

ferentiated function during a period of cell replication or to different effects on separate types of target cells.

The effects of the bone-derived growth factor are similar to those previously described for the somatomedins (9-11). We found that the factors derived from cell cultures, tested at a variety of concentrations, did not stimulate the incorporation of ^{35}S into costal cartilage as the somatomedins do and did not have detectable somatomedin activity on radioreceptor assay (12). The fraction derived from organ cultures which eluted with chymotrypsinogen and stimulated thymidine incorporation did not have detectable sulfation activity. However, the fraction of lower molecular weight increased ^{35}S incorporation in hypophysectomized rat cartilage at 2 to 20 $\mu\text{g}/\text{ml}$ and contained 1.1 to 1.4 ng of Somatomedin C per microgram of protein as determined by radioreceptor and radioimmunoassay (13). Thus, one of the activities appears to be related to the somatomedins while the others do not. Cortisol seems to play a permissive role in the expression of the effect of conditioned medium on collagen and NCP synthesis. This is consistent with the observation that glucocorticoids can enhance the differentiated function of osteoblasts in organ culture (14).

The presence of a growth factor in cultured fetal rat calvaria could provide a mechanism for stimulation of rapid skeletal growth during late fetal life. If similar factors are produced by adult bone, they could play a role in local responses to mechanical stress and in the coupling of new bone formation to prior bone resorption.

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Retinal Tumor Induced in the Baboon by Human Adenovirus 12

Abstract. *Three of 21 newborn baboons injected intraocularly with human adenovirus type 12 developed an intravitreal mass 12 to 36 months later. Two of the masses were indistinguishable from human retinoblastoma, a retinal tumor that afflicts children. To our knowledge this is the first time a retinoblastoma-like tumor has been induced experimentally by adenovirus type 12 in a nonhuman primate.*

Although the nature and morphology of human retinoblastoma, the most common tumor of the retina in infancy and childhood, are well established (1), neither spontaneously occurring nor experimentally induced retinal tumors in nonhuman primates has ever been described (2). Because baboons are among the most intensively studied of all nonhuman primates, and because the time sequence of their growth and aging parallels that of humans in many biologic respects (3), we attempted to induce retinal tumors in baboons. We used human adenovirus serotype 12 (Ad. 12) because of the results of two earlier studies: the pioneering work of Albert *et al.* (4), who demonstrated that young adult hamsters' neural retina cells could be transformed in vitro by Ad. 12; and our own production of retinoblastoma-like neoplasms in rodents with Ad. 12 (5).

Twenty-one baboons were each given a single intraocular inoculation of Ad. 12 (Fig. 1, A and B), 0.05 to 0.1 ml of 10^8 TCID₅₀ (50 percent tissue culture infective dose) per 0.1 ml in human embryo kidney (HEK) cells, in the right eye through a No. 30 G1/2 hypodermic needle. Their left eyes were not treated. The baboons were inoculated within 24 hours after birth; at this stage, baboons' retinas are well differentiated (Fig. 1C). At approximately 12 months after inoculation, one of the baboons exhibited a peculiar light reflection in its virus-inoculated eye (Fig. 1D). Two more baboons later showed the same reflection: one at 18 months after inoculation, the other at 36 months. Ultrasonographic scanning showed that all three baboons had an intravitreal mass. Twenty-four

months after inoculation, a grayish-red mass was removed from the enucleated right eye of the first baboon. Part of the mass was fixed in 2.5 percent glutaraldehyde in phosphate buffer, pH 7.4, at 4°C for electron microscopy; part was fixed in Formalin for light microscopy; and part was frozen for tumor (T) antigen detection. Cell impressions on cover slips were also made for the T-antigen test. Paraffin sections 7 μm thick were cut from both glutaraldehyde- and Formalin-fixed specimens and stained with hematoxylin-eosin, thionin, periodic acid-Schiff, Luxol fast blue, and Holmes' silver impregnation.

Under low magnification, the intravitreal mass appeared to be composed of densely packed cells (Fig. 1E). A closer examination revealed dense, uniformly distributed retinoblastoma-like tumor cells with rosettes of various forms (Fig. 1F). In some areas, "Flexner-Wintersteiner" true rosettes predominated, but most transformed cells had the morphologic appearance typical of the neuroblastoma-like cells we observed in tumors induced by Ad. 12 in rodents (Fig. 1G).

