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ULTRASTRUCTURAL STUDIES ON BOVINE HORN CANCER

By

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SOMVANSHI, R., S. C. MUKHERJEE and CLAES REHBINDER: *Ultrastructural studies on bovine horn cancer.* Acta vet. scand. 1983, *24*, 252—258. — The ultrastructure of bovine horn cancer (squamous cell carcinoma) of Indian cattle is described. The tumour was composed of pleomorphic epithelial cells. The nuclei were enlarged with membrane invaginations and peripherally distributed heterochromatin and pleomorphic nucleoli. The cytoplasm contained bundles of tonofilaments and keratohyalin granules. In addition a majority of the cells revealed marked degenerative changes of the cytoplasmic organellae and intracellular oedema. Desmosomes were rarely observed. Budding of virus-like particles from the cell membrane of a few cells was demonstrated.

horn cancer; bovine; ultrastructure; virus.

Bovine horn cancer is an important neoplastic condition, affecting zebu cattle (Bos indicus), mainly ageing bullocks, in India (*Naik et al.* 1969, *Kaul & Kalra* 1973, *Naik & Randelia* 1978).

The condition has also been reported from Indonesia (*Burgraaf* 1935), Brazil (*Rezende & Neves* 1976) and Iraq (*Zubaidy* 1976). Histopathologically it is a keratinizing type of squamous cell carcinoma.

Although light-microscopic, epidemiological and immunopathological studies (*Kuchroo et al.* 1980, *Dhingra et al.* 1982) have been carried out in India, no efforts have been made to study the ultrastructure of the tumour except for a preliminary observation (*Somvanshi et al.* 1982).

The study was undertaken in order to characterize the ultrastructural features of this ailment.

MATERIALS AND METHODS

Clinical cases of horn cancer were regulary attended at the Operational Research Project Centre, Rithoura and various state veterinary hospitals in and around Bareilly, Uttar Pradesh, India. Six cases have been used for this study.

The affected horns were amputed surgically. Material was immediately collected from the tumours. Tissues were fixed in 2.5 % glutaraldehyde, buffered with 0.1 mol/l sodium cacodylate (pH 7.2) at 4°C for 1—2 h. Tissues were then dehydrated in ascending grades of alcohol and cleared through 2 changes of propylene oxide. After infiltration was carried out, for 1 h in 1:1 mixture of Araldite 502 embedding resin and propylene oxide, the tissues were embedded in the resin mixture. Polymerisation was carried out overnight at 45°C and then at 60°C for another day.

Thick and thin sections were cut using a LKB Ultratome III. Thick sections were stained with toluidine blue and examined in light microscope. Thin sections were loaded on to 300 mesh copper grids and dried overnight in a dust free chamber. Sections were stained in 1 % uranyl acetate followed by Reynold's lead citrate and examined at various magnifications in a Philips Electron Microscope, EM-301, at 30—60 k.v.

For histopathological studies material was collected from the same site, the tissues were fixed in 10 % formaldehyde, cut into 5 μm thick sections and stained with haematoxyline-eosin.

LIGHT MICROSCOPIC OBSERVATIONS

All examined cases were typical squamous cell carcinoma characterized by whorls and cords of squamous cells, epithelial pearls and keratinized centres. Single keratinized cells were also observed. The whorls and cords of squamous cells were occasionally separated by wide bands of connective tissue.

The neoplastic cells contained enlarged, hyperchromatic nuclei with prominent multiple nucleoli. Mitotic figures were frequently present. All tissues revealed numerous blood vessels. The periphery of the neoplastic tissue exhibited varying degrees of degenerative changes and also necrosis along with polymorphonuclear and monenuclear cellular infiltration.

ELECTRON MICROSCOPIC OBSERVATIONS

Ultrastructurally, the cancerous cells depicted marked pleomorphism with irregular cell membranes, characterized by numerous protrusions and invaginations and also inter- and intracellular oedema. Some of the cells appeared large, oblong and columnar with comparatively translucent cytoplasm (Fig. 1). Desmosomes or junctional complexes were rare and could be seen only occasionally.

