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Light and Electron Microscopic Investigations of Nasopharyngeal Carcinomas with Regard to the Viral Etiology of these Tumors*

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Summary. Five carcinomata of the nasopharynx (four lymphoepithelial carcinomata of the Regaud type and one squamous cell carcinoma) were examined light and electron microscopically. In addition to the familiar histological and cytological features of these tumors, and because of an increased antibody titer against Epstein-Barr virus in all five patients, all those cytoplasmic and nuclear inclusions were examined which could be interpreted as indicative of a virus contact.

The following structures were found:

1. Particles and microtubules which correspond in diameter, shape, and location to Corona viruses.

2. Particles surrounded by a double membrane and resembling in form and diameter Oncorna viruses.

3. Tubulo-reticular, coil-shaped cytoplasmic inclusions interpreted as an unspecific reaction of the host cell to viral attack.

4. Spherical nuclear bodies, which are frequently observed in tumors and in viral infections.

5. Intranuclear particles which correspond in diameter, structure, and distribution to viruses of the herpes type such as have been described in cell cultures of Burkitt lymphoma and nasopharyngeal carcinoma.

The fifth group particularly was discussed in detail with regard to differentiation between those particles and other structures which could simulate a virus structure.

Together with the appearance of increased ribosomes and of particular chromatin distribution within the tumor cell nuclei, the particles we discussed have been interpreted as morphological indications of a virus etiology of the examined tumors.

Key words: Lymphoepithelial Carcinoma – Corona-Viruses – Tubulo-reticular inclusions – Oncorna-like viruses – Nuclear bodies – Herpes-like viruses

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Carcinomata of the nasopharynx occur in greatly varying geographic locations. This fact has led to much speculation about the significance of racial-genetic, hormonal, and exogenous pathogenetic factors.

There exist a large number of casuistic publications and general reports concerning the morphological differentiation of nasopharyngeal carcinoma. Since the first descriptions of lymphoepithelial carcinoma of the tonsils and in the nasopharynx put forth by Schmincke [63], Regaud and Reverchon [60], and Reverchon and Coutard [61], the transitional cell carcinoma, defined for the first time by Quick and Cutler [56], as well as anaplastic carcinoma or indifferent cell carcinoma have been classified separately from keratinizing or non-keratinizing squamous cell carcinoma of the nasopharynx [13, 17, 18, 64]. Yeh [72], after having evaluated 1000 biopsies, rejected the definition of lymphoepithelioma as a separate entity and classified them as transitional cell carcinomata which infiltrate local lymphatic tissue. In concurrence with Wang et al. [70], Yeh could see no better healing process or prognosis for nasopharyngeal carcinoma with lymphoepithelial differentiation. In contrast to these observations, von Ilberg et al. [41] ascertained that different clinical processes and a better prognosis exist for lymphoepithelial carcinomata than for other nasopharyngeal carcinomata. Chiang and Jung [14], who classified 159 cases as squamous cell carcinoma, transitional cell carcinoma (lymphoepithelial type carcinomata were included in this category), or anaplastic carcinoma, saw no correlation between histological type and tumor spread.

The first electron microscopic investigations of nasopharyngeal carcinomata were carried out by Svoboda et al. [68] in American and Chinese patients. They were able to identify with the aid of electron microscopy the squamous epithelial components of these tumors; in their tumor differentiation they found no distinctions based on race. Döhnert [19] in a comprehensive study using light microscopic, cytochemical, electron microscopic and immunological methods of investigations as well as tissue cultures and taking into consideration the clinical processes and therapy results, could point out clearly, that a lymphoepithelioma of the nasopharynx is a carcinoma which represents an independent tumor entity with special histological and biological features. An early infiltrative growth and a rapid formation of lymphogenic metastases are prominent characteristics of the spreading of these tumors.

As causal factors immunologic defects are being discussed as well as genetic and hormonal factors [58, 73]. Since increased antibody titers against antigens from Epstein-Barr virus were detected in numerous patients with nasopharyngeal carcinoma [15, 36, 42, 58, 67, 71, 75], viruses have been increasingly often discussed as the triggering agent of these tumors. An interaction of the carcinogens and dispositional factors involving to some extent the simultaneous presence of different viruses, have also been mentioned in this regard.

The electron microscopic evidence of various intracytoplasmic and nuclear particles present in the nasopharyngeal carcinoma with predominantly lymphoepithelial structures of five patients having positive antibody titers against Epstein-Barr virus antigens has led to present report.

Material and Methods

The titer assessments of the five patients under examination were gratefully carried out by Prof. Dr. H. Zur Hausen/Freiburg.

Tumor tissue specimens from patients with a histologically identified carcinoma of the nasopharynx were excised again for study under the electron microscope. Fixation occured by immersion in 3.6% buffered glutaraldehyde and then in 1% buffered osmium tetroxide. The material, fixed, and contrasted en bloc with uranyl acetate was dehydrated and then embedded in Epon. The thin sections were contrasted with lead citrate.

For the light microscopic examinations mainly semi-thin sections of the Epon embedded material were used. For purposes of comparison, paraffin sections were used treated with the following stains: hematoxylin and eosin, Giemsa, PAS, ironhematoxylin-picrofuchsin van Gieson combined with resorcin; in addition, silver impregnation according to Gomori was applied.

Findings

In all five patients the serum titer analysis relative to EBV IgG/IF showed distinctly elevated values (1:2048).

Light Microscopic Finding

Semi-thin sections proved to be of value for the tissue examinations because they made possible a better definition between epithelial tumor components and lymphatic elements and moreover allowed a differentiation within the lymphatic tissue between lymphocytes, immunocytes, and immunoblasts. Four of the examined tumors presented the typical picture of a lymphoepithelioma of the Regaud type with development under the epipharynx epithelium, tendency to spread downwards into the adjacent connective tissue and a relatively sharp definition between the epithelial part of the tumor and lymphatic tissue (Fig. 1 a). The tumor cells were arranged in the form of wide bands with pointed extensions. They were often bordered by a row of narrow connective tissue cells, not however by a distinct basal membrane. Within the cell bands the atypical squamous epithelium was characterized by the following: cytoplasm of varying extent with occasional marked lightening of the cytoplasmic ground substance, polymorphous, chromatin dense nuclei, as well as some large and bubble-like nuclei and the presence of one or two large dark nucleoli (Fig. 1 a, b). Cells with two nuclei or with a single huge nucleus also occured. Mitosis with partly atypical figures were frequent (Fig. 1 a). Only single lymphocytes and immunocytes could be recognized directly between the epithelial tumor cells. The lymphatic tissue beside the epithelial bands consisted for the most part of immunocytes (Fig. 1 a). Only in one case, an epithelium type having spindly extensions had developed in places (Fig. 1 b).

A squamous cell carcinoma with low differentiation and in which the atypical squamous cell strands greatly outweighed the lymphatic tissue was in contrast to the four lymphoepithelial carcinomata described above. In places, only fibrocytic stroma had developed between the tumor cell bands (Fig. 1 c). Keratinisations could not be detected with the light microscope. In places, a distinction from the smaller cell type of the transitional cell carcinoma was difficult to make.

In all five cases, the spreading was similarly downwards, the surface epithelium of the epipharynx remained substantially intact.

