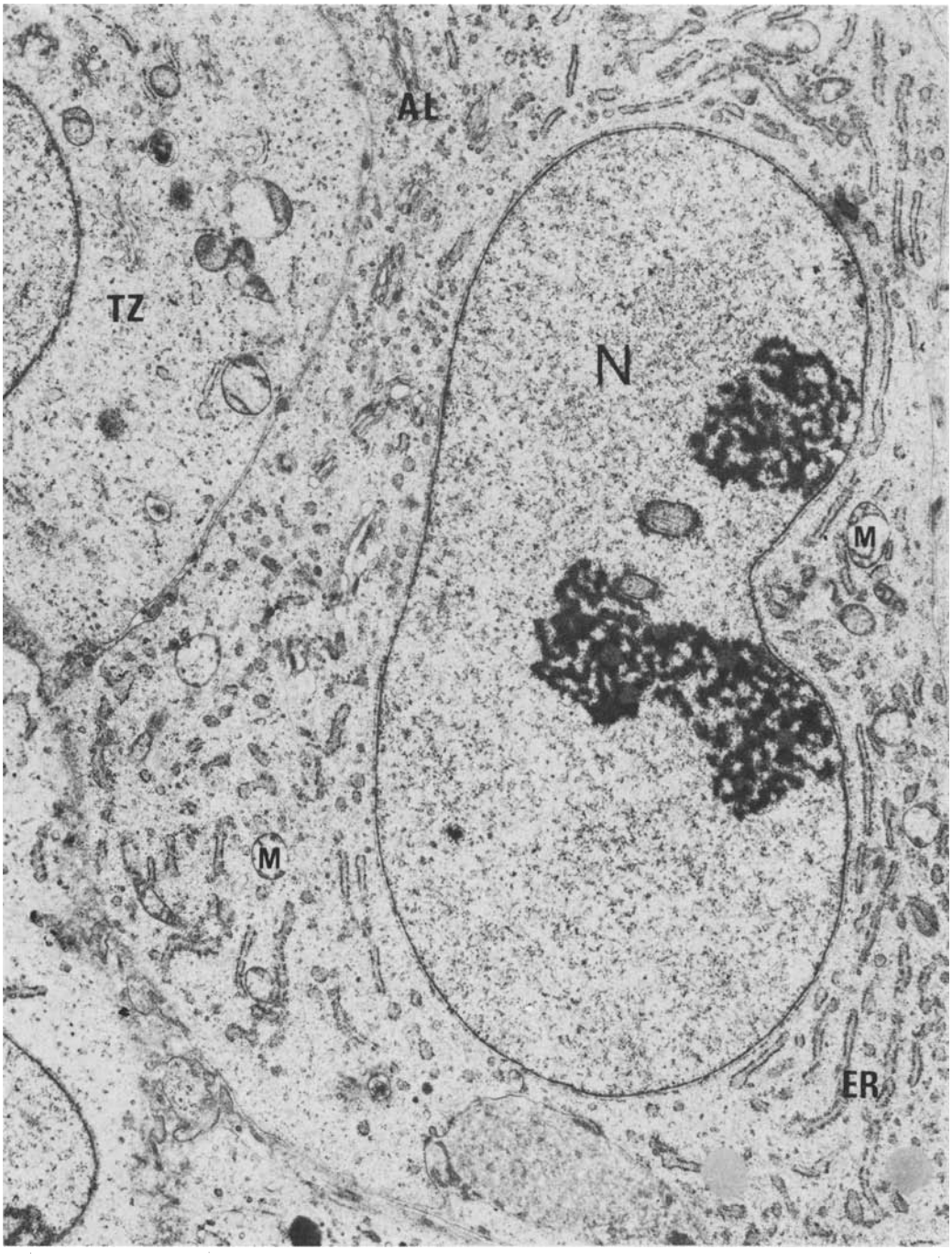

Fig. 8. Ultrastructure of clear tumor cells: tumor cell with a large nucleus (*N*) and nucleoli, numerous membranes of the rough endoplasmic reticulum (*ER*), anulated lamellae (*AL*) and altered mitochondria (M); tumor cell with only a few organelles (*TZ*). ×14,500