Wide intercellular spaces filled with low electron dense material and cellular components like vesicles and free ribosomes were general features (Figs. 1 and 2). Slightly flocculent intracellular fluid of low electron density was also evident in most cells. Many cells revealed a rough endoplasmic reticulum with strongly and irregulary dilated cisternae and loss of ribosomes (Fig. 3).

The cytoplasm also showed diffusely scattered or clumps of tonofilaments, keratohyaline granuales and ribosomes (Figs. 2 and 4), degranulated endoplasmic reticulum and intracellular oedema. In some cells, densely packed tonofilaments and keratohyaline granuales were noted along the cell membrane. Numerous collagen fibres were seen in the intercellular space in some areas (Fig. 4).

Vesicles filled with fluid of varying electron density, probably deriving from smooth endoplasmic reticulum or degranulated rough endoplasmic reticulum or being swollen vesiculated mitochondria, were regular findings. The shape, size and number of mitochondria varied. Some showed increased electron density, some were swollen with disrupted cristae, and a few were devoid of their inner structure appearing completely vacuolated (Fig. 3).

Shape, size and structure of the nuclei and nucleoli showed great variation. Some nuclei were irregular in shape with numerous invaginations and peripherally distributed heterochromatin and some were enlarged having regular nuclear membrane, multiple nucleoli and comparatively translucent chromatin. Pronounced pleomorphism of nucleoli and sometimes fusion with dense chromatin was a common finding (Figs. 1, 4 and 5).

The endothelial cells of the capillaries depicted various changes of mostly degenerative character from almost normal to necrosis (Fig. 6).

Occasional neoplastic cells revealed thickening and cresent-like projections of the plasma membrane. The projections con-

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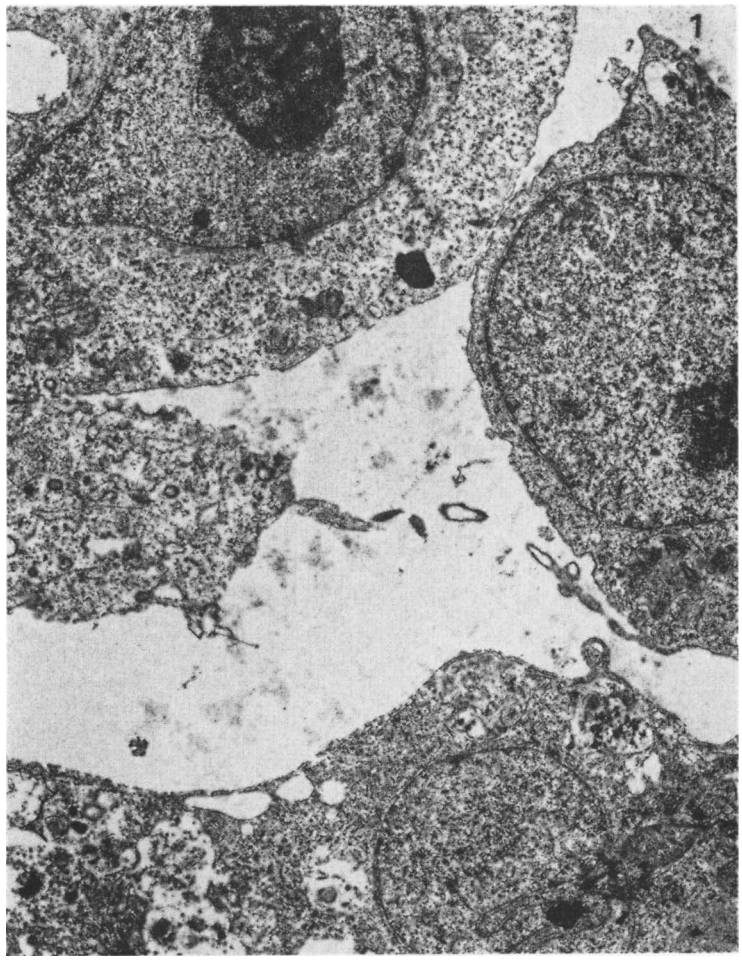

