Path. Res. Pract. 191, 535–540 (1995)

Unusual Intracytoplasmic Inclusions in Metastatic Carcinoma*

Discussion of their Possible Significance

U. J. G. M. van Haelst and M. Pruszczynski
Department of Pathology, Medical Faculty, Nijmegen, The Netherlands

SUMMARY

Unusual filamentous inclusion bodies in the cytoplasm of metastatic tumor cells are described. Their presence (intermingled with zymogen granules) seems rather restricted to cells of primary or metastatic acinar cell carcinoma of the pancreas, acinic carcinoma of salivary gland (parotid gland) and Paneth cells (neoplastic or in zinc deficiency state). For the time being, the real nature of these inclusions (deranged zymogen granules?) is unclear.

This case also emphasizes the value of electron microscopy in solving the problem of the occult primary tumor and avoiding the misdiagnosis of an endocrine tumor (e.g. islet cell tumor or carcinoid), or a duct cell tumor with eosinophilic granular cytoplasm or in recognizing the foci of acinar cells in a mixed variant of carcinoma of the pancreas.

Introduction

Correct pathological diagnosis is the most important cornerstone in patient therapy and management. However, metastatic tumors of occult primary origin is a repeatedly occurring challenge for the clinician or pathologist[7]. In the last decade enormous progress has been achieved in resolving those diagnostic problems with the advent of immunohistochemistry, but the results are not always straightforward or conclusive. In this paper we present and discuss the diagnostic value and significance of peculiar cell organelles in metastasis detected by electron microscopy.

* Dedicated to the memory of Prof. Dr. Pierre Dustin (1914–1993).

Results

Histopathology

On light microscopy the lymph node was almost completely replaced by predominantly solid cords and sheets of tumor cells with eosinophilic granular cytoplasm and centrally located mildly pleomorphic nuclei with small single nucleoli (Fig. 1). Mitoses were numerous. A glandular or ductular growth pattern was noted in different areas (Fig. 2). Alcian blue and mucicarmine stains were slightly positive in the lumina but intracellular mucin was never detected. Small PAS positive granules resistant to diastase digestion could be observed in scattered individual neoplastic cells. There was no necrosis nor lymphocytic infiltrate in the metastatic areas.

Immunohistochemistry

The cytoplasm of numerous tumor cells exhibited positive staining for polyclonal cytokeratin (Fig. 3).