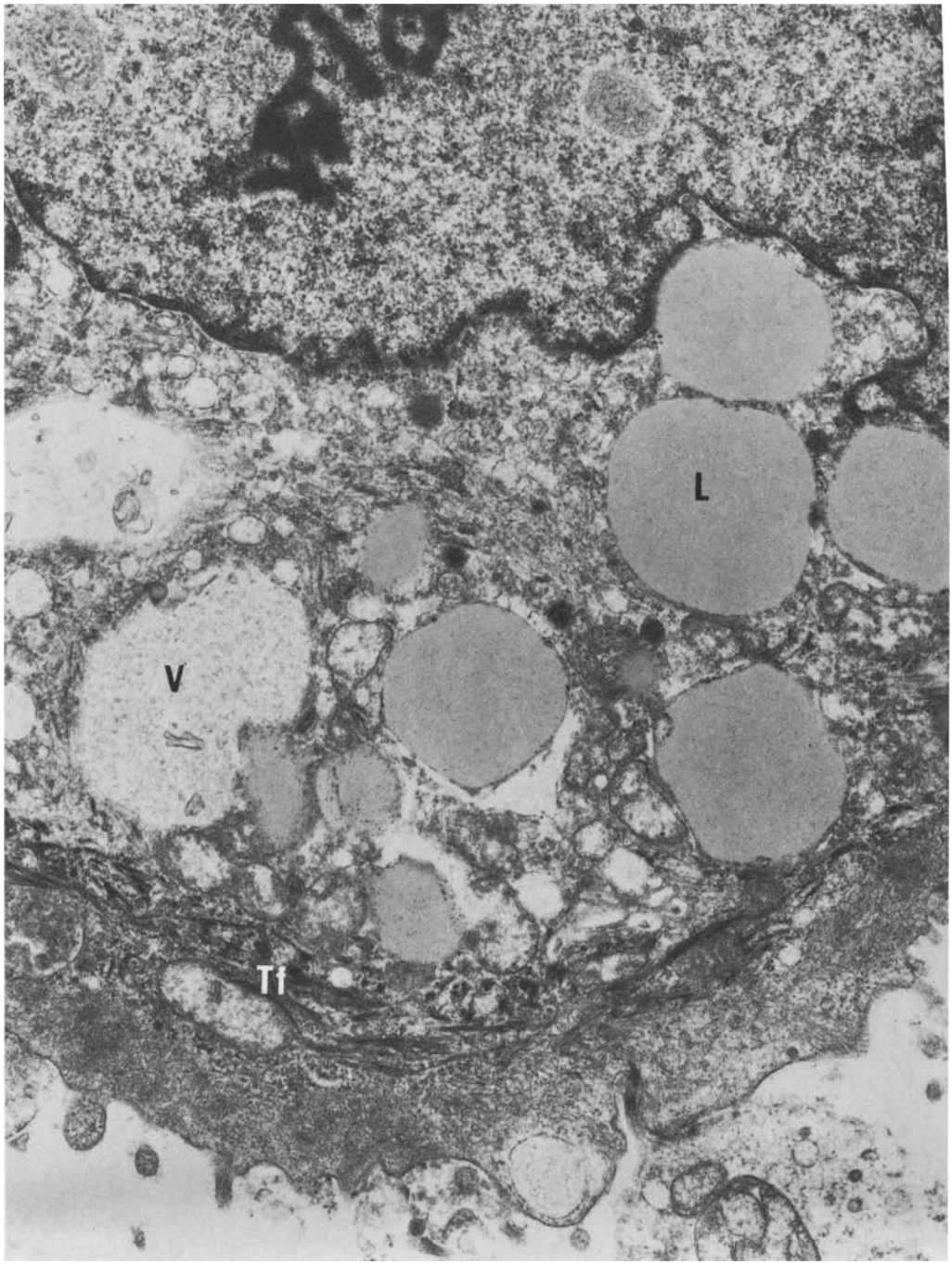

Fig. 9. Ultrastructural details of a dark tumor cell: tonofilaments (*Tf*) and microfibrils, lipid drops (*L*) and vacuoles with granulated material (*V*). × 16,400

