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Efforts to produce retinoblastoma experimentally have so far been unsuccessful. However, human adenovirus Type 12, a unique DNA-type virus, has been found to produce neurogenic tumors in the peripheral nervous system, as well as medullo-epitheliomatous tumors in the central nervous system of hamsters and mice. Our recent experiments with rats have indicated that neuronal precursors in the rat brain are far more susceptible to this virus than those in the hamster brain. These results prompted us to test the possible affinity of the virus for undifferentiated neuronal cells in the rat retina. This preliminary report describes the first experimental production of retinoblastoma-like tumors in animals, using human adenovirus Type 12.

The method of preparation of the virus has been described previously. Timed pregnant C-D-strain rats were obtained from the Charles River Breeding Lab (North Wilmington, Mass.); the rats gave birth in our laboratory. Within 24 hours after birth, 35 newborn rats received a single injection of 0.01 ml. of the virus fluid into the vitreous from a fine hypodermic needle (No. 30, Metropolitan Supply, Cambridge, Mass.) connected to a microsyringe with a polyethylene tube. The supernatant of nonvirus-infected HeLa cells was injected into the vitreous of control animals.

The first animal produced a massive intraocular tumor 204 days after injection of the virus. By Day 300, three of the 35 rats produced similar intraocular malignant tumors. None of the fifteen control rats produced tumors.

Case 1. The tumor was recognized on Day 204 and observed for the ensuing week. It appeared to have infiltrated the anterior chamber and cor-
Fig. 1. Section of tumor-bearing eye, 215 days after a single virus inoculation. The tumor mass fills the vitreous and infiltrates the optic nerve beyond the lamina cribrosa (double arrow). Hemorrhagic necrosis is prominent (single arrow).

ne, which showed prominent hemorrhagic necrosis. The solid tumor tissue filled the vitreous cavity and showed marked infiltration of the optic nerve.

Case 2. At 215 days after virus inoculation, a solid intraocular tumor was observed through the transparent cornea. The tumor mass filled the vitreous cavity almost entirely and invaded the anterior chamber and optic nerve head. Part of the tumor appeared to have undergone hemorrhagic necrosis (Fig. 1).

Case 3. The intraocular tumor was diagnosed 228 days after injection. Although the tumor mass filled the vitreous cavity, one part of the retina remained well preserved.

Perfusion of the whole body with a formaldehyde-glutaraldehyde fixative was performed in all tumor-bearing rats. All tumors were soft and grayish-pink. Histologically, they appeared indistinguishable from each other. The major part of each tumor was composed of small hyperchromatic undifferentiated cells that formed perivascular wreaths or rosettes (Fig. 2A). Most of the tumor cells showed irregularly shaped, round, or slightly elongated hyperchromatic nuclei and small amounts of poorly defined cytoplasm. Mitotic figures were abundant.

All tumors showed a tendency to undergo hemorrhagic necrosis in which degenerated tumor cells encircled nondegenerated cells located around the blood vessels. In such areas, a trabecular or papillary appearance was one of the characteristic features. Tumor cells had a marked tendency to form incomplete rosettes throughout the tissue. No so-called Flexner-Wintersteiner rosettes were found. Most of the retina was overrun by the tumor cells. Some of the remaining retinal tissue showed marked degeneration due to compression of the tumor. No intracranial metastases were observed.

Electron microscopically, tumor cells showed very few intracellular junction complexes and terminal bars. The cytoplasmic organelles were poorly differentiated with a number of mitochondria, while free ribosomes were abundant (Fig. 2B). In the apical region of the cytoplasm in many tumor cells, only one cilium which showed a typical concentric pattern of nine pairs of peripheral tubules without central axial pairs (a 9 + 0 pattern) was observed (Fig. 2B, arrow).

Since there is no report of comparable man-made retinoblastoma in the literature, no unequivocal criteria exist for designating these retinal tumors. However, it seems reasonable to apply the diagnostic criteria and nomenclature established for human retinoblastoma. We could also refer to the animal tumors induced by adenovirus Type 12 in the central nervous system and the retrobulbar adnexa.5-7

Adenovirus-induced intraocular tumors are remarkably uniform and basically identical to those tumors produced by the same virus in different organs. Adenovirus-induced brain tumors in hamsters and rats have been designated as a medulloepitheliomatous tumor derived from neuroepithelial periventricular zone.5, 1 It has been postulated that sensory neuronal precursors constitute the susceptible target cells in adenovirus tumorigenesis.