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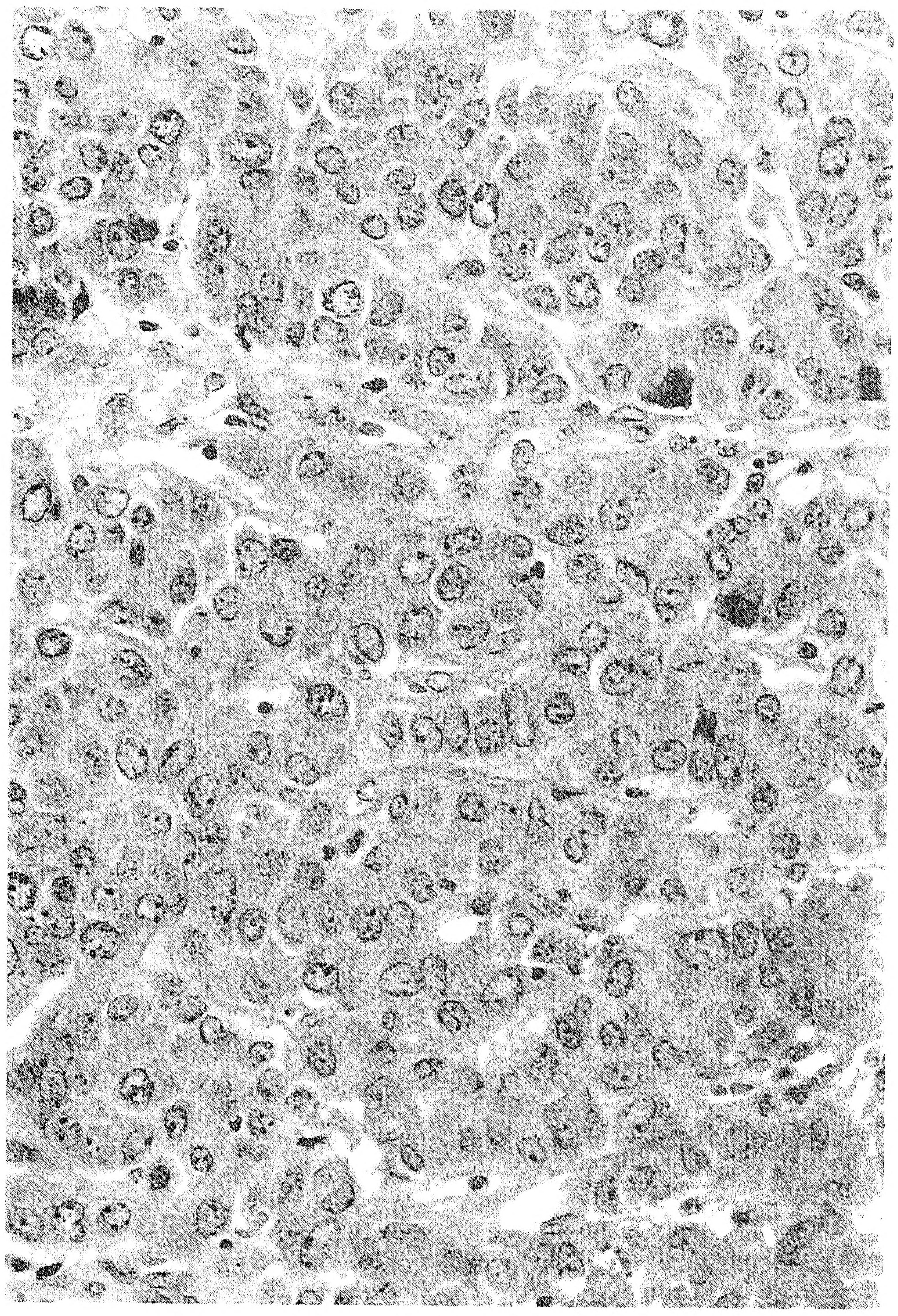
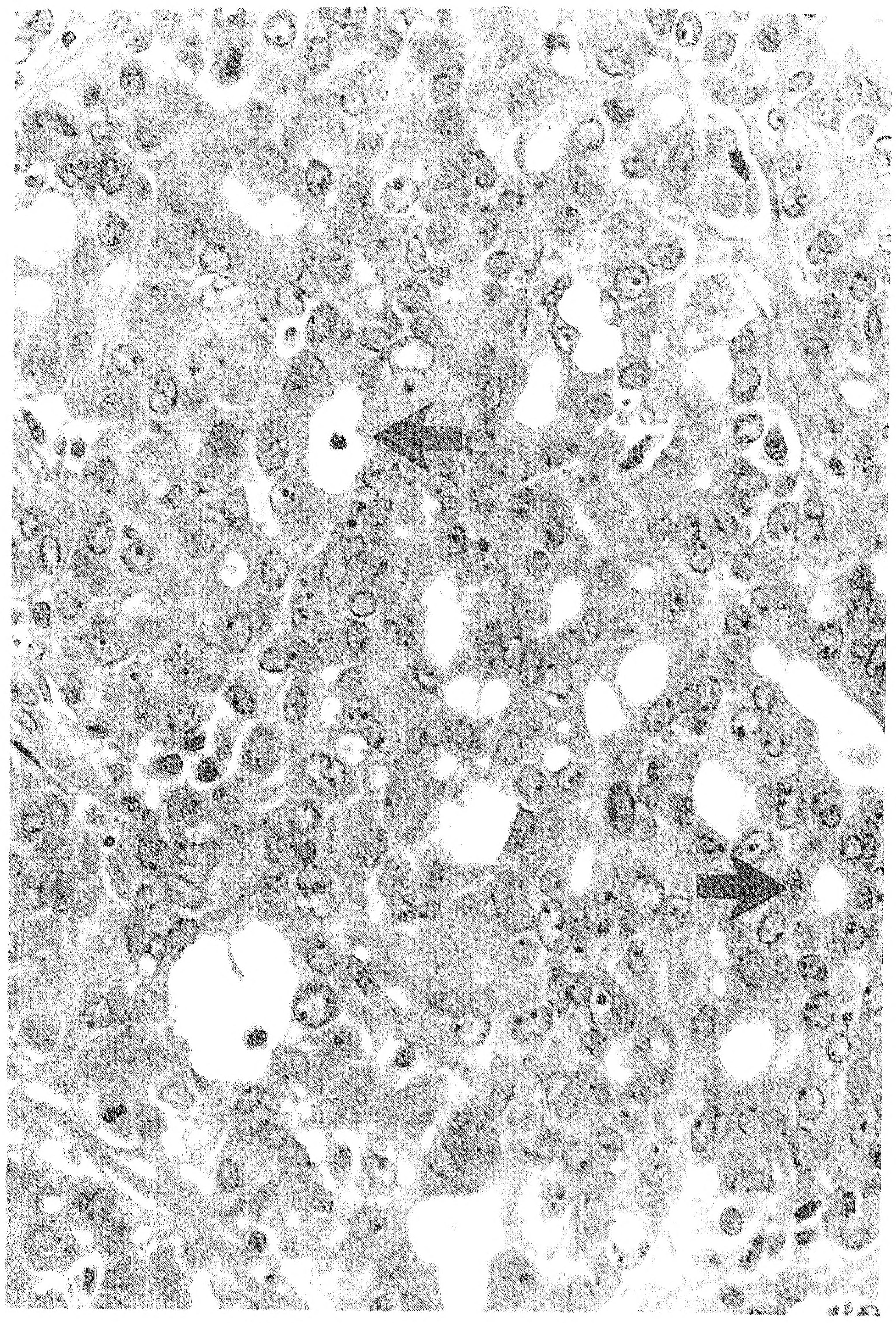