Figure 1. Pleomorphism, irregular cell membranes and inter- and intracellular oedema. Note also irregular nuclei, large irregular nucleoli (with "pars amorpha" and dense "fibrous centers"). Observe also lack of desmosomes and uneven cell membrane with numerous protrusions and invaginations. × 13,500.

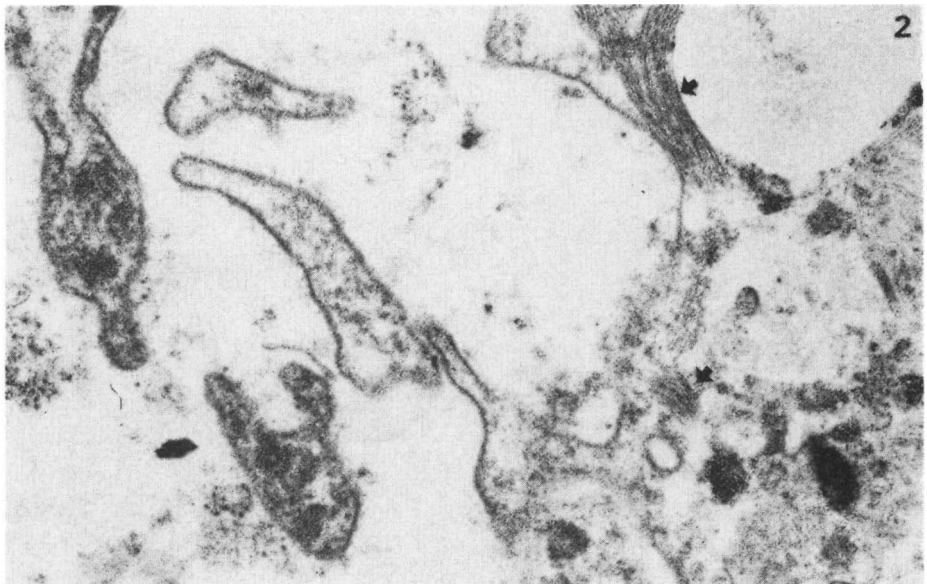

Figure 2. Inter- and intracellular oedema, vesicles with slightly flocculent material and dense packed tonofilaments. → The cell membranes are irregular. In the intercellular space is seen cellular debris in a slightly flocculent fluid. × 57,600.