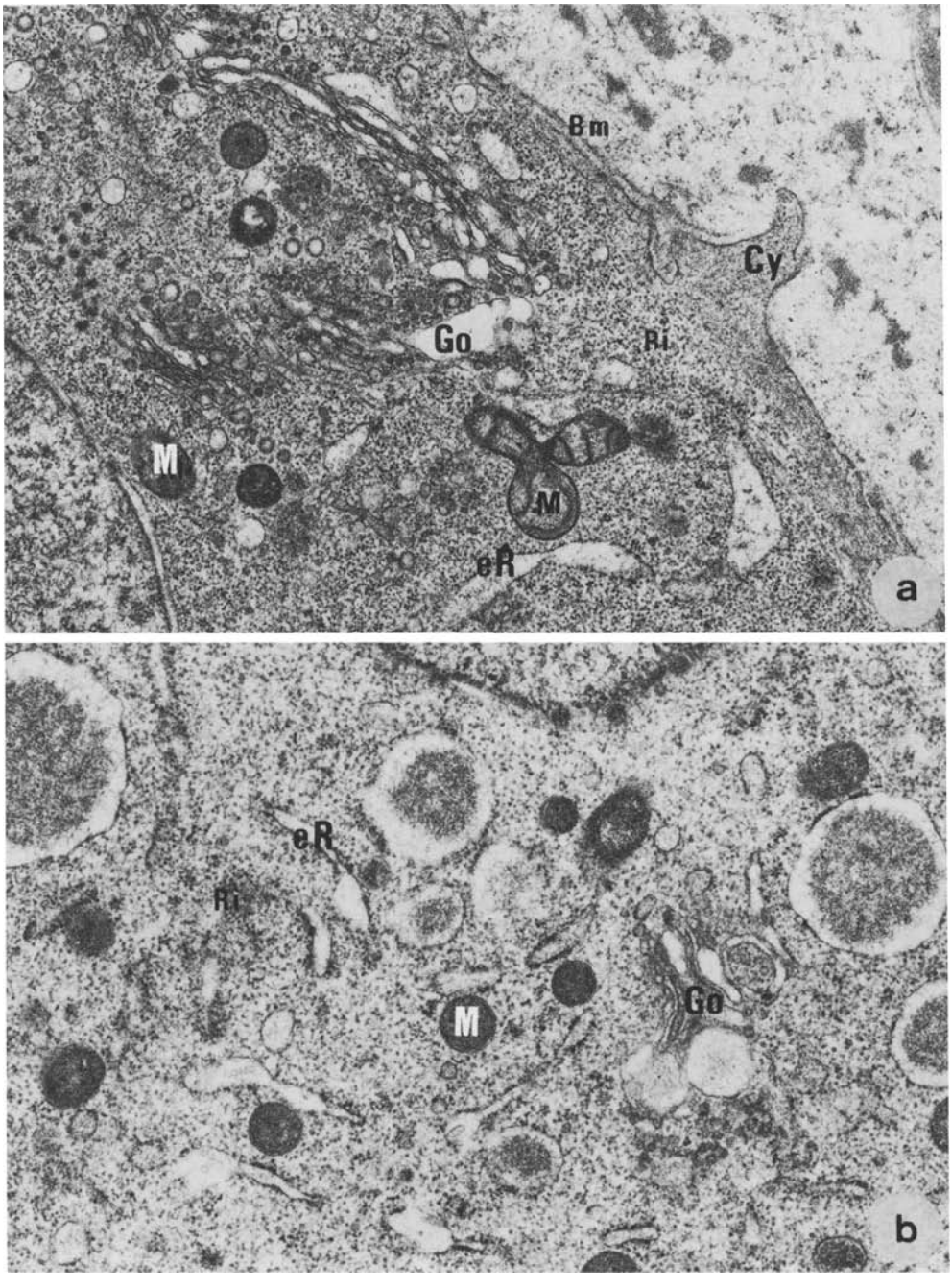

Fig. 10a, b. Ultrastructural details of dark tumor cells: **a** peripheral tumor cell with incomplete basal membrane (*Bm*), cytoplasmic process (*Cy*), large Golgi-area (*Go*) and few other cell organelles (*eR* = rough endoplasmic reticulum, *M* = mitochondria, *Ri* = free ribosomes). **b** Perinuclear Golgi-area (*Go*), numerous vacuoles with granulated material (*Va*) and some other organelles (*eR* = rough endoplasmic reticulum, *M* = mitochondria, *Ri* = free ribosomes). × 18,400