Fig. 1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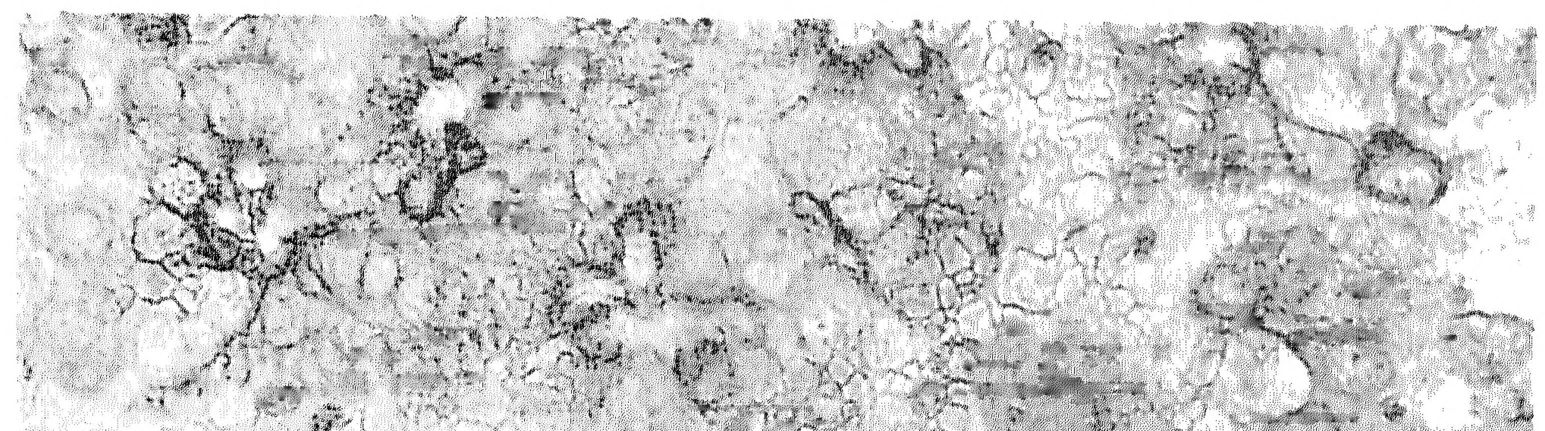
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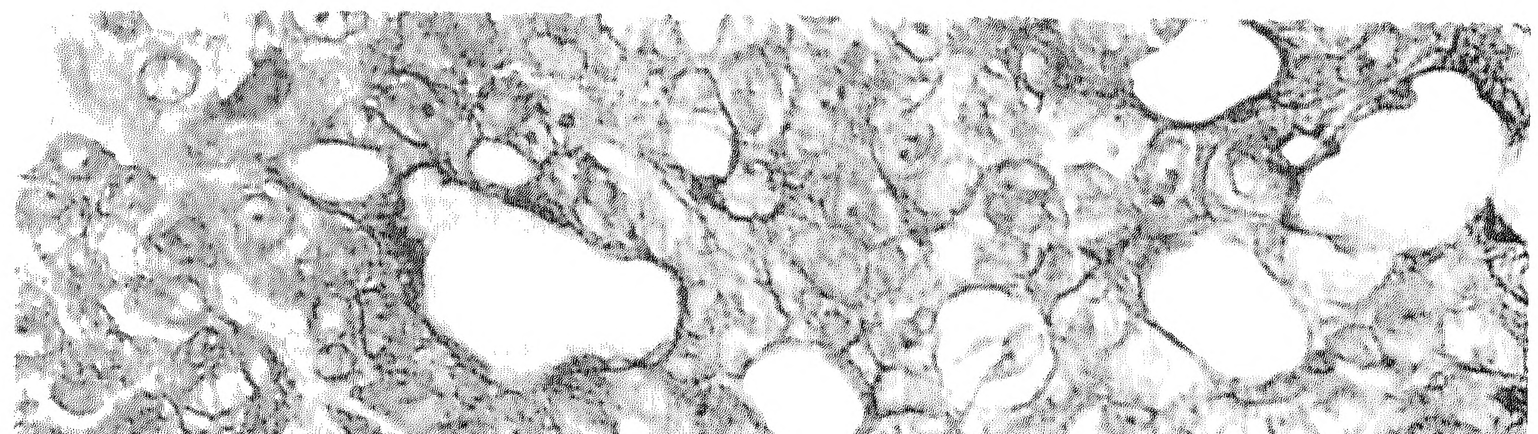
Fig. 4