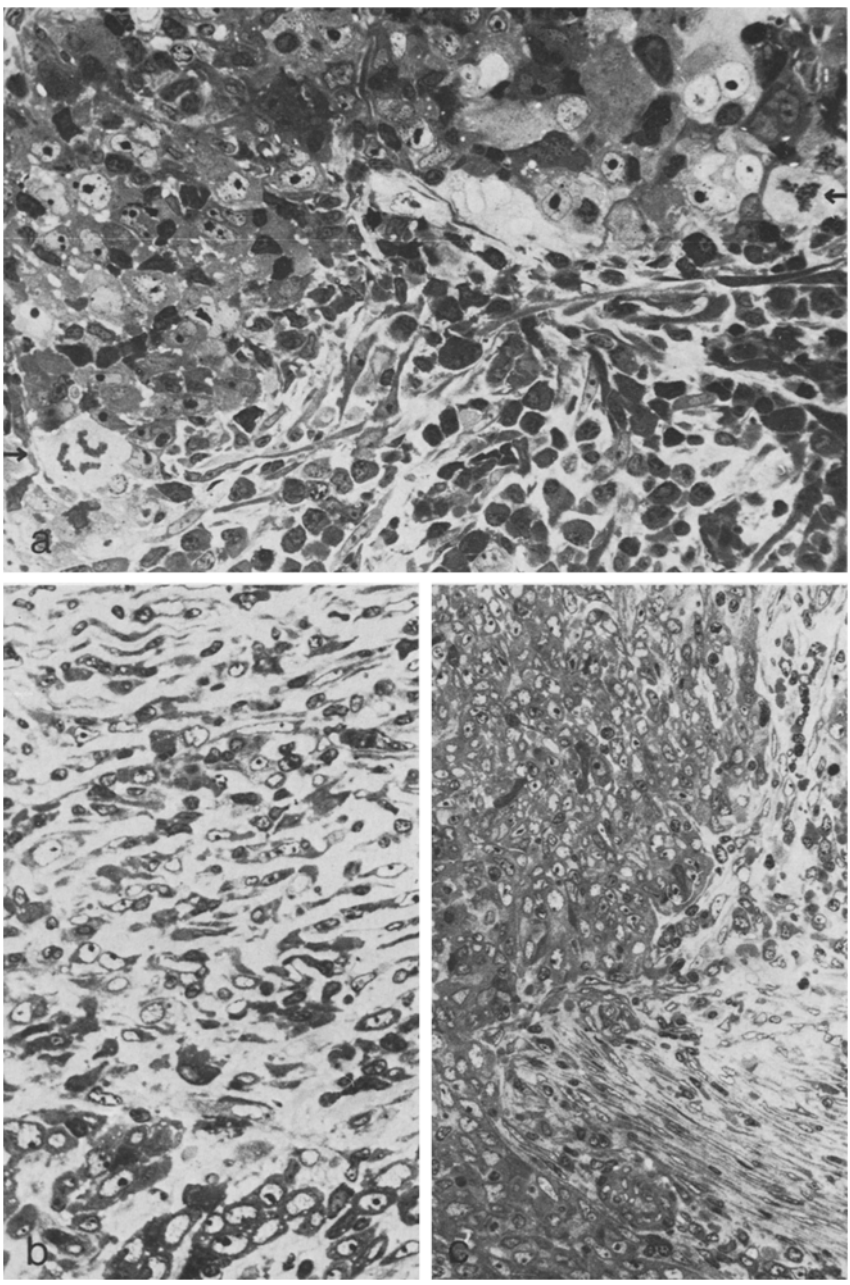

Fig. 1 a. Lymphoepithelial carcinoma of the Regaud type with wide strands of a polymorphous atypical epithelium as well as stroma infiltrated by immunocytes and lymphocytes. In the epithelial complexes atypical mitotic figures (arrows). Semithin section, 400×. **b** Spindle cell marking of the epithelium in a lymphoepithelial carcinoma. Semithin section, 400×. **c** Low differentiated squamous cell carcinoma with fibrocytic stroma and sparse lymphocytic reaction in the stroma. Semithin section, 250×

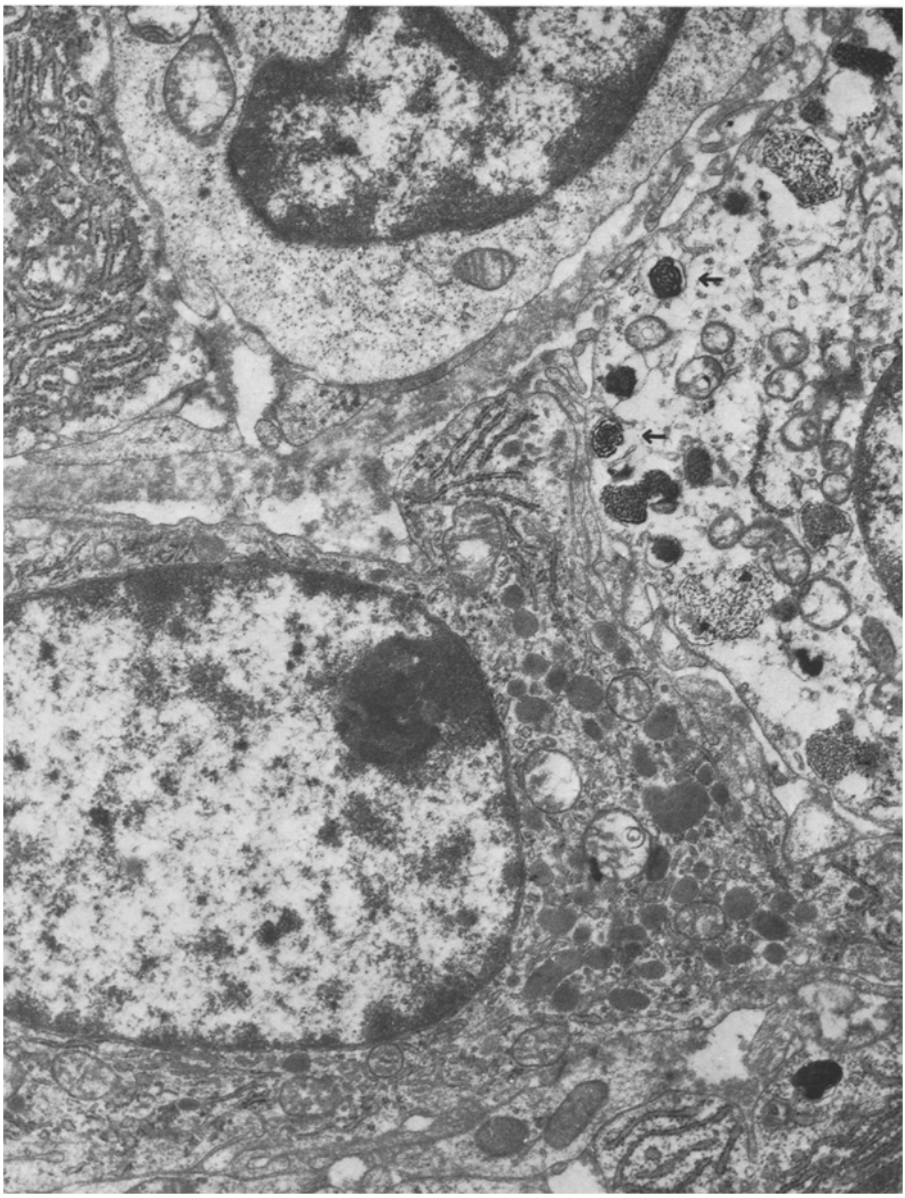

Fig. 2. Two epithelial tumor cells, one part of an immunocyte, and a mast cell with typical granules (arrows). Basal membrane like material in the interstitium. 23 500 ×

Electron Microscopic Findings

Also upon electron microscopic examination of lymphoepithelial carcinoma of the Regaud type is it possible to clearly distinguish the large epithelial cells with their projecting cytoplasm and large light nuclei from the surrounding immunocytes, lymphocytes, and mast cells (Figs. 2–4). Occasional interdigitations of tu-