Integration of Ad. 12 viral DNA into the transformed retinal cells was demonstrated by the presence of immunofluorescent Ad. 12-specific T antigens (Fig. 1H). Electron microscopy showed that the neoplastically transformed retinal cells had oval nuclei with evenly distributed chromatin and a scanty rim of cytoplasm with poorly organized intracellular organelles (Fig. 1I). Ciliated tumor cells (with a 9 + 0 tubule pattern) were common. These ultrastructural characteristics of the Ad. 12-induced

retinal tumor cells agree with well-established criteria of human retinoblastoma (1).

We have recently removed and examined an intraocular tumor from the second baboon. (The tumor was removed 46 months after inoculation with Ad. 12.) Final results on the transplantability of tissue culture explants on this tumor are not yet available, but the morphologic characteristics of this tumor are nearly identical to those of the retinoblastoma-like tumor removed from the first baboon.

The ultrastructural findings on both tumors confirm that we have established a model of retinoblastoma in a nonhuman primate.

Human retinoblastomas fall into two categories. About 60 percent of them are undifferentiated neuroblastic neoplasms that form only incomplete (Homer

Wright-type) rosettes. The other 40 percent are more differentiated retinoblastomas with true (Flexner-Wintersteiner) rosettes. These true rosettes are considered a neoplastic mimicry of photoreceptor (rod and cone) differentiation.

In earlier models of retinoblastoma in rodents (5), Ad. 12 produced retinoblastoma-like tumors with incomplete rosettes only. To induce these tumors, we inoculated the animals within 24 hours after birth, a stage of development during which rodents' retinas are immature and actively replicating. We hypothesized that these actively replicating cells were the target of transformation by Ad. 12 (5).

In contrast, newborn baboons such as those we used in the present work have mature retinas, with no actively replicating neurons (Fig. 1C). Nevertheless, after being injected into the

eyes of newborn baboons, Ad. 12 produced retinal tumors with the complete rosettes characteristic of photoreceptor differentiation.

Our findings thus imply that whereas types of retinoblastoma characterized by true rosette morphology result from neoplastic differentiation of rods and cones, retinoblastoma with incomplete rosette morphology (as we have demonstrated in rodents) may result from neoplastic transformation, in developing retinas, of sensory neurogenic precursor cells, which would otherwise be destined for the inner nuclear layer.

Neuroblastoma-like embryonic neoplasms with Homer Wright rosettes have been induced in the cerebrum, cerebellum, and peripheral nervous system of rodents by human adenovirus (6). Thus a question for further research is whether similar tumors could be induced in primates' brains by human adenovirus, as has been done by London *et al.* (7) in owl monkey brain using papovavirus JC, another DNA virus of human origin.

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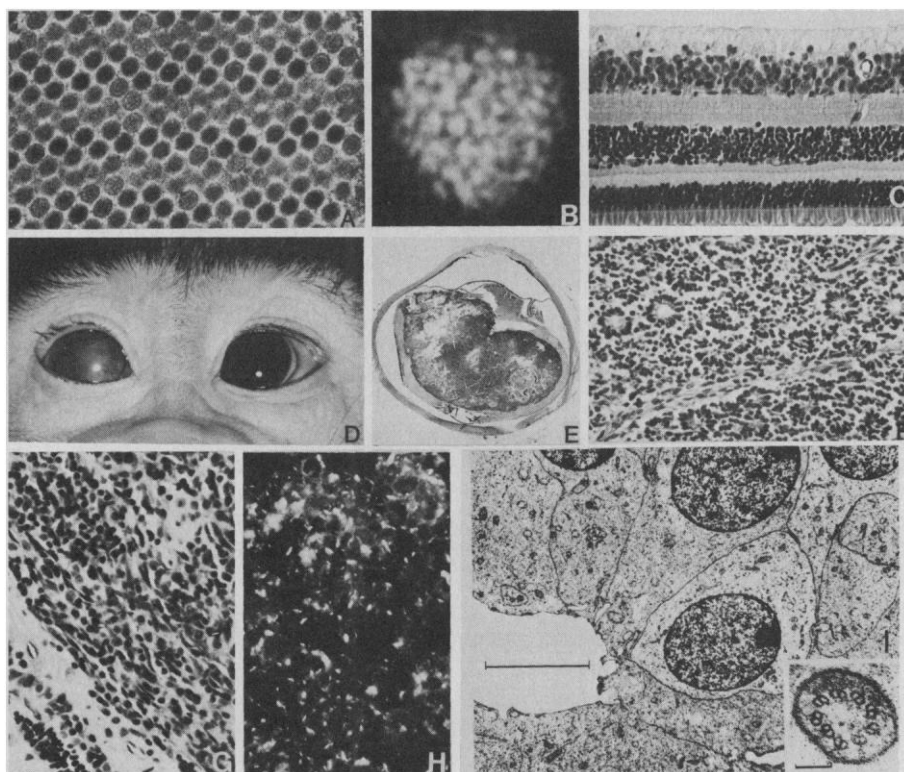