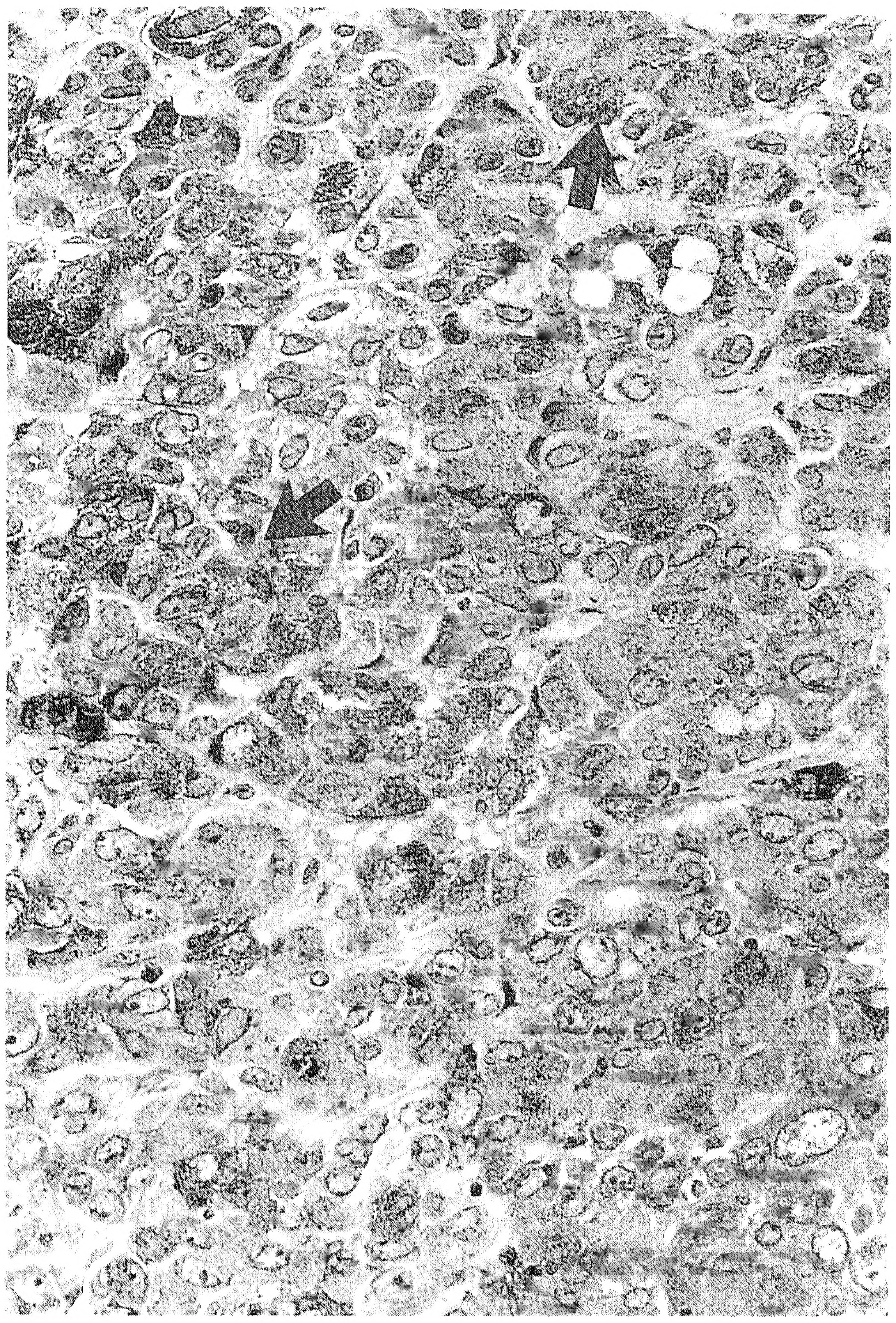

Fig. 5. An intense diffuse cytoplasmic reaction (arrows) could be observed for muramidase. × 410.

CAM 5.2. was generally more positive on the apical cell membranes than in the cytoplasm (Fig. 4). No immunoreactivity was identified for vimentin, NSE, CEA, PSA, S-100 protein, chromogranin and synaptophysin. Muramidase was focally strongly positive in the cytoplasm (Fig. 5). EMA decorated especially the apical sides of the tumor cells comparable to CAM 5.2 (Fig. 4).

Electron Microscopy

On ultrastructural examination, dark and clear neoplastic cells showed mostly a tubular arrangement and exhibited inter- as well as intracellular luminal spaces with numerous apical blunt microvilli. Each tumor cell contained a large number of homogenous electron-dense spherical granules resembling zymogen granules that were often crowded within the apical cytoplasm and under the lateral cell membranes (Fig. 6). In addition, many cells displayed variable numbers of pleomorphic but mostly oval or slightly curved inclusions with electron-dense fine filamentous internal structure with no apparent periodicity (Fig. 7). These inclusions were not or only partly surrounded by a membrane, and lay free in the cytoplasm outside the parallel arrays of well-developed rough endoplasmic reticulum (Fig. 8) and between the numerous mitochondria. Cells with endocrine features were not identified.

Discussion

When these electron microscopical findings were presented years ago as a case for the panel in Ultrastructural Pathology[6] we suspected metastases of a poorly differentiated adenocarcinoma with zymogen granules and abundant developed rough endoplasmic reticulum. Therefore, the primary site or origin was considered to be acinar cells of the pancreas, of the salivary gland or of Paneth cells in the intestine. At that time, and to the best of our knowledge, the association of filamentous inclusions or bodies, which constitute the second most important component and finding in the tumor cells of our case, with acinar cell carcinoma was never explicity reported. Several studies have recently described pleomorphic, membrane bound inclusions that contain filaments in this respect: nine cases of acinar cell carcinoma of the pancreas[11, 18], in cases of malignant mixed exocrine-endocrine tumor of the pancreas[8, 12], in the neoplastic Paneth cells of a carcinoma of the ascending colon but not in the adjacent non-neoplastic Paneth cells[20] and in altered serous granules in acinar cells of human parotid gland[17].