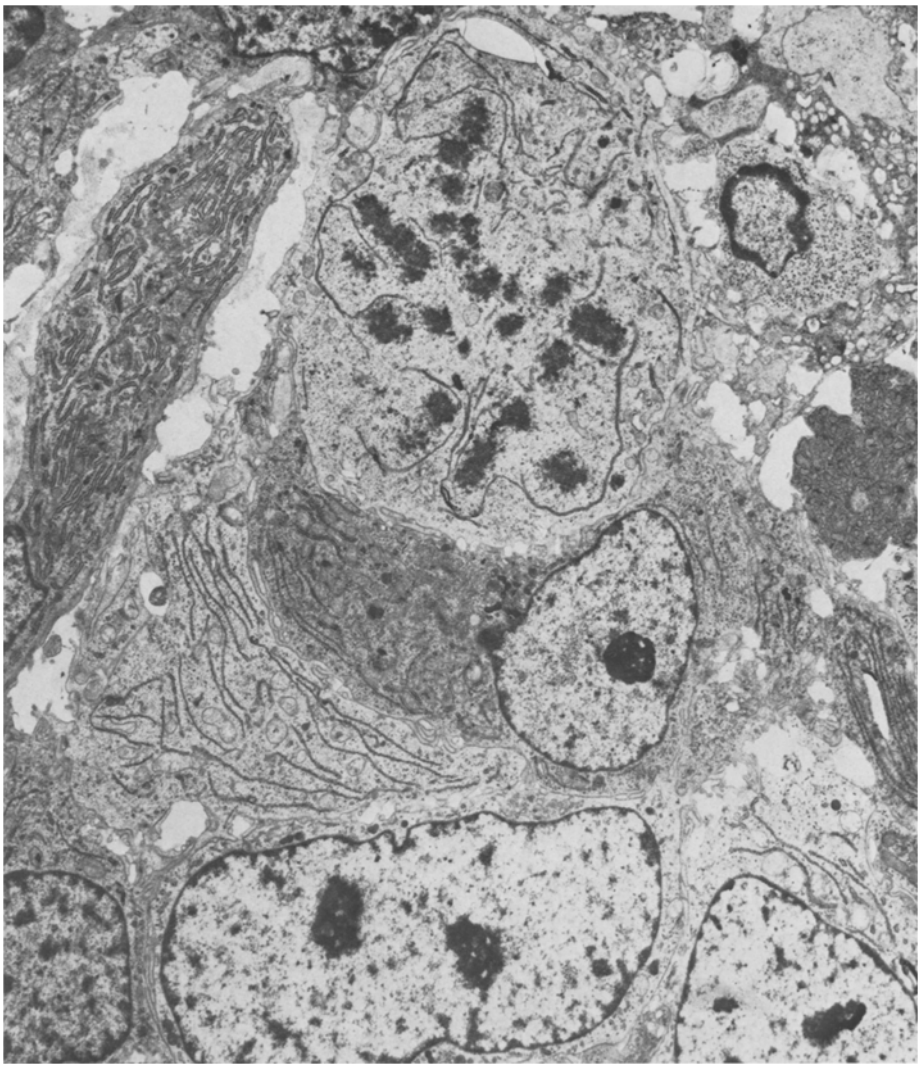

Fig. 3. Tumor cells with mitosis in close contact to immunocytes. 14 500 ×

mor cells appear. In other places, the interstitium is relatively wide and streaky structures resembling basal membrane material (Fig. 4) with concentrations near the cell membrane are found there as well as collagenous fibrils. The tumor cells can be in very close contact with the surrounding immunocytes, lymphocytes, and mast cells with their typical mature granules (Fig. 2). The tumor vessels are uncharacteristic.

Structures Within the Cytoplasm of the Tumor Cells

The cytoplasm of the epithelial tumor cell has, in places, enlarged ergastoplasmic tubules, in which lie, together with a dense homogenous to fine granular content, isolated myelin figures, which also appear in nuclear sections (Fig. 5a) and in

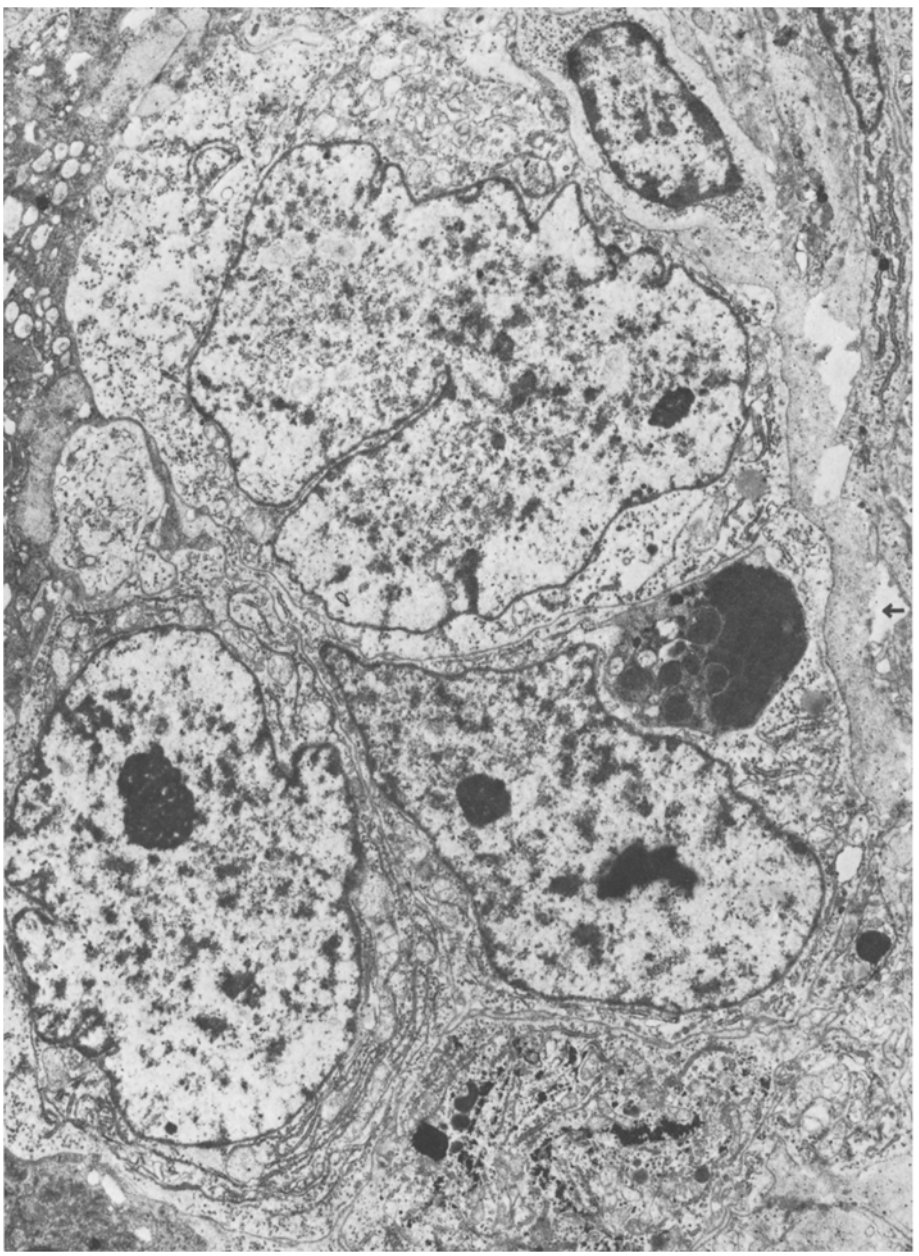

Fig. 4. Tumor cell complex with characteristic light polygonal nuclei, rich in ergastoplasm, and surrounded by a basal membrane like material (arrow). 14500 ×

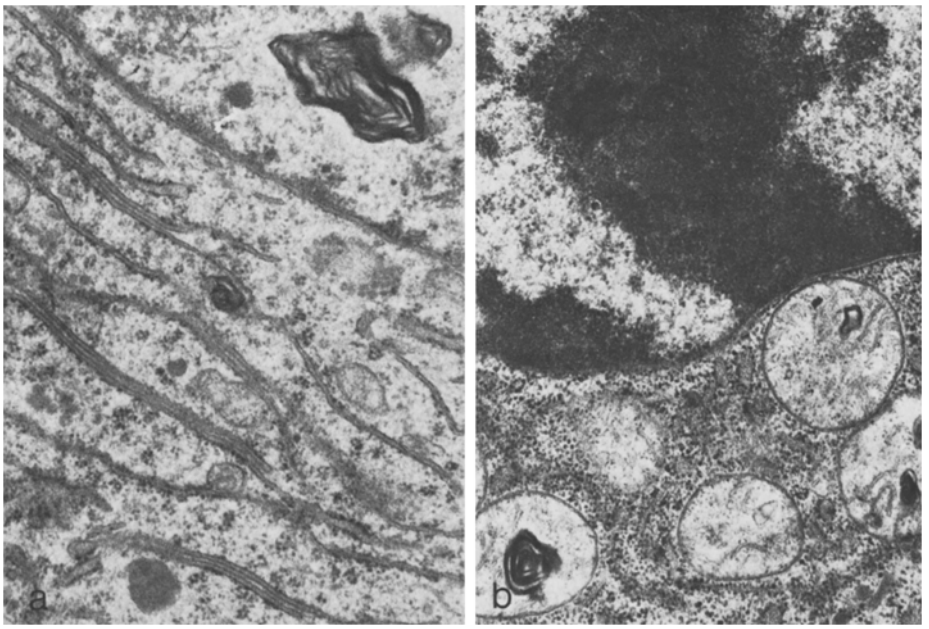

Fig. 5. a Nuclear section of a tumor cell with myelin figure. In the cytoplasm doublings of the ergastoplasmic membranes. 23500 b. **b** Tumor cell section with myelin figures in mitochondria. 23500 ×

mitochondria (Fig. 5 b) of the tumor cells. They are surrounded by many double figures of the endoplasmic reticulum (Fig. 5 a) which are studded with ribosomes only along the outer membrane lines. In addition, there appear numerous free ribosomes and, to some extent, ribosomal groups in the form of rosettes. Other coil-like, fine filamentary structures in the cytoplasm of the tumor cells reveal no relationship to other membranes. Moreover, the tumor cells contain some special cytoplasmic inclusions which we would like to divide into three groups:

(a) Short tubular formations, often with a dumb-bell shaped appearance, are regularly observed in the tumor cells, sometimes lying free in the cytoplasm, sometimes surrounded by membranes of the endoplasmic reticulum (Fig. 6 b, d). Also, particles with a double membrane and a diameter of 80–160 nm (Fig. 6 a, c) can be found lying in the form of small groups freely in the ground substance or in enlarged tubules of the ergastoplasm. Many of these particles are studded on their surface with very fine "granules". Particles of this size range also appear frequently between the microvilli of the overlying mucous membrane cells, mainly on the transitional epithelium. These particles, some roundish, some tubular correspond therefore in their structure and localisation to the Corona viruses.

(b) A second cytoplasmic particle type is also found either free in the ground substance (Fig. 7 a) or surrounded by membranes (Fig. 7 b), but, never within the ergastoplasm. The particles can be clearly distinguished from Golgi vesicles or multivesicular bodies. Their diameter is a constant 60 nm, in which respect they differ from the Corona viruses. They have a double membrane, of which the inner ring is thicker than the outer, they often reveal a dense core.

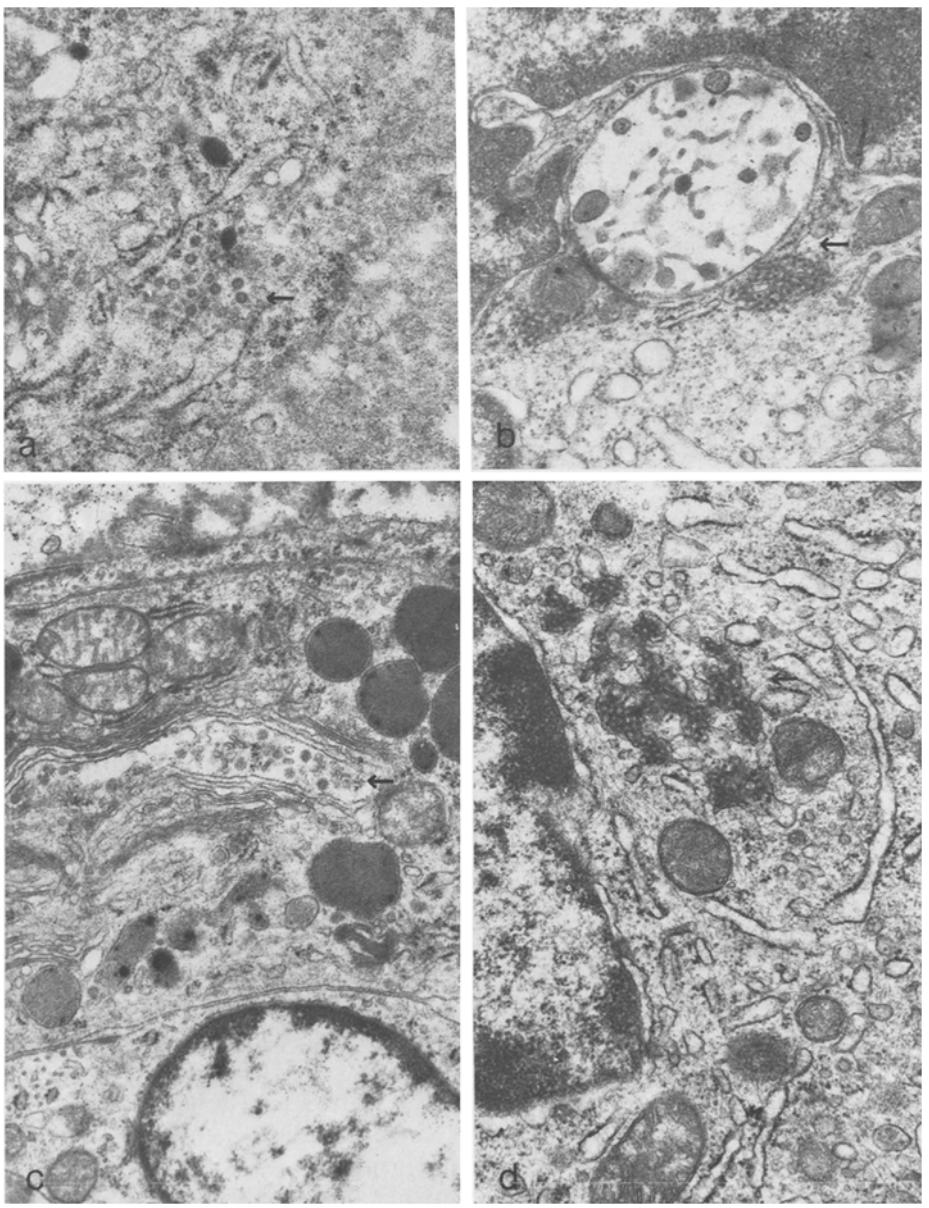

Fig. 6.a Corona viruses with typical radiating surface in the cytoplasm of a tumor cell (arrow). 23 500×. **b** Corona viruses in the form of tubular conglomerations beside a large vacuole with dumbbell shaped particles (arrow). 23 500×. **c** Typical Corona viruses in enlarged cisterns of the ergastoplasm of a tumor cell. 23 500×. **d** Paracristaline form of Corona viruses in enlarged cisterns of the ergastoplasm (arrow). 23 500×

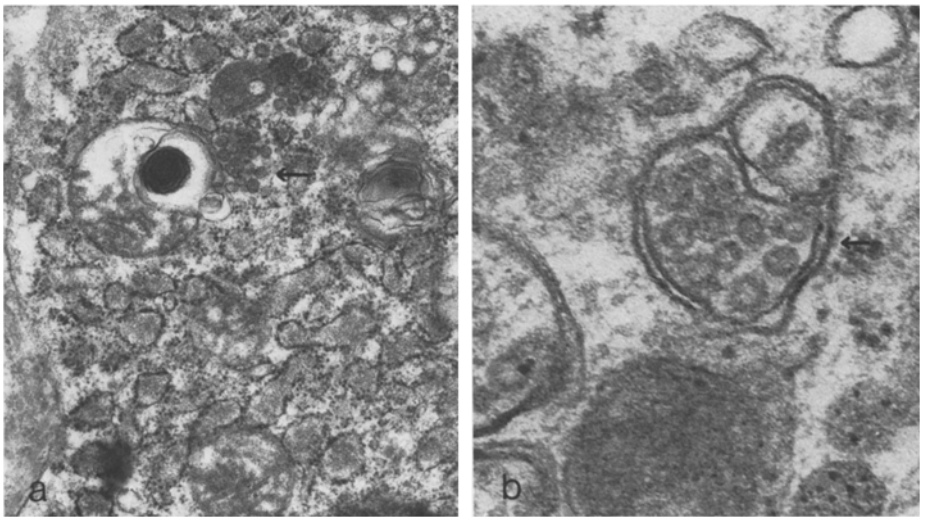

Fig. 7. a Groups of equal sized small vesicular particles surrounded by a double membrane and lying freely in the cytoplasm of a tumor cell (arrow). 23500×. **b** A 75000× enlargement of the same particles within a vacuole (arrow)

(c) In the cytoplasm of tumor cells but also in the cells of blood vessel walls are found complexes of intertwinned tubules, whose diameter measures 12–15 nm. These complexes are surrounded by ergastoplasmic membranes (Fig. 8). The fine tubular coils show a great similarity to tubulo-reticular structures which are often found in lymphocytes and adventitial cells of virus infected tissue and in lupus erythematosus.