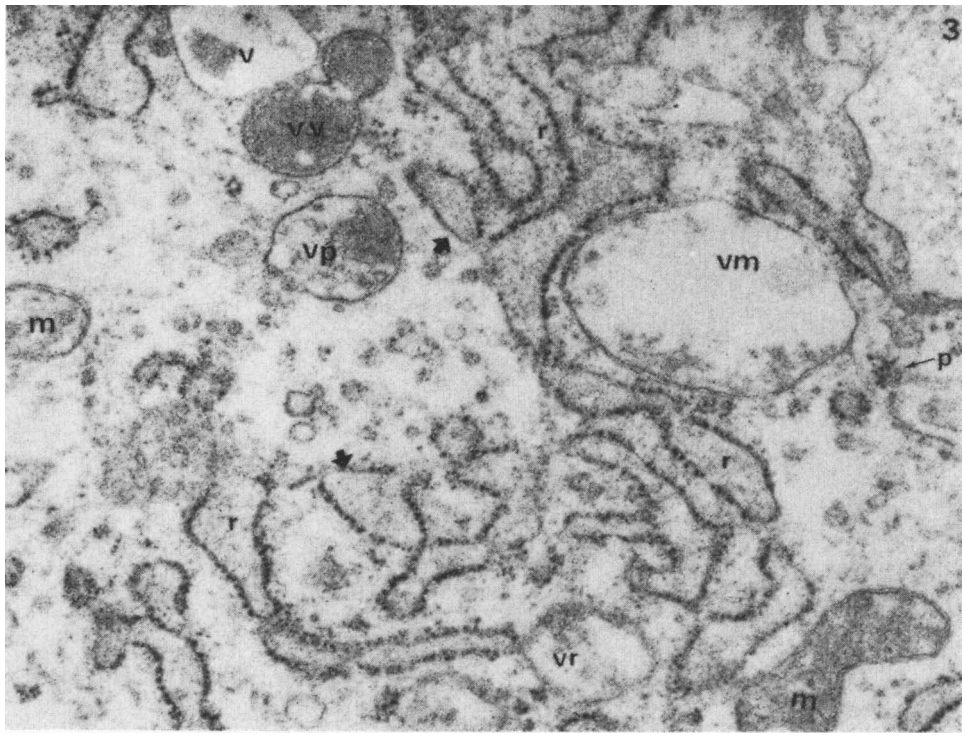

Figure 3. Intracellular fluid, slightly flocculent, of low electron density; the rough endoplasmic reticulum is strongly and irregularly dilated (r), segments show loss of ribosomes →. Note also dense irregular mitochondriae with disrupted cristae (m). Numerous vesicles are present some filled with fluid of different electron density (v, vr & vm) probably deriving from smooth endoplasmic reticulum (v) or degranulated rough endoplasmic reticulum (vr) or being swollen vesiculated mitochondriae (vm). Some vesicles are filled with electron dense material (vv) or partly filled (vp). Note also aggregation of ribosomes →. × 57,600.

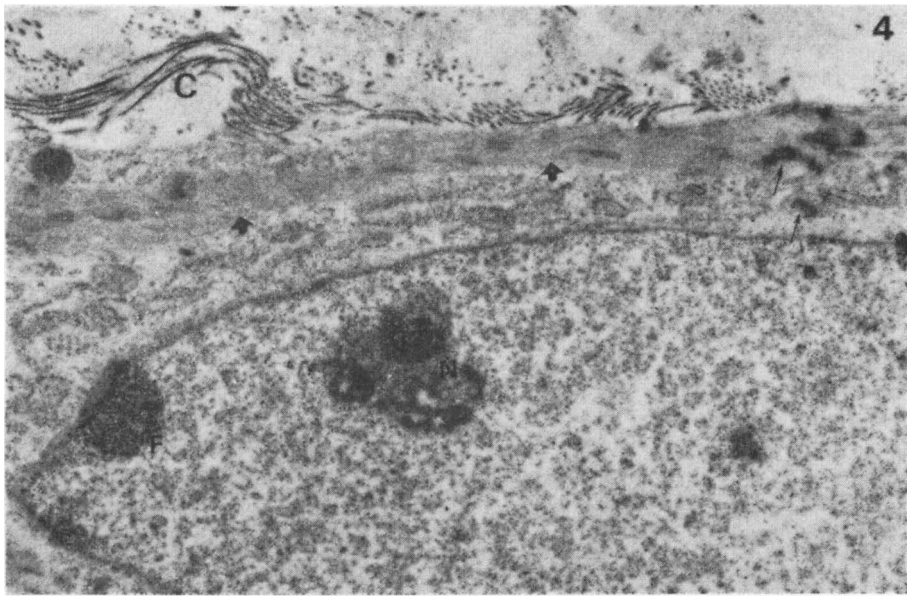

Figure 4. Dense packed tonofilaments along the membrane (thick arrow) → and possible keratohyaline granules (thin arrow) →. At (N) nucleolus with pronounced pleomorphism. At (F) focal condensation of chromatin. At (C) collagen fibres. × 19,200.