For those who have written-off the discipline of TEM as a diagnostic technique (for discussion see Erlandson 1994[15]), this current case proves the contrary. Ultimately, the ultrastructural analysis of the metastasis of a clinically occult primary tumor provided the final or prompt diagnosis of an unusual acinar cell carcinoma. In addition, on the basis of the partition and the polarity and size of the zymogen granules within the tumor cells, the distinction from mixed acinar-endocrine carcinoma (MAEC), mixed pancreatic tumor with ductal differentiation and mucin production, islet cell tumor, or carcinoid, can be made by the electron microscopical study[12, 16, 14, 8]. Moreover, filamentous inclusions, as found in our case, have not been identified in the latter two forms of tumors[18].

◀ Fig. 1. Solid growth pattern of neoplastic cells with granular, eosinophilic cytoplasm and centrally located nuclei. H.E.; × 410. – Fig. 2. A glandular architecture (arrows) with dispered luminal spaces is focally present. H.E.; × 410. – Fig. 3. Apical staining for polyclonal cytokeratin is visible on immunohistochemical staining. × 410. – Fig. 4. Strong positive staining for CAM 5.2. × 410.

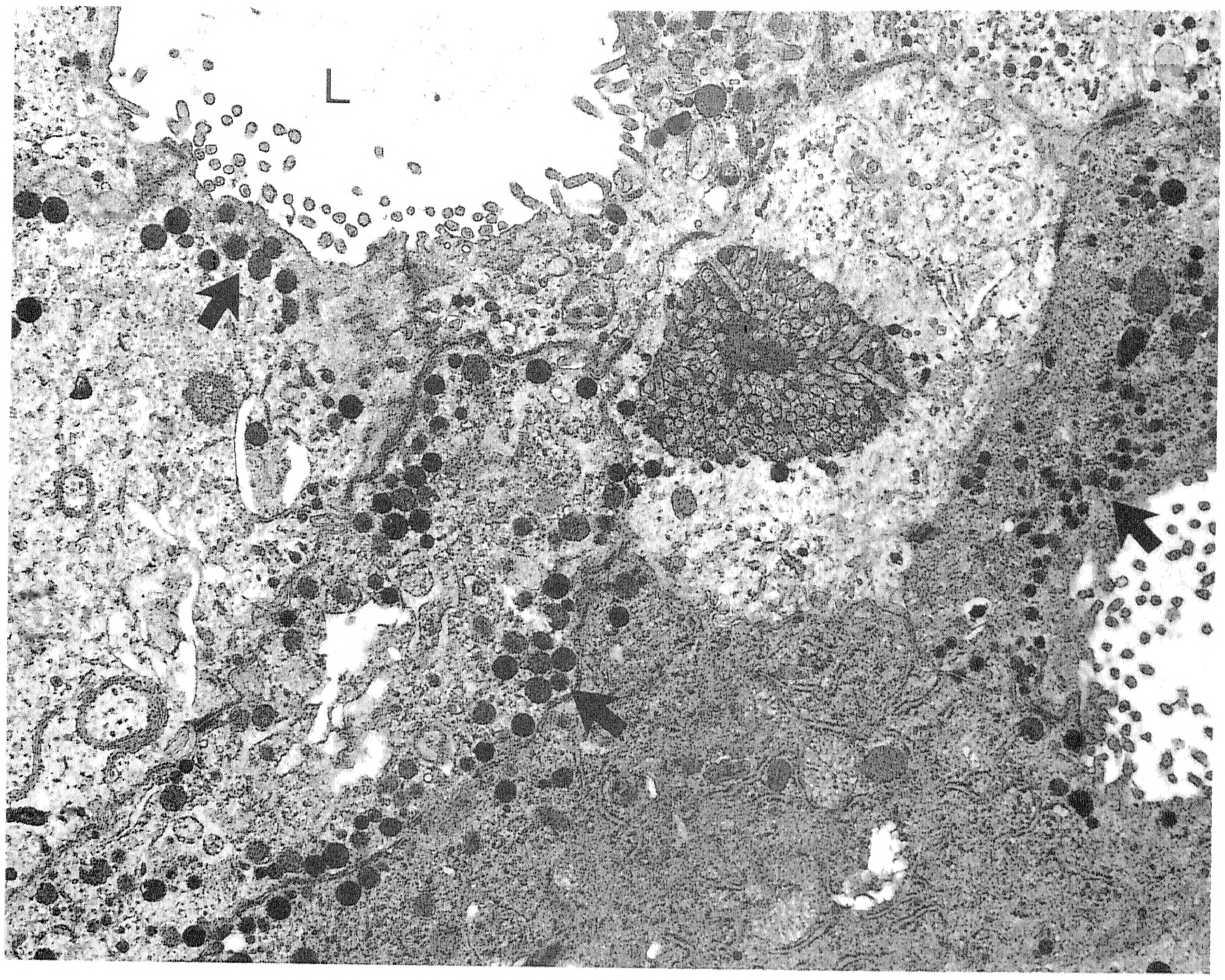

Fig. 6. Electron microscopic appearance of part of a glandular structure. The apical luminal (L) spaces are lined by short microvilli. Electron-dense zymogen granules (arrows) are congregated near the lumina and the lateral cell membranes. × 13 300.

