Bovine Ocular Squamous Cell Carcinoma. I. Tissue Culture Studies of Plaque.* (24685)

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The pathological anatomy of bovine ocular squamous cell carcinoma has been fully described(1). This disease includes benign hyperplasia (plaque) and pappilloma, which often precede development of carcinoma. Eosinophilic intranuclear inclusion-like bodies, not seen in eyes of apparently disease-free cattle, have been described in cells of benign and malignant lesions(1). This observation, combined with bilaterality, disappearance and reappearance of the benign precursor lesions, plaque and papilloma, suggested that a virus, among other factors, may play a part in origin of this disease(1). Tissue culture studies were undertaken, as a first step, to determine whether a viral agent could be implicated in the origin of benign or malignant lesions of the disease.

Materials and methods. The first lesion to be investigated was plaque, one of the benign precursor lesions. Plaque lesions were removed under local anesthesia induced by retro-ocular inoculation of 20-30 ml of 2% procaine. The method of collection of specimens in the field which gave a high percentage of successful primary cultures was as follows: After removal, a portion of the lesion was put in Bouin's fixative, the remainder placed in screw-capped vial with Eagle's basal medium containing 20% inactivated calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and stored overnight at 4°C. The following day the tissue was minced with scissors in a 60 mm Petri dish, and treated for 20 minutes at ambient temperature with minimal volume of trypsin solution (0.25% Difco Bacto Trypsin in Ca and Mg free Hanks' B.S.S.)(2). The tissue mince, suspended in trypsin solution was then placed in 10-15 ml of complete culture medium in screw-capped test tube and stored at 4°C during transportation. Tissue fragments were resuspended in 7-14 ml of fresh medium and the suspension placed into one or more T-30 culture bottles (Kontes Glass Co., Vineland, N. J.) The cultures were left undisturbed at least 4 days. Thereafter medium changes were made every 4 days irrespective of pH. Primary outgrowth was observed to occur within 4-30 days. The cells were too thin for detailed observations by bright-field microscopy. Such observations were made possible by use of chambers specially constructed for phase microscopy(3). These chambers also allow study of the same culture after staining.

Results. Phase microscopy of cells from plaque lesions grown in the chambers, confirmed early observations made by bright-field microscopy on stained cells in T-30 flasks(4). As already reported, 2 types of cells have been observed in tissue cultures of plaque. One type of cell is large and contains one or more large nuclei; the other is considerably smaller and as a rule contains a single small nucleus (Fig. 1). Some of the larger cells may be seen at somewhat higher magnification in Fig. 2. Well marked tono-fibrils have been observed in the larger cells (Fig. 3). Characteristic cell with large cytoplasm and strongly basophilic granular material in the perinuclear region may also be seen. Cytoplasm of the larger cells has frequently been found highly vacuolated (Fig. 4). Vacuolization of cytoplasm occurs with equal frequency in larger and smaller size cells (Fig. 5). Vacuoles in the cytoplasm have often been found to contain strongly basophilic material (Fig. 6). Nuclei contained a number of nucleoli variable in size and shape, and show frequent margination of chromatin and vacuolization (Fig. 6).

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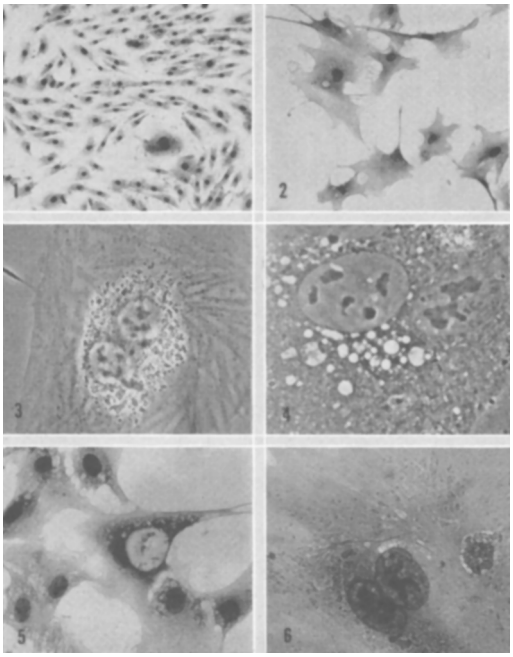

FIG. 1. Cells derived from Plaque, 21st passage, showing small and large cell types. May Grünwald-Giemsa stain. × 38.

FIG. 2. Cells similar to those shown in Fig. 1 at higher magnification. May Grünwald-Giemsa stain. × 60.

FIG. 3. Phase contrast micrograph of a large cell showing tono-fibrils and granular material in perinuclear zone. × 150.

FIG. 4. Phase contrast micrograph of perinuclear region of a large cell showing vacuolization. × 263.

FIG. 5. Vacuolated cells, one showing a large vacuole containing small discrete aggregations of basophilic material. May Grünwald-Giemsa stain. × 150.

FIG. 6. Portion of a large cell with vacuoles containing strongly basophilic granular material. May Grünwald-Giemsa stain. × 225.

Vacuolar degeneration of cytoplasm has been observed in scattered foci of cells in cultures of plaque gradually extending towards the periphery. The changes appear in cultures of fifth or subsequent passages and lead to loss of the culture even after frequent subcultures are made. Cultures of a number of plaque lesions have been maintained for more than 45 sequential passages, for more than 30 weeks. One hundred plaque lesions were put into tissue culture; 53 were maintained 1-5 weeks, 7 for 5-10 weeks, 28 for 10-20 weeks, and 5 for 20-40 weeks. Seven cultures grew for less than 1 week. Cells of 18 plaque lesions are presently in culture, 3 have been in culture for 30 weeks and 45 passages, 15 for 10 weeks and 7 passages.

Studies are in progress to determine whether the observed changes are due to a viral agent.

Summary. The method of collection and cultivation *in vitro* of specimens of plaque, one of the benign precursor lesions of bovine ocular squamous cell carcinoma, is described. Primary outgrowths were obtained in 98% of collected specimens. Cells derived from plaque lesions, following a number of consecutive passages, show nuclear and cytoplasmic changes which may appear as early as the 5th passage. These changes lead to the eventual loss of the culture. Some specimens of plaque lesions in which changes have as yet not been observed are now in the 46th consecutive passage. The nature of the observed changes is being investigated.

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