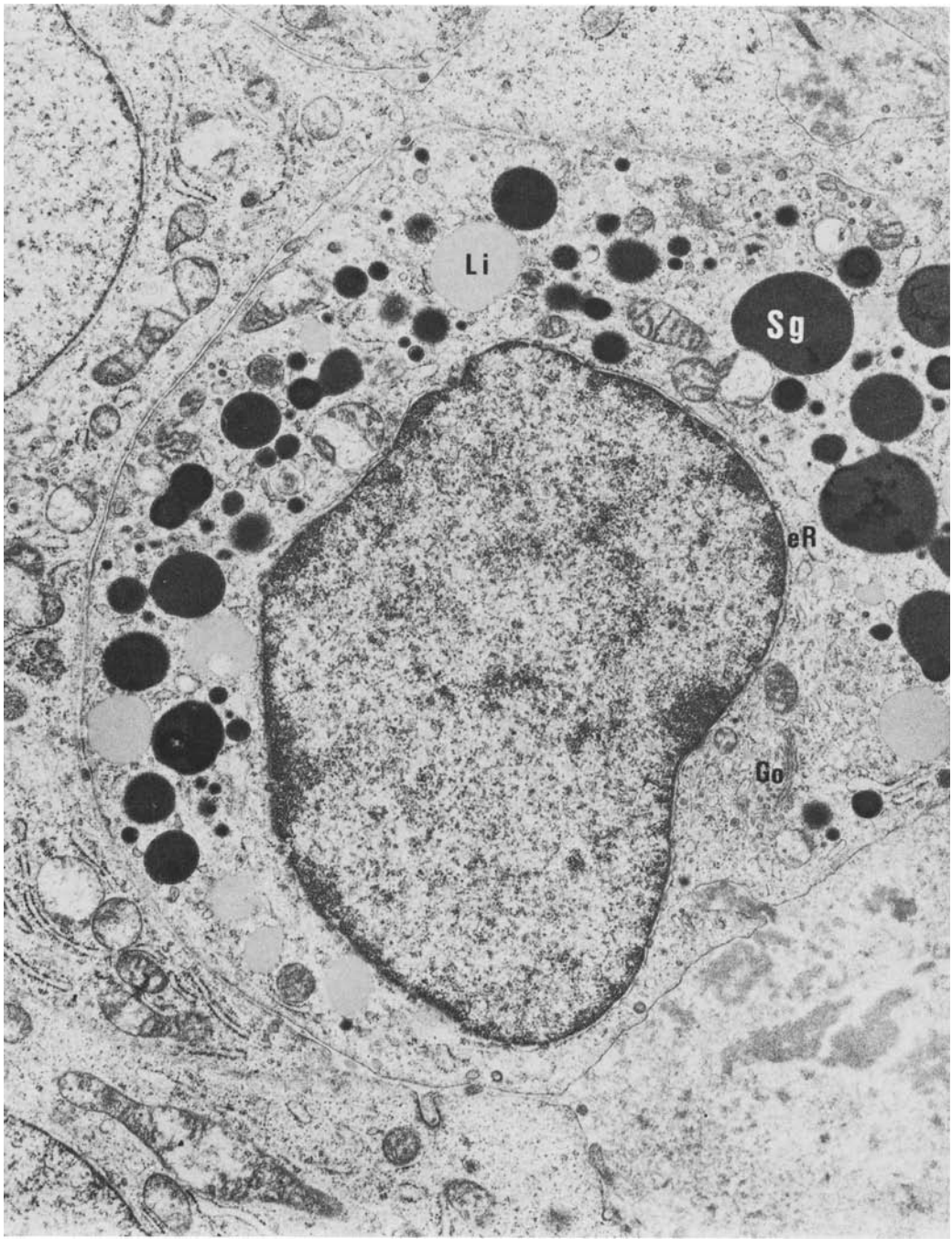

Fig. 11. Ultrastructure of an acinic tumor cell: numerous secretory granules (*Sg*), small Golgi-area (*Go*), isolated lipid drops (*Li*) and little rough endoplasmic reticulum (*Er*). ×12,500