The cytoplasmic structures within the cell strands of the low differentiated squamous cell carcinoma differ distinctly from those of the previously described lympoepithelial carcinoma cells. There appear numerous bundles of tonofilaments (Fig. 9a), desmosomal connections to neighboring cells (Fig. 9a) and also tight junctions. Occasionally, numerous keratohyalin granules can be detected in the cytoplasm of these cells (Fig. 9b).

Nuclei of the Tumor Cells

As the electron microscopy showed no substantial differences between the nuclear structures in carcinoma cells of the lymphoepithelial tumors and the squamous cell carcinoma, the findings will be presented jointly.

In contrast to the dark nuclei of the surrounding lymphocytes and the more coarse, lumpy chromatin of the adjacent immunocytes, the tumor cell nuclei are large and considerably paler; concentrations of chromatin are found only along the edges of the nuclear membrane. Large nuclear pores with a diameter of up to 480 nm are observed in those tumor cells with a closed nuclear membrane (Fig. 10b). In these pores often lie rosettes of ribosomes which correspond to a release from the nucleoplasm. Occasionally, extensions of the outer nuclear membrane to the cell membrane are visible (Fig. 10a). In mitotic dividing structures,

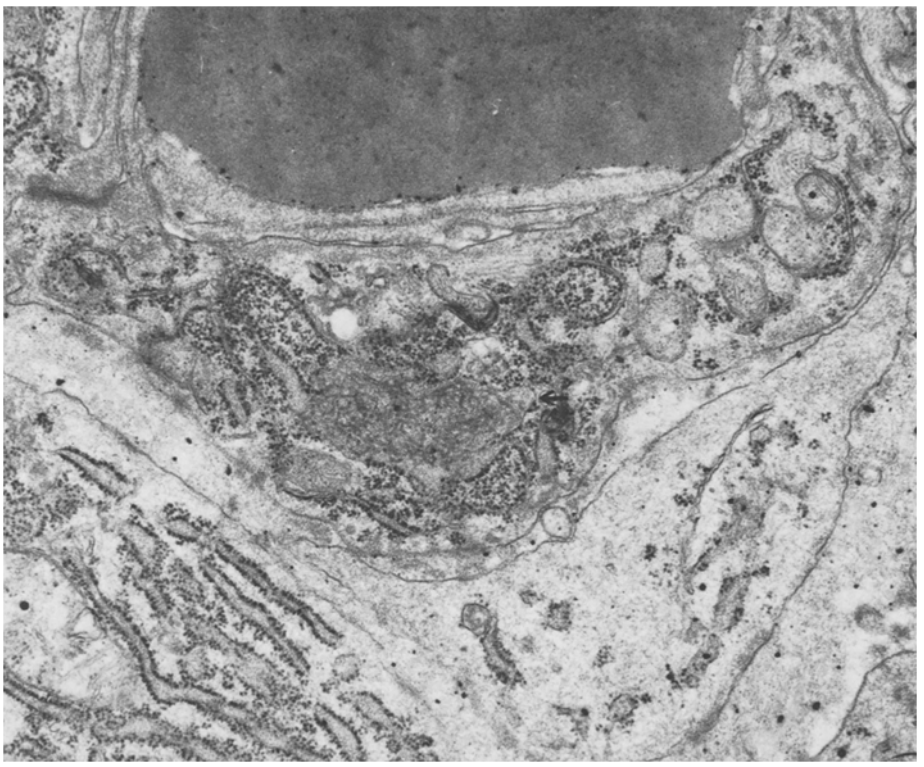

Fig. 8. Tubulo-reticular aggregation within enlarged ergastoplasm of a blood vessel wall cell (arrow). 23 500 ×

lumpy chromosomal concentrations partially enclosed by osmiophilic double membrane fragments are found irregularly distributed in the cytoplasm (Fig. 3). In the late anaphase and in the telophase it is also possible to observe between the membraneless or only partially enclosed chromatin conglomerations, radiating formations of elongated tubules with a diameter of 12–18 nm and sections of the centriol, both resembling mitotic spindles (Fig. 11). In telophase nuclei (Fig. 12) but also in other nuclear sections of the tumor cells, round particles surrounded by a light area are conspicuous. They have a double membrane and in many cases contain a core. These particles occur in approximately every third to fifth tumor cell. They appear spread diffusely about the nucleoplasm as well as in concentrations in the vincinity of the nuclear membrane. Even when they are situated between the nuclear membrane and the concentrations of chromatin, they exhibit a double membrane and a denser core. Occasionally they are located in groups near the nucleus (Fig. 13). They are uniform in size (Figs. 12, 14). The outer diameter of the particles measures approximately 100 nm. The inner ring diameter measures 18 nm and that of the central core 24 nm. The distance between the inner and outer membrane of the particles is a constant 12 nm.

In the nuclei of the squamous epithelium carcinoma cells, the same particles are present as well as spherical bodies and isolated myelin figures (Fig. 5a).

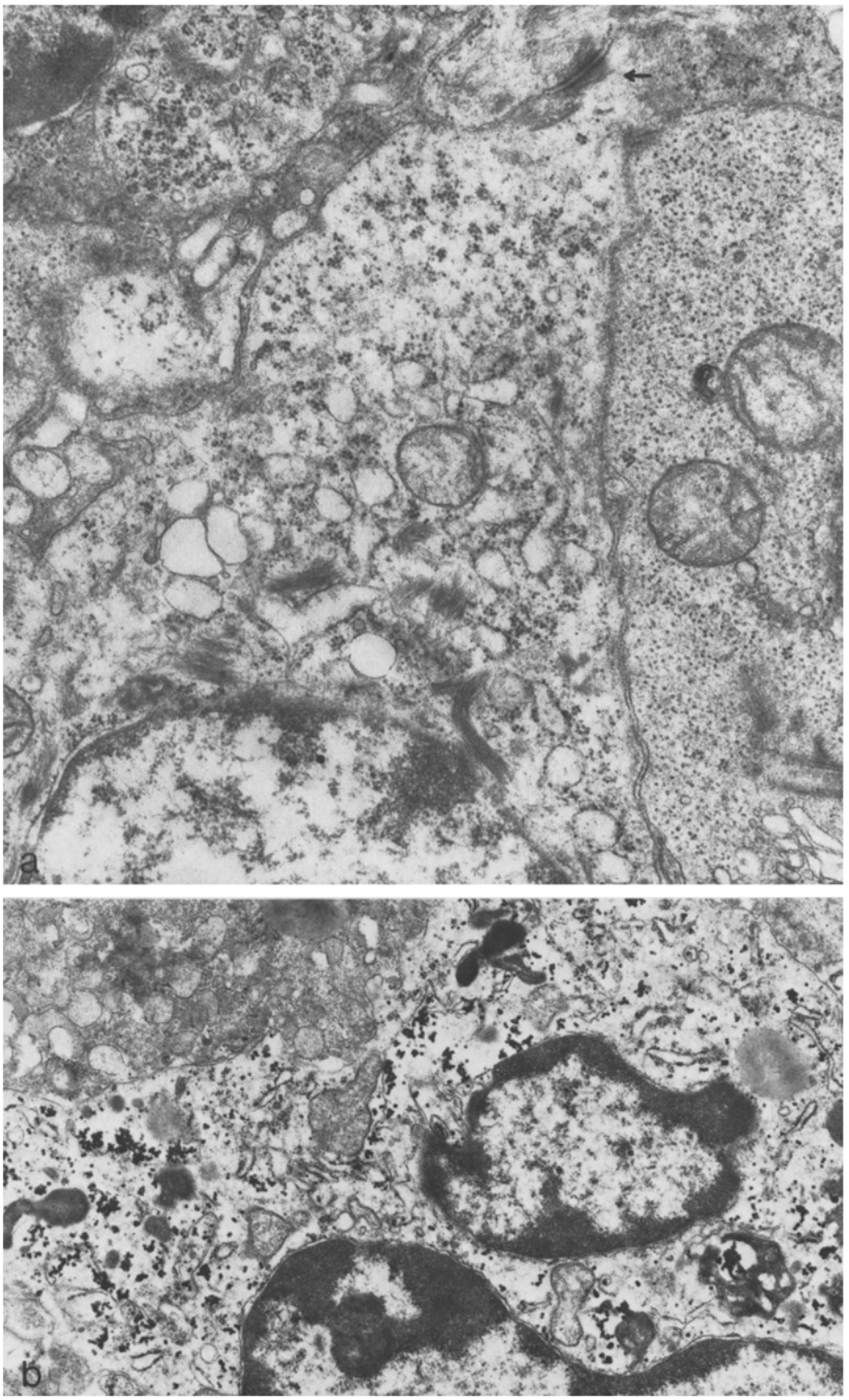

Fig. 9.a Squamous epithelial carcinoma cells with characteristic bundles of tonofilaments and desmosomal connections to the next cell (arrow). 23 500×. **b** Squamous epithelial carcinoma cell with keratohyalin granules. 14 500×