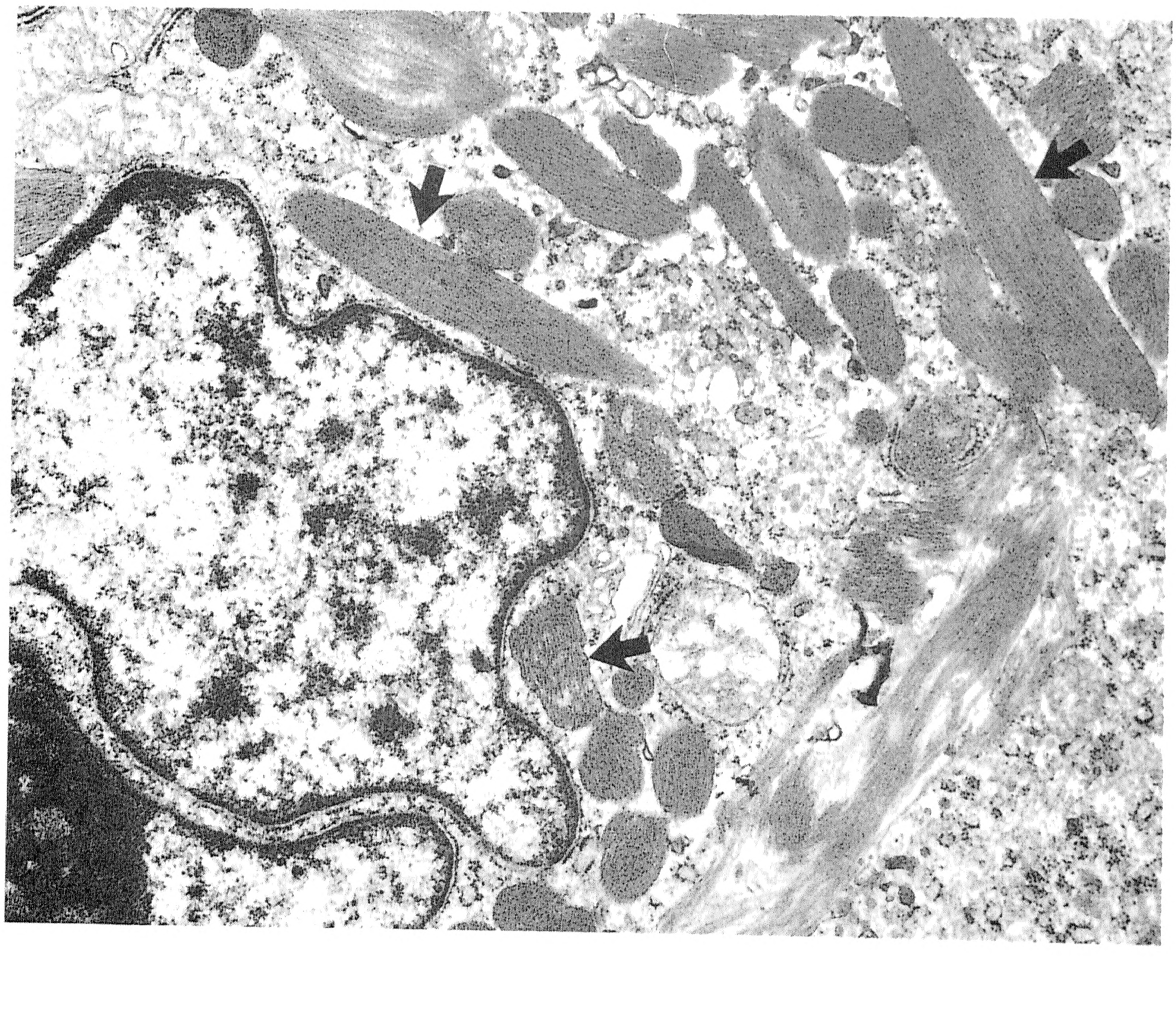

Fig. 7. Part of a neoplastic cell displays many irregular, mostly elongated granules (arrows) with fibrillary internal structure. × 18 700.