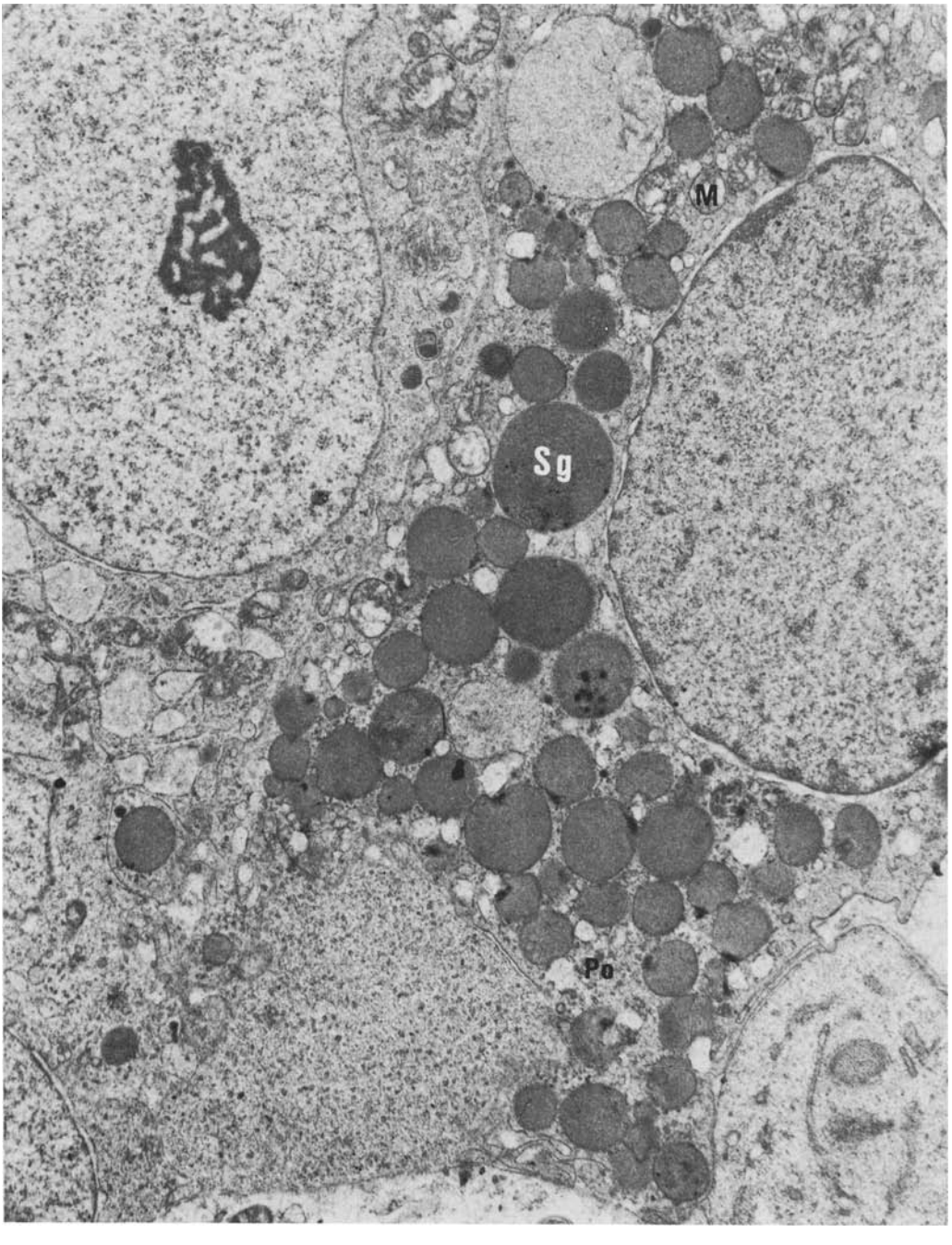

Fig. 12. Ultrastructure of an acinic tumor cell: clear secretory granules (*Sg*), numerous mitochondria (*M*) and polysomes (*Po*). ×10,500

Immunhistochemistry. The reaction with anti-amylase was only positive in some tumor cells (Fig. 6). CEA (carcino-embryonic antigen) could be demonstrated in small areas of high differentiated tumor cell complexes (Fig. 7). Anti-CEA failed to react in other solid, medullary or acinous differentiated areas. The distribution of keratin was similar to CEA. Lactoferrin and TPA (tissue polypeptid antigen) were not demonstrated.

Ultrastructure. The solid tumor areas consisted of pale and dark cells in a scanty connective tissue matrix. Some cells were clearly defined by a basal lamina. Lymphocytes were not present in the tumor or in the connective tissue. Fibroblasts and collagenous fibers were sparse.

Based on cytological and histological criteria, four types of epithelial cells could be differentiated.

The first type included large pale cells with only a few organelles and a large oval nucleus (Fig. 8). These cells were arranged in clusters between groups of dark cells. Single tumor cells did not adhere to each other by desmosomes. The cytoplasm of the pale cells contained short lamellae of rough endoplasmic reticulum, annulated lamellae, free ribosomes, mitochondria and some lipid droplets. The surface of the nucleus was smooth. Two or three reticulated nucleoli were present.