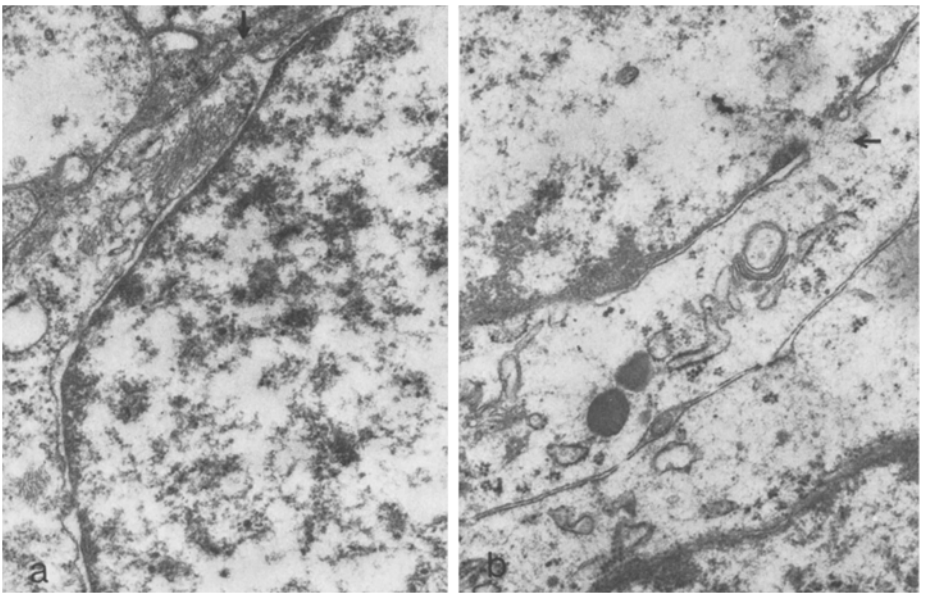

Fig. 10. a Tumor cell nucleus with wide nuclear pores and extension of the perinuclear cistern reaching to the cell membrane (arrow). 23 500×. **b** Tumor cell nucleus with wide nuclear pore (arrow) and polyribosomes in the pore opening. 23 500×

Discussion

In light microscopy the lymphoepithelial carcinoma displayed bands of a polymorphous epithelium with either large bubble-like or somewhat smaller chromatin dense nuclei as well as numerous, partly atypical mitoses between a marked lymphocytic-immunocytic stroma. In the squamous cell carcinoma a more fibrocytic stroma with only few lymphocytes and immunocytes had developed between the solid epithelial strips.

Upon electron microscopic examination certain histological and cytoplasmic characteristics emerged which supplement the first submicroscopic investigations concerning these tumors reported before [19, 30, 46, 51, 67, 68]. In the case of the lymphoepithelial carcinoma, the tumor cells showed varying features; besides interlocking structures of tumor cells wider interstitial gaps with a basal membrane like substance and collagenous fibrils were observed. The immunocytes with their marked ergastoplasm frequently lay cap-like against the epithelial elements. The occasionally enlarged ergastoplasm of the tumor cells contains isolated myelin figures, doublings of the membranes also appeared. Coil-like, intertwinned, fine filamentary cytoplasmic inclusions could not be interpreted. In mitotic figures, even during the telophase, extended spindle forms with extraordinary long tubules could be observed. Those particle inclusions which could be easily distinguished from multivesicular bodies will be reserved for the second part of the discussion. In the low differentiated squamous cell carcinoma there also appeared many short bundles of tonofilaments and occasional keratohyalin

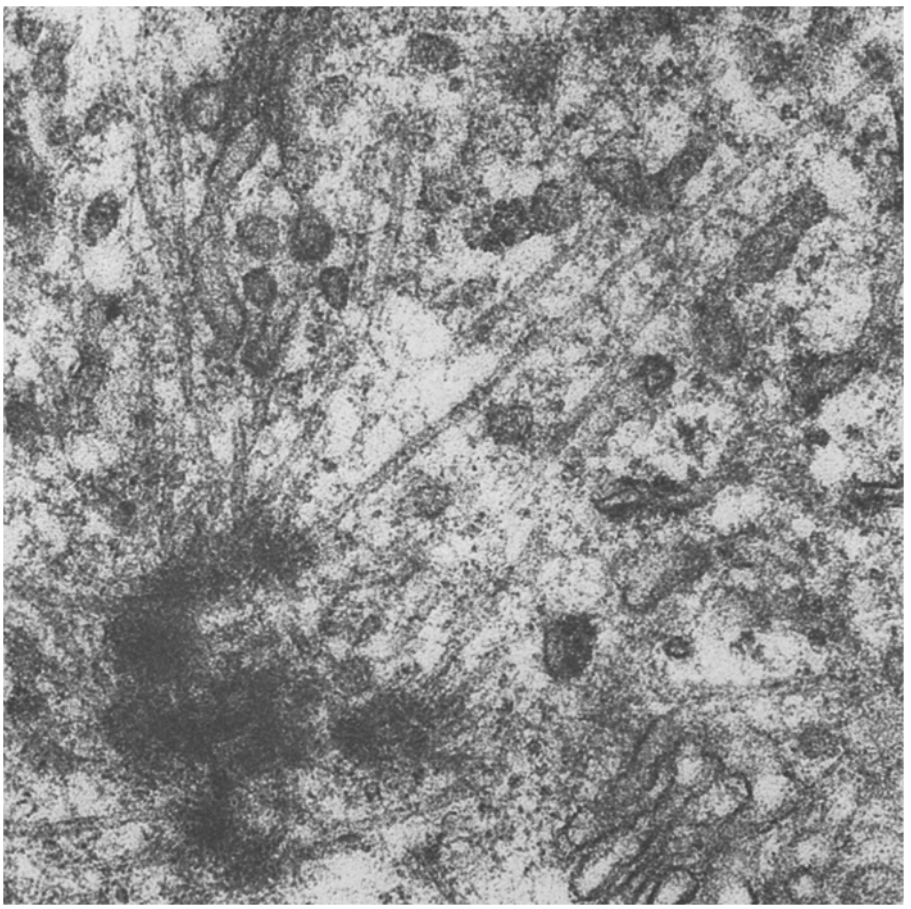

Fig. 11. Magnification of a centriol section with radiating mitotic spindles. 85000×

granules. Moreover, the cells of the atypical squamous epithelium often exhibited desmosomal connections and infrequent tight junctions. Such interconnecting structures rarely occured among the cells of the lymphoepithelial carcinoma.

The carriers of the five carcinomata examined electron microscopically showed an increased antibody titer against the Epstein-Barr virus, which is regarded as significant for the growth of nasopharyngeal carcinoma, although such titer increases have also been determined in Burkitt lymphoma, infectious mononucleosis and leukemia. Schmauz et al. [65] saw themselves in a position to estimate the therapeutic success for nasopharyngeal carcinoma by considering the different titer levels. This control possibility appears all the more significant due to the fact that is difficult to detect the spreading of these carcinomata by clinical-morphological methods as they frequently show primary invasive growth and no ulceration of the surface mucous epithelium.