Figure 5. Nucleus with indentations and marginal condensation of chromatin. Note also electron dense mitochondriae with disrupted cristae (m) and vesicles with different degree of electron dense material (v). At the arrows → vesicular structures (most probable of rough endoplasmic reticulum) containing cytoplasmic matrix. At (C) collagen fibres. × 38,400.

Figure 6. Cell of capillary (A) with only minor changes. At thick arrow →, dilated golgi, at thin arrows → dilated cisternae and vesicles of rough endoplasmic reticulum. The cell B shows more alterations wiz. dilated rough endoplasmic reticulum (e) and golgi apparatures (g), vesiculated mitochondrium, (m) a swollen nucleus (B) and free ribosomes (double arrows ⇉). Note that tight junctions or zona occludens (O) appear normal.
× 38,400.

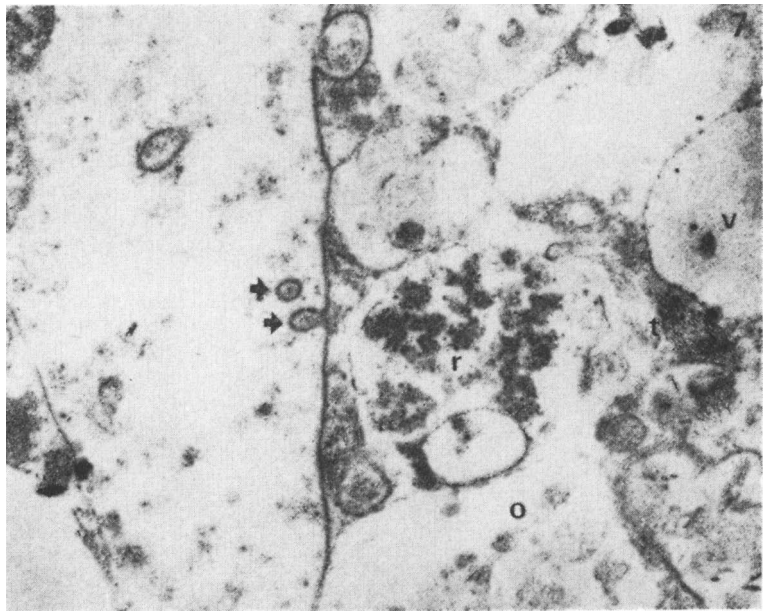

Figure 7. Budding virus-like particle and free virus-like particle →. Note the severe vesiculation of endoplasmic reticulum (v), tonofilament bundles (t) free ribosomes in flocculent material (r) and intracellular oedema (O). × 57,600.

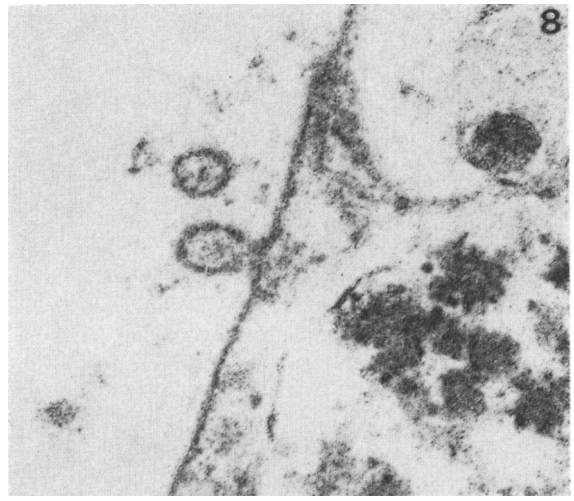

Figure 8. Still higher magnification of budding and free virus-like particles. × 134,000.

tained electron dense spots and appeared to form buds and free particles 80—90 μm in size (Figs. 7 and 8). A pronounced intracellular oedema with a severe vesiculation of the endoplasmic reticulum was present in these cells and in addition bundles of tonofilaments and free ribosomes (Fig. 7).