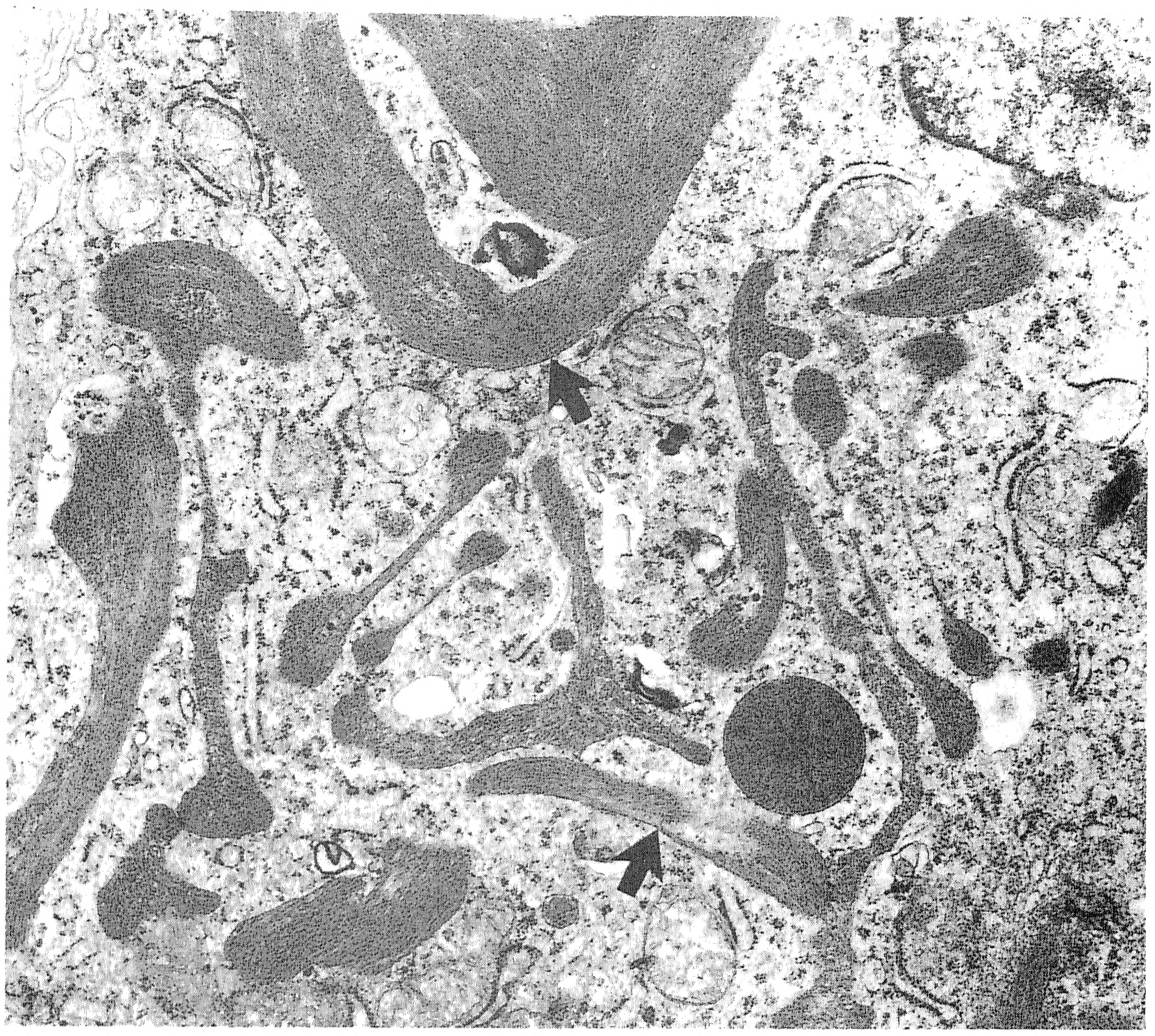

Fig. 8. Numerous fibrillary inclusions. A limiting membrane seems to surround partly some of the inclusions (arrows). × 16600.

The significance and genesis of the dense fibrillar inclusions has been a subject of debate. One could speculate that the polymerization of proteins form either filamentous or crystalline structures of various resident or secretory long-chain molecules (proteins or mucin) lying free in the cytoplasm. Many examples of such secretory products have been described in various normal and neoplastic tissues[9]. If so, this would cast doubt on the specific character of these inclusions. The assumption of crystallized proteins seems less probable if, as stated by Tucker et al.[18] and Hassan et al.[8], the inclusions are totally or at least partly membrane bound. There is no indication that the structures are located within the cisternae of the rough endoplasmic reticulum. Do they represent products of degeneration (e.g. of keratin filaments) or perhaps the products of an abnormal synthesis of keratin and keratohyaline[10] as is suggested for the filamentous bodies described in squamous cell carcinoma[19]? Tucker et al.[18], with well illustrated ultrastructural findings, stress the suggestion of an apparent transition from zymogen granules to those inclusions, or that the latter represent abnormal or deranged zymogen granules. The latter opinion is favoured by the rare labeling of the large irregular fibrillary granules with a chymotrypsin antibody as shown by immuno-gold electron microscopy[12]. On the other hand, how could this explain the view of the great variability in size or form of the bodies observed in different cells? The filamentous instead of lamellar or microtubular ultrastructure on cross-sections clearly demonstrated by Tucker et al.[18], speaks against the origin of so-called angulate lysosomes (formerly called the angulate bodies) as originally described by Dingemans et al.[2]. As it is known, the angulate bodies are an integral part of the ultrastructural appearance of so-called granular cell tumors and their presence is of major importance in the diagnosis of this tumor[1]. In our case there is no indication of metastases of a granular cell tumor as the primary tumor.

Unfortunately, autopsy was not permitted in our case excluding a more thorough examination of the pancreas. Theoretically, we should consider intestinal Paneth cells as the second best possibility for the cell of origin of this malignant tumor. This presumption stems from the fact that alongside zymogen type granules, neoplastic Paneth cells can contain bizarre cytoplasmic fibrillary inclusions. Moreover, their similarity to those associated with zinc deficiency or zinc metabolism disorder has been reported in normal intestinal Paneth cells in cases of acrodermatitis enteropathica[13, 15], in (metaplastic?) Paneth cells in chronic inflammatory bowel disease (e.g. Crohn's disease[3, 4]) and in experimental zinc deprivation. In our patient there was no direct clinical evidence of abnormal zinc metabolism. No further examination in this concern was performed after the diagnosis of metastatic disease was made.