The second cell type varied in size and had a dark cytoplasm. The irregularly formed nuclei contained little heterochromatin and reticulated nucleoli. The cytoplasm revealed numerous lipid drops, microfilaments (7–11 nm), tonofilaments, mitochondria, ribosomes, small vesicles and cytoplasmic vacuoles, filled with a fine flocculent material (Fig. 9). Numerous dark cells contained a large Golgi-area, little rough endoplasmic reticulum, abundant free ribosomes and small dark mitochondria. Some peripheral cells had cytoplasmic processes. The cell surface was incompletely covered by a dark basal lamina (Fig. 10). Other dark cells had numerous vacuoles, which contained a flocculent material (Fig. 10). Their membranes varied in thickness. Similar vacuoles were seen close to the Golgi-apparatus. Ribosomes were irregularly distributed on the lamellae of the rough endoplasmic reticulum.

The third cell type was likely to be equivalent to acinic (or serous) cells. The large nucleus was centrally located. The cytoplasm contained numerous zymogen-like granules of various sizes, mitochondria, free ribosomes, sparse rough endoplasmic reticulum, one or two Golgi-areas and some lipid droplets (Fig. 11). Pale granules, filled with fine granular material and some dense cores, could be demonstrated on other acinous cells.

The fourth cell type was only found in less extent and showed epidermoid differentiation. Desmosomes were widely present binding these cells together.

Discussion

Embryonal tumors occur as 'congenital' tumors or they often present in the first year of life. If they grow slowly or if they derive from embryonal

remnants, they can be observed in childhood or adolescence. Embryonal carcinomas of the salivary glands have not yet been reported, and the common salivary gland tumors in childhood are hemangiomas and pleomorphic adenomas, with the most important malignant tumors being mucoepidermoid tumors, acinic cell tumors, adenoid cystic carcinomas and adenocarcinomas (Lit.: Kaufman and Stout 1963; Seifert 1965; Castro et al. 1972; Schuller and Maccabe 1977; Catania and Bozzetti 1977). Undifferentiated carcinomas in the newborn have occasionally been described (Dick 1954; McKnight 1939), Vawter and Tefft (1966) mentioned two congenital parotid gland tumors with ductular and trabecular structures where the similarity to embryonal salivary tissue led them to call these tumors 'embryomas'.

Embryonal tumors as defined above have to be distinguished from undifferentiated carcinomas. These include primitive, low differentiated, solid epithelial tumors which are difficult to classify, light microscopically. Previous studies describe small cell and large cell carcinomas which originate from the salivary duct system (Koss et al. 1972; Blanck et al. 1974; Wirman and Battifora 1976; Dubois et al. 1977; Brocheriou et al. 1977; Kumar et al. 1979; Donath et al. 1982; Nagao et al. 1982; Yaku et al. 1983). A special problem represent undifferentiated parotid gland carcinomas with a lymphoid stroma (Wassef et al. 1982; Nagao et al. 1983), which are difficult to distinguish from metastasis of a primary nasopharynx carcinoma. Electronmicroscopically, the undifferentiated carcinomas showed structures which can be found in the embryonic development of the duct system (Donath et al. 1978; Donath et al. 1982) including undifferentiated duct cells, epithelial cells with secretory activity and myoepithelial cells. Primitive epidermoid cells with desmosomes and tonofilaments were also present. On the basis of the ultrastructural findings, these carcinomas have to be classified as very low differentiated squamous cell carcinomas or salivary duct carcinomas. The tumors probably derive from regenerative cells of the duct system which can differentiate along many paths including epidermoid cells. However, they should not be called 'embryonal carcinomas' as they do not show acinar structures.

The ultrastructural examination of this case revealed not only cells characteristic of embryonal salivary ducts, but also acinar structures with amylase-containing secretion granules, typical of the parotid gland and acinic cell tumors (Hübner et al. 1968; Kay and Schatzki 1972; Ellis and Corio 1983). A parallel finding is reported by Benjamin and Wright (1980), who described two carcinomas of the pancreas in children. Ultrastructurally, these tumors showed ductular differentiated cells (microvilli, tonofilaments and desmosomes) and acinar structures with typical enzyme granules.

In immuncytochemistry the presented tumor reacted positively with anti-amylase, anti-CEA and anti-keratin, particularly in better differentiated areas of the tumor. The tumor cells failed to react with lactoferrin and tissue polypeptid antigen. These immunhistochemical findings show that poorly and well differentiated areas exist in embryonal carcinomas.

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