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Bovine Ocular Squamous Cell Carcinoma II. Tissue Culture Studies of Papilloma.* (24879)

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Studies on pathological anatomy of bovine ocular squamous cell carcinoma demonstrated eosinophilic, cytoplasmic inclusion-like bodies in cells of both benign precursor and malignant lesions(1). Plaque and papilloma, benign precursor lesions, exhibited bi-laterality, disappearance and reappearance, which suggested a virus may be involved in the etiology of this disease. Tissue culture studies of cells derived from plaque lesions(2) have shown characteristic changes suggestive of a filterable agent. As a result of these preliminary findings, tissue culture studies of papilloma were undertaken to determine whether similar changes also occur in cells from papilloma grown *in vitro*.

Materials and methods. The method of removal of the lesions, treatment for transportation to the laboratory, subsequent treatment in laboratory and methods used for detailed study of cellular outgrowths from lesions have already been described(2,3,4).

Results. Phase contrast microscopy of cells derived from papillomata growing in specially designed chambers(2) has confirmed and extended preliminary observations on stained cultures in T-30 culture flasks examined at low magnifications by bright field microscopy. The cells derived from papillomata are epithelioid in character, relatively uniform in size measuring 0.01 mm in diameter, and frequently multinucleated (Fig. 1). Occasional large cells are seen as much as 0.03 mm in diameter; these cells are always multinucleated. The cells exhibit well marked tonofibrils, mitochondria long and interlacing are well developed (Fig. 2). Pinocytosis is marked, many cells showing cytoplasmic fimbriae (Fig. 3). Cells frequently exhibit vacuolisation of cytoplasm. Such cells occur in scattered foci. Vacuoles are found in the perinuclear zone and contain dense homogeneous material (Fig. 4). Cytoplasmic inclusions, irregular in outline, and not associated with presence of vacuoles also occur (Fig. 5). The nuclei contain multiple nucleoli of variable size and shape, and occasionally show margination of chromatin (Fig. 6). As in cells derived from plaque lesions, vacuolisa-

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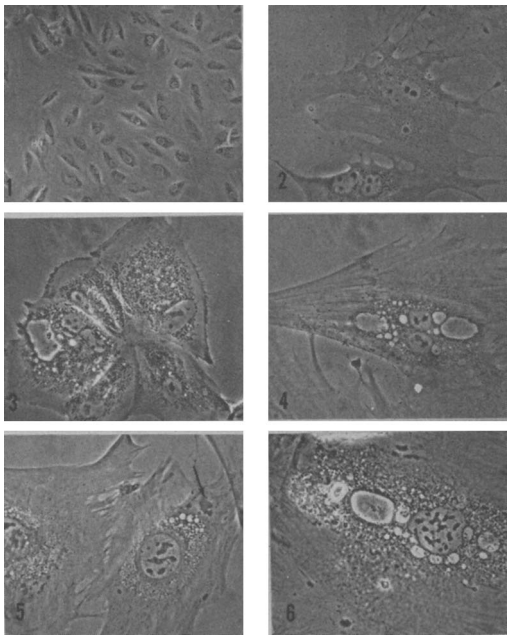

Photographs were made with the phase optics on a Zeiss Microscope, Model No. GFL-65, using the 35 mm camera attachment.

FIG. 1. Cells derived from papilloma, a precursor lesion of bovine ocular squamous-cell carcinoma. 22nd passage, 11 day S-M chamber culture. × 70.

FIG. 2. Cell showing well developed mitochondria and cytoplasmic inclusion. 5th passage, 3 day S-M chamber culture. × 195.

FIG. 3. Cells showing well developed cytoplasmic fimbriae. 22nd passage, 8 day S-M chamber culture. × 350.

FIG. 4. Part of a cell with perinuclear vacuolisation and early inclusion formation. 22nd passage, 11 day S-M chamber culture. × 350.

FIG. 5. Cells showing perinuclear vacuolisation and cytoplasmic inclusions. 12th passage, 7 day S-M chamber culture. × 350.

FIG. 6. Part of a large cell with nucleus showing multiple nucleoli and early margination of chromatin. Perinuclear vacuoles containing early inclusions are also seen. 22nd passage, 9 day S-M chamber culture. × 350.

tion of cytoplasm, when it occurs, spreads peripherally to adjacent cells and leads to loss of culture. Vacuolisation is less frequent and occurs later in cell cultures derived from papillomata than in cultures derived from plaque lesions. It has been observed in cells of cultures of seventh and subsequent passages. Cultures of a number of papillomata have been maintained for more than 50 sequential passages over 30 weeks. Thirty-three papillomata have been put into culture: 4 were maintained for 4 weeks or less; 17 for more than 8 weeks; 9 for more than 18 weeks and 3 for more than 30 weeks. Cells derived from 7 papillomata are presently in culture, 3 have been in culture for more than 30 weeks, 4 for more than 12 weeks. Studies are in progress to determine the nature of changes which lead to loss of cultures.

Summary. Properties of cells derived from papillomata lesions of bovine ocular squamous-cell carcinoma grown *in vitro* are described. The cells have been observed, after 7 or more passages, to exhibit cytoplasmic changes which commonly result in loss of the culture. In some cultures changes have not been observed after more than 50 passages. The nature of the changes is being investigated.

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