Materials and Methods

A 65-year-old man was presented with vague pain in the right upper abdomen for approximately one month. Palpation, x-ray examination and computed tomography revealed a large retroperitoneal mass measuring 10 × 13 cm localized behind the stomach and duodenum and infiltrating the mesentery, right colon and inferior vena cava; serum lipase: 90 (nl. value: 10–190 U/l); amylase: 115 (nl. value: max. 65 U/l). At surgery, this tumor appeared to be irresectable. The pancreas was described as normal intraoperatively. A biopsy was taken of one of the enlarged para-caval lymph nodes. The patient died shortly after the operation. Autopsy could not be performed.

Tissue obtained at surgery was fixed in 10% neutral buffered formalin and embedded in paraffin for light microscopy. Sections were stained with hematoxylin-eosin, periodic acid-Schiff (PAS) with and without diastase pretreatment, mucicarmine and alcian blue. Immunohistochemical staining was performed on the sections of the same paraffin-embedded material for polyclonal cytokeratin, CAM 5.2, vimentin, CEA, NSE, PSA, S-100 protein, EMA, chromogranin, synaptophysin and muramidase. Small fragments were fixed in glutaraldehyde (2.5%), postfixed in osmium tetroxide (1%) and processed routinely for electron microscopy. The ultra-thin sections were stained with uranyl acetate and lead citrate and examined using a Siemens Elmiskop 101.

References

[1] Carstens PHB, Yacoub O (1993) Importance of angulate bodies in the diagnosis of granular cell tumors (schwannomas). Ultrastruct Pathol *17:* 271–278

[2] Dingemans KP, Mooi WJ, van den Bergh-Weerman MA (1983) Angulate lysosomes. Ultrastruct Pathol *5:* 113–122

[3] Dvorak AN (1980) Electron microscopy of Paneth cells in Crohn's disease. Arch Pathol Lab Med *104:* 393–394

[4] Dvorak AN, Dickersin GR (1980) Crohn's disease: Transmission electron microscopical studies. I. Barrier function. Possible changes related to alterations of cell coat, mucous coat, epithelial cells, and Paneth cells. Hum Pathol *11:* 561–571

[5] Erlandson RA (1994) Diagnostic Transmission Electron Microscopy of Tumors. Raven Press; New York

[6] Haelst van UJGM, Pruszczynski M (1990) Unusual intracytoplasmic inclusions in metastatic tumor cells in retroperatoneal lymph nodes of a man with unknown primary tumor. Case for the panel. Ultrastructural Pathol *14:* 101–106

[7] Hammar S, Bockus D, Remington F (1987) Metastatic tumors of unknown origin: an ultrastructural analysis of 265 cases. Ultrastruct Pathol *11:* 209–250

[8] Hassan MO, Gogate PA (1993) Malignant mixed exocrine-endocrine tumor of the pancreas with unusual intracytoplasmic inclusions. Ultrastruct Pathol *17:* 483–493

[9] Henderson DW (1990) Comments by the panel. Ultrastruct Pathol *14:* 102–106

[10] Ioachim HL (1990) Comments by the panel. Ultrastruct Pathol *14:* 102–106

[11] Klimstra DS, Heffess CS, Oertel JE, Rosai J (1992) Acinar cell carcinoma of the pancreas. Am J Surg Pathol *16:* 815–837

[12] Klimstra DS, Rosai J, Heffess CS (1994) Mixed acinar-endocrine carcinomas of the pancreas. Am J Surg Pathol *18:* 765–778

[13] Lombeck I, von Bassewitz DB, Becker K, Tinschmann P, Karstner H (1974) Ultrastructural findings in acrodermatitis enteropathica. Pediat Res *8:* 82–88

[14] Nonomura A, Kono N, Mitzukami Y, Nakanuma Y, Matsubara F (1992) Duct-acinar-islet cell tumor of the pancreas. Ultrastruct Pathol *16:* 317–329

[15] Polanco I, Nistal M, Guerrero J, Vazquez C (1976) Acrodermatitis enteropathica, zinc, and ultrastructural lesions in Paneth cells. Lancet *i:* 430

[16] Schron DS, Mendelsohn G (1984) Pancreatic carcinoma with duct, endocrine, and acinar differentiation. A histologic, immunocytochemical, and ultrastructural study. Cancer *54:* 1766–1770

[17] Tandler B, Riva A (1990) Altered serous granules in acinar cells of a human parotid gland. Ultrastruct Pathol *14:* 11–19

[18] Tucker JA, Shelburne JD, Benning TL, Yacoub L, Federman M (1994) Filamentous inclusions in acinar cell carcinoma of the pancreas. Ultrastruct Pathol *18:* 279–286

[19] Tuma B, Damjanov I (1988) Case for the panel: Filamentous bodies in a squamous cell carcinoma. Ultrastruct Pathol *12:* 667–671

[20] Watson PH (1990) Fibrillary cytoplasmic inclusions in neoplastic Paneth cells. Histopathology *16:* 69–74

Received January 20, 1995 · Accepted April 10, 1995

Key words: *Filamentous inclusion bodies – Metastasis – Acinar cell carcinoma – Pancreas – Paneth cells-ultrastructure*

Prof. Dr. U. J. G. M. van Haelst, Department of Pathology, Medical Faculty, University of Nijmegen, Geert Grooteplein Zuid 24, 6525 GA Nijmegen, The Netherlands