The morphological findings which pathogenetically link up an EBV infection with human tumors are based on the evidence of particles similar to herpes virus,

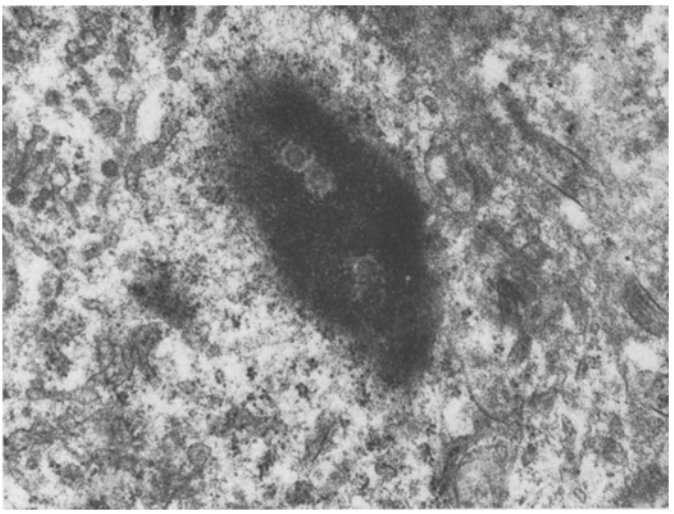

Fig. 12. Tumor cell nucleus with herpes virus like particles, some with central core. 23 500 ×

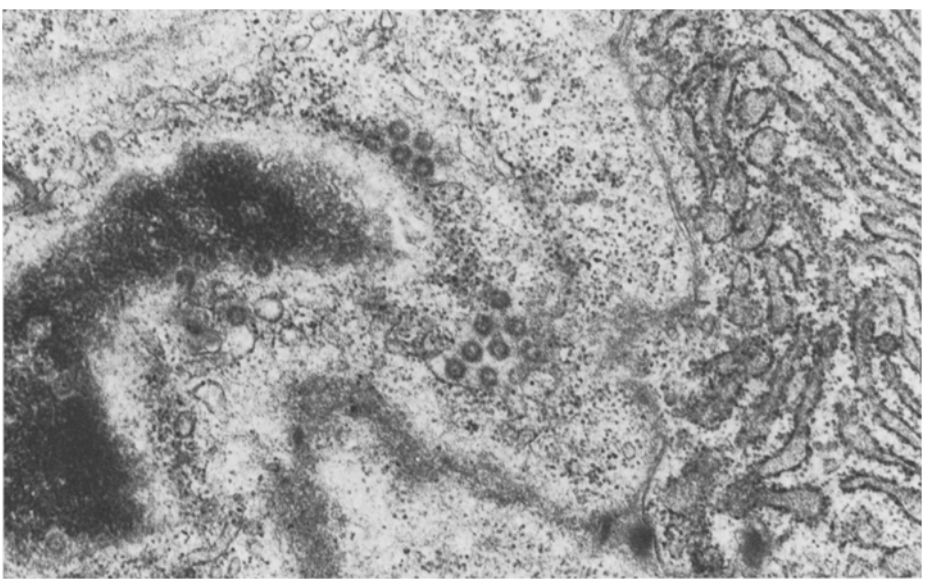

Fig. 13. Tumor cell nucleus with virus like particles lying in the nucleoplasm and on the periphery of the nucleus, most with a central core. 23 500 ×

which were found with the aid of electron microscopy in transformed lymphocytes from tissue cultures of Burkitt lymphoma and of nasopharyngeal carcinoma. With the exception of one report [52] there exists up until now no morphological evidence of virus particles in biopsy material of the nasopharyngeal carcinoma. This absence of morphologically detectable virus particles has been explained in various ways: According to Epstein (1962) as well as to Rapp and

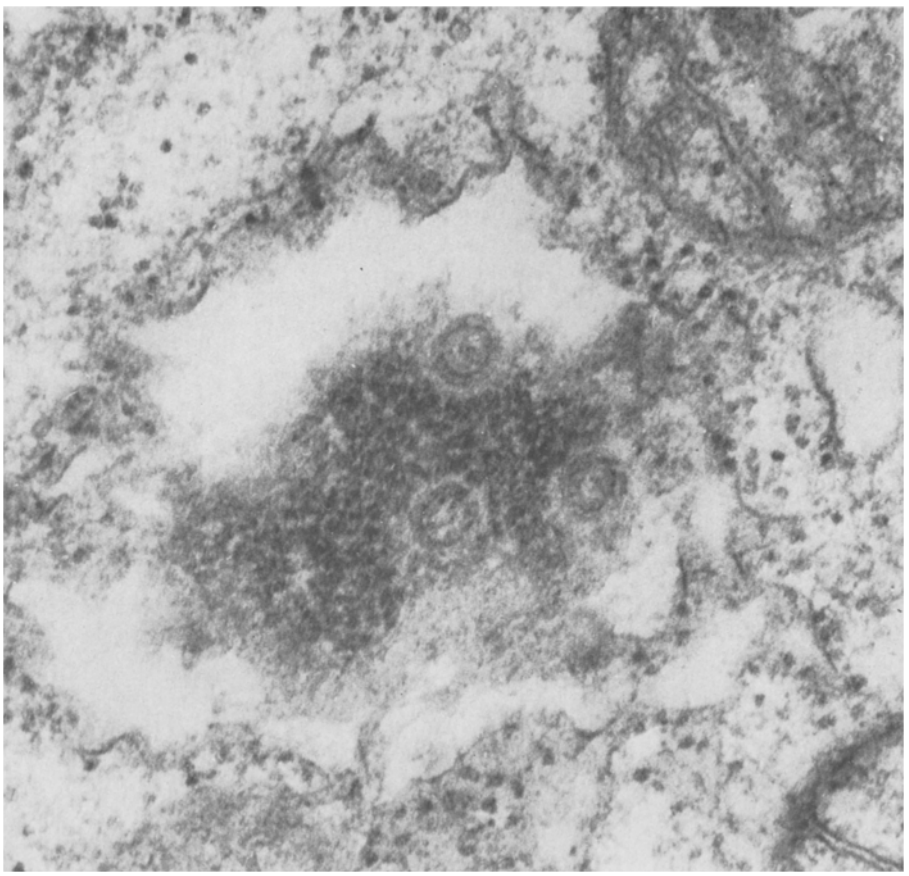

Fig. 14. Magnification of a tangential nuclear section of a tumor cell with three particles resembling herpes virus particles. 83000×

Duff [57], the infection with the oncogenous virus brings about a malignant transformation of the normal cell, with incorporation of the viral genom into the cellular DNA, whereby a neoplasm without viral replication is induced. An increased serum antibody titer against Epstein-Barr virus is one of the "fingerprints" of the virus contact. A viral capsid protein is suspected as antigen [33, 36]. However, the fact that sufficient capsid protein is subsequently produced to maintain the serum antibody level or cause it to increase, is not explained by this theory. But, apart from this, there exist examples of neoplastic diseases in animals as well as in humans triggered by DNA viruses in where viral particles, including those of the herpes type have been found in tissue [2, 5, 12, 20, 24]. Nadol [52] explained the lack of morphological virus evidence in tissue from nasopharyngeal carcinoma by stating that the concentration of viral particles in tumor tissue is so minimal that they cannot be detected by electron microscopy. Moreover it is possible that the sensitive DNA viruses could lose their morphological visible-

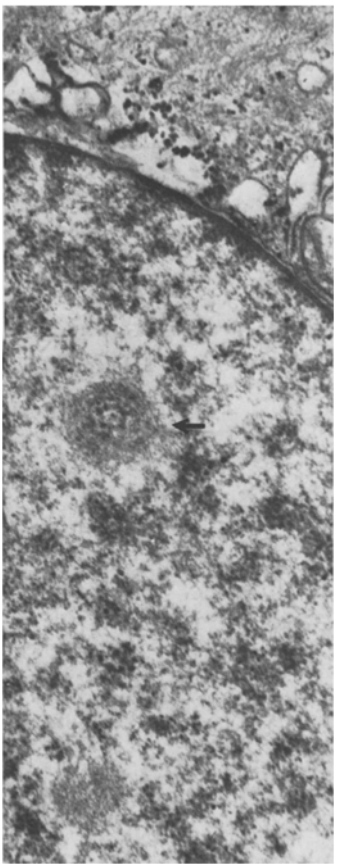

Fig. 15. Nuclear bodies (arrow) in the nucleoplasm of a squamous epithelium carcinoma cell. 23 500 ×

ness during the time required for biopsy excision and fixation [20, 44, 47]. Thus it would be understandable that virus particles were found in transformed lymphocytes of tissue cultures from Burkitt lymphoma and from nasopharyngeal carcinoma [15, 22] as it is assumed that the propagation of virus particles is promoted within the tissue culture [36]. Arnold et al. [5] were also of the opinion that DNA viruses could be detected morphologically especially when the viral DNA either accidentely or because of cellular death is ejected again from the cellular DNA.