DISCUSSION

The findings of the present ultrastructural investigation differ in some aspects from the findings by *Pospischil et al.* (1976), on squamous cell carcinomas of ethmoidal tumours in cattle who observed large cytoplasmic areas with bundles of tonofilaments and numerous desmosomes in well differentiated squamous cell carcinomas.

In the present study a marked reduction in the number of desmosomes and a loss of structural cohesion were obvious which is more in agreement with the findings of *Vandrewalla & Sirsat* (1963) and *Chevile* (1967).

Squamous cell carcinomas are characterized by the presence of tonofilaments which are more frequently encountered and better developed in well-differentiated squamous cell carcinomas (*Ghadially* 1980).

The observation, however, of fewer tonofilaments in anaplastic tumours were not observed in the present study of these apparently rather well differentiated tumours, in which a decrease in tonofilaments was noted only in severely degenerated cells. The absence or scarcity of junctions in malignant epithelial tumours may be related to the diminshed adhesiveness of the tumour cells and the lack of intercellular communication (*Martinez-Palomo* 1970). Cell degeneration itself may also attribute to the loosening of cell contacts (*Sugar* 1968).

The loss, however, of structural cohesion, found in the investigated tumours, underlines their carcinomatous character (*Martinez-Palomo* 1970) while the findings of tonofilaments in connection with keratohyaline granules are indicative of their squamous keratin forming origin (*Brody* 1959, *Ghadially* 1980 & 1982). Neither membrane coating granules (*Matolsy & Parakkal* 1965), myelinoid figures (*Robinson & Gregory* 1981) nor intra nuclear microspherules, observed in bovine ocular squamous cell carcinoma (*Hod & Perk* 1972) were found.

Mitochondriae of varying morphology were present.

Some cells contained swollen mitochondriae with disorganized cristae, while others were devoid of their inner structure appearing completely vacuolated. Some of these changes were recorded in ethmoid squamous cell carcinomas of cattle (*Nair* 1980) and human oesophageal squamous cell carcinomas (*Ghadially* 1982, *Robinson & Gregory* 1981).

The degenerative changes of the cytoplasmic organellae could be associated with a rapid cell proliferation and abnormal metabolism of the cells but also to disturbed nutrition of the neoplastic cells as indicated by the findings of degenerative changes of the endothelial cells lining the capillaries. The changes found in the nuclei and nucleoli are well known unspecific features of tumour cells (*Ghadially* 1982).

One notable feature of this investigation was the presence of virus-like particles budding from the cell membrane. These particles were present in very small number and found only after thorough scanning. The etiology of horn cancer is still obscure and the possibility of involvement of virus can not be ruled out (*Pachauri & Pathak* 1969). The possible role of a virus in the, most probable, multifactorial etiology of horn cancer is obscure and the presence of the found budding organisms could be attributed to a passenger virus.

Earlier, viruses have been demonstrated in bovine ocular squamous cell carcinomas by *Hod & Perk* (1973) and *Ford et al.* (1982), in bovine ethmoidal tumours (squamous cell carcinoma) by *Nair et al.* (1981), and in squamous cell carcinomas in sheep by *Vanselow et al.* (1982).

The virus-like particles found in this investigation are not similar in their structure to the viruses described by the above mentioned authors. The finding of virus-like particles in cases of horn cancer could indicate the possibility of a partly viral etiology and be of value for further studies.

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SAMMANFATTNING

Ultrastrukturella studier av bovin horncancer.

Ultrastrukturen hos horncancer (plattepitelcarcinom) från Indisk nötboskap beskrives.

Tumörerna bestod av pleomorfa epiteliala celler. Kärnorna var förstorade med membraninvaginationer, perifert lokaliserat heterochromatin och pleomorfa nucleoler.

Cytoplasman innehöll tonofilamentbuntar och keratohyalinkorn. Därtill uppvisade huvuddelen av cellerna markanta degenerativa förändringar av cellorganellerna samt intracellulärt ödem. Desmosomer förelåg sparsamt. Hos enstaka celler sågs knoppning från cellmembranen av virusliknande partiklar.

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