The ultrastructural identification of the characteristic cilium and its associated centrioles, which are highly reminiscent of the connecting sensory cilia in the retinal receptors, has been well-documented in human retinoblastoma cases.8, 9 Extracranial orbital tumors produced by the same virus are also indistinguishable from human neuroepithelioma, which is presumably derived from the precursor cells of the ciliary ganglia.10

Based on these data, it is reasonable to assume that the malignant tumors produced by adenovirus Type 12 are derived from neuronal precursor cells of the developing retina. We therefore propose that the tumors produced by adenovirus Type 12 be considered a counterpart of retinoblastomas without rosettes in the human.

The incidence of tumors in the rat eye is lower than in the central nervous system. One of the probable reasons is the technical difficulty presented by the size of the vitreous cavity in newborn rats. According to Yabe, Trentin, and Taylor,10 the incidence of tumors induced is directly proportional to the dose of virus injected. The limited dose of virus which can be injected into the vitreous cavity together with the presumably
Fig. 2A. Small hyperchromatic cells form many poorly differentiated rosettes. Arrow shows necrotic area. (Hematoxylin-eosin stain, ×100.)

Fig. 2B. Electron microscopic picture shows poorly differentiated cytoplasmic organelles and solitary cilia (arrow, a 9 + 0 pattern) in the apical area of a tumor cell. (Direct ×2,300.)
lower population of susceptible precursors in the retina would account for the present low incidence of tumor production. Detailed studies of nine intraocular retinoblastoma-like neoplasms obtained to date will be published soon.

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From the Wesley C. Bowers Laboratory of Pharmacology and Experimental Pathology, Department of Retina Research, Retina Foundation, Boston, Mass. 02114. This work was supported by the Massachusetts Lions Eye Research Fund, Inc., by Public Health Service Research Grants EY-00227 and CA-12180, and by grants from Research to Prevent Blindness, Inc. S. Kobayashi was the recipient of a Grant-in-Aid from Fight for Sight, Inc., New York. Manuscript submitted for publication July 19, 1973; manuscript accepted for publication Aug. 28, 1973.

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An in vitro evaluation of tetrahydrotriamcinolone. JOHN F. BIGGER, HARRY A. ZINK, PAUL F. PALMBERG, AND BERNARD BECKER.

A major side effect of corticosteroid therapy for ocular inflammation is the increased intraocular pressure which occurs in susceptible individuals. The therapeutic efficacy of corticosteroids, and the intraocular pressure effect, have been shown to vary with the relative potency of the drug used, its concentration, frequency and duration of usage, and its ocular penetration.

Attempts to dissociate the anti-inflammatory and ocular pressure effects of corticosteroids have led to the evaluation of several new compounds in recent years. One such compound is tetrahydrotriamcinolone (THTA), a derivative of triamcinolone. THTA has been shown to only rarely elevate intraocular pressure, however, its potency as an anti-inflammatory drug is controversial.1, 2

Recently, Bigger, Palmberg, and Becker3 reported that differences in individual sensitivity to corticosteroids could be detected using a peripheral blood lymphocyte transformation inhibition assay and that the systemic sensitivity correlated closely to the results of ocular testing. The purpose of the present study was to ascertain whether differential sensitivity of lymphocytes to THTA occurs, and to determine the potency of THTA relative to prednisolone-21-phosphate.

The method of assay used in the present study has been reported previously3 and will be only briefly described. Suspensions of lymphocytes are prepared from freshly drawn heparinized venous blood and incubated for 48 hours in the presence of phytohemagglutinin-P (PHAP) after preincubation with various concentrations of the corticosteroid compound to be tested. Following this incubation tritiated thymidine is added and the lymphocyte suspensions are allowed to incubate for an additional sixteen hours at which time they are harvested for scintillation counting.

In the present study, for each patient we prepared two unstimulated blanks, six PHAP-stimulated controls, quadruplicates of each of four concentrations of prednisolone-21-phosphate with PHAP, and quadruplicates of each of five concentrations of tetrahydrotriamcinolone (THTA) with PHAP. In each case the prednisolone-21-phosphate or THTA was added to the cell suspension and allowed to incubate at 37° C. for one hour prior to the addition of PHAP.

For each patient two dose-response curves for the inhibition of tritiated thymidine incorporation into PHAP-stimulated lymphocytes were obtained from the same sample of blood; one for inhibition by prednisolone-21-phosphate, and the second for inhibition by THTA. Counts per minute incorporation corrected for the nonstimulated blank was plotted vs. log concentration of the drug. Regres-