After incorporation of the virus DNA into the chromosomes of the host cell, the viral DNA components behave as cellular genes during subsequent cell divisions and are multiplied in synchronization with the genes. The transformed cells exhibit new virus-specific macromolecules such as multiple viral gene copies, viral m-RNA, a virus-specific tumor antigen in the cell nucleus and the virus-specific transplantation antigen [52]. However, it is also conceivable that in a cell infected with DNA virus, the viral DNA is ejected again or becomes independent especially when incomplete or atypical mitoses occur, there being many indications of this happening in nasopharyngeal carcinoma.

In view of these theoretical possibilities to explain the lack of definite viral particles in the nasopharyngeal carcinoma, we have focussed our attention on the numerous cytoplasmic and nuclear particles in the epithelial tumor cells under investigation. These particles can be divided into groups and at least partially identified as follows:

(a) Bubble shaped particles with a double membrane and with a star-shaped, fine granular coating on the surface are found freely in the cytoplasm but also within tubules of the ergastoplasm. The particles have a diameter of 80–160 nm. In addition, tubular formations, also enclosed by membranes some of which appear entwinned, others in the form of short tubules or dumb-bell shaped, often containing a light core can be observed. Because of the characteristic structures, this particle group can be classified as Corona viruses [54]. This classification is all the more justified as these particles can also be observed on the surface epithelium and as these viruses have been identified in cases of viral air passage infections.

(b) Round particles having a double membrane and with a constant diameter of 60 nm were also found, some lying freely in the cytoplasm, others surrounded by membranes. They were however never found in tubules of the ergastoplasm. They can be clearly distinguished from vesicles of the Golgi apparatus as well as from multivesicular bodies. It is not possible to classify these with certainty into a known particle group. However, they do have a certain similarity to the Oncorna viruses as described by Rapp and Reed [58] and others, but on the strength of our findings we are not in a position to classify them as belonging to this virus group.

(c) Fine tubular coils, the individual tubules having a diameter of 12–15 nm were located in some isolated tumor cells and in the wall cells of some tumor blood vessels. Such cytoplasmic inclusions have been described as occuring in similar shape and to a comparable extent in the lymphocytes of circulating blood during various diseases and in the cells of virus induced tumors [4, 7, 8, 10, 31, 32, 34, 39, 69]. They were often confused with tubular or cristaline structured viruses as have been observed in viral diseases such as distemper, herpes simplex encephalitis and papillomas [8, 9]. At present, these structures are being interpreted as an unspecific reaction of the host cell to the viral attack.

(d) In the tumor cell nuclei occasionally spherical bodies were detected. These have appeared as an uncharacteristic structure in various types of cells but have also frequently been found in virus infections and in tumor cells [6, 11, 37, 38, 45]. Bouteille et al. [11] were able to present five different nuclear bodies of spherical shape, which had a comparable appearance in the investigated tumor specimens.

(e) For the first we were able to detect in the tumor cell nuclei of both the lymphoepithelial and the squamous cell carcinoma, particles, primarely located in telophase nuclei, having an outer diameter of 100 nm, a double membrane and frequently a central core with a diameter of 24 nm. These particles were scattered diffusely over the entire chromatin as well as in the chromatin concentrated along the edges. They were seldom found extranuclearly in the cytoplasm near the nucleus. The particles also revealed a double membrane and often an electron dense core when they were located between the nuclear membranes and the chromatin concentrated on the edges. Comparable particles within biopsy material of nasopharyngeal carcinoma have so far only been observed located within

the cytoplasm [52]. The particles are morphologically identical in shape and size to virus particles of the herpes type which have been demonstrated before [40, 42, 74]. Moreover, they differ very little from the virus particles which were found in cell cultures of Burkitt lymphoma and nasopharyngeal carcinoma [16, 22, 23, 67].

Given evidence of this type of particle located primarely within the nucleus, it would be desirable to prove the identity with herpes or Epstein-Barr viruses by the fact that the localisation of the particles corresponded on a immunologically-cytochemical basis with the positive fluorescence on the virus capsid antibodies. Such proof is at this time hardly possible because of the method used to prepare the tissue of the tumors under discussion for examination under the electron microscope (Zur Hausen, personal communication).

While discussing the viral nature of the demonstrated particles various nuclear structures must be taken into consideration, as they could lead to a misinterpretation [35]. On a differential diagnostic basis, perichromatin granules can definitely be eliminated because of the constant size of the particles we described and because of their differentiated structure. Hypertrophied ribonucleoprotein granules can also be easily distinguished from the particles. However, the morphological distinction from nuclear pores with their many shapes especially within mitotic figures and pathological mitoses can present difficulties. Numerous reports about the pores of interphase nuclei have been available [1, 21, 25, 28, 50, 66], but no pertinent investigation results were available to us concerning the size of nuclear pores in the prophase of mitoses and above all within pathological nuclear dividing figures such as appear in the tumors. Even the data about pore diameters vary considerably according to the animal species or tissue type examined before [26, 28, 29, 48, 49]. With regard to the number of nuclear pores, perhaps comparisons can be drawn to the nuclei of HeLa-cells, in which [11, 24] nuclear pores per μ^2 were counted [49]. The number of particles in the nuclei of tumor cells of the examined neoplasms is 40–45 per μ^2. Occasionally, pores with a diameter of 85–90 nm are found in the nuclear sections. With the exception of some passing ribosomal groups no central dense area could be detected within the pore lumen. Therefore tangential sections of such pores would essentially have to produce the picture of a double ringed figure, whose diameter should measure approximately 90 nm. The thin membrane, which spans the pore opening can not simulate the picture of a central core in tangential cut. Moreover, it must be noted that in tangential sections of nuclei with the usual thickness of approximately 60 nm only unsharply defined chromatin substance will be displayed, as too little of the nuclear membrane material is struck as the rays pass through to allow a sharp depiction of a membrane structure. Thus, one arrives at the picture of a "membraneless" nuclear section (Vogell, personal communication). This means that in the case of tangential sections of nuclear pores the pores can also appear as unsharply defined openings on the edge of the nuclear chromatin. Based on these reconstruction considerations of sections, it is our opinion that a distinction can be made between nuclear pores and the virus like particles which we have presented. In this connection it must once again be pointed out that the particles in some nuclei were so diffusely distributed over the nucleoplasm, that considering the constant particle size, the discussion about pores of the nuclear membrane can be abandoned.

If we assume that the intranuclear particles we have presented are in fact the electron microscopic morphological correlate of the Epstein-Barr virus, then the question remains open, why these viruses, against which approximately 90% of all healthy adults in central Europe have antibodies [27], lead in a few cases to nasopharyngeal carcinoma. In this regard, many authors have already pointed out the importance of individual immunological defects, racial and constitutional factors. From a morphological point of view we would like to pursue speculatively a thought expressed by Zur Hausen [76] about this question. Zur Hausen considered it possible that facultatively oncogenous viruses such as the Epstein-Barr virus can enter into cells through infection of these cells with another virus by means of a carrier mechanism. Structures which we have detected in the same tumor cells offer support for such a pathogenetic consideration. Some of these structures were classified as Corona viruses, others resembled structures of the Oncorna viruses. Finally, it must be pointed out again that other cytoplasmic nuclear inclusions indicate a participation of viruses in the transformed cells: namely a multiplication of ribosomes [62], a distribution of the chromatin on the edges of the nucleus [53], intracytoplasmic tubulo-reticular inclusions [3, 8], and the appearance of spherical "nuclear bodies" [11]. We interpret our findings as substantiation for the thesis that viruses have a pathogenetic and probably even an etiologic significance in the formation of nasopharyngeal carcinoma.

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