Fig. 1. (A) Human adenovirus type 12 propagated intranuclearly in HEK cells. Note the crystalloid arrays (original magnification, $\times 36,000$). (B) Single virion approximately 70 nm in diameter. Virion possesses characteristic capsomeres demonstrated with negative stains (original magnification, $\times 73,000$). (C) Baboon retina at a perinatal stage. The retina appears almost totally differentiated, with no replicating neurogenic cells adjacent to the external membrane (hematoxylin-eosin; $\times 250$). (D) Appearance of baboon after the development of an intraocular mass in the right eye. (E) Section of intraocular mass attached to the thickened retinal tissue (hematoxylin-eosin; loupe). (F) Histopathologic section of the tumor cells, showing their close resemblance to their human counterpart, retinoblastoma cells with various transitional forms of rosettes (Hematoxylin-eosin; $\times 250$). (G) Most undifferentiated neuroblastomatous cells maintain continuity with the inner nuclear layer of the retina. Note relatively well preserved outer nuclear layer (thionin; $\times 400$). (H) Numerous intensely luminescent filamentous and crescent-shaped T-antigen-positive particles in tumor cells (immunofluorescein T-antigen test; $\times 600$). (I) Electron micrograph of adenovirus-induced retinal tumor cells surrounding the lumen of the rosettes. Note cilium-like projections (bar, 5 μm ; original magnification, $\times 4063$). Inset: Cross section of cilium showing a 9 + 0 tubule pattern highly reminiscent of the connecting cilia of normal photoreceptors (bar, 100 nm; original magnification, $\times 47,000$).

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Isolation of O1 Serovars of *Vibrio cholerae* from Water by Serologically Specific Method

Abstract. *Vibrio cholerae* bacteria of the serological variety O1 were consistently isolated from water samples by passing the water with added Tween 20 through columns packed with polystyrene beads coated with antibodies against the O1 antigenic determinants. The beads from the columns were washed, transferred to alkaline peptone broth, and incubated. The O1 serovars were isolated and identified by established procedures.

In the summer of 1978 there were 11 cases of cholera among residents of southern Louisiana. The causative agent was *Vibrio cholerae* biotype El Tor, serological variety (serovar) Inaba. The source of the bacterium for the first cases of cholera was traced to cooked crabs that had been returned to the containers used for transporting the live crabs. *Vibrio cholerae* bacteria were subsequently isolated from infected patients and sewage during the summer and fall of 1978 (1).

It is generally accepted that cholera is transmitted by food, water, and fomites that are polluted with human feces from acute, subclinical, or carrier cases of cholera (2). Only the O1 Inaba and O1 Ogawa serovars, which possess the A antigen in common and specific C or B antigens, respectively, are considered capable of causing severe disease or epidemics. A third serovar, O1 Hikojima, contains all three somatic antigens A, B, and C. Because cholera is not endemic to Louisiana, an important concern is whether the O1 serovar responsible for the Louisiana cholera cases was imported from an endemic area by an infected traveler or immigrant or is a member of the normal bacterial flora of Louisiana waters. Bashford *et al.* (3) and Colwell *et al.* (4) have provided evidence that O1 serovars and the non-O1 serovars of *V.*

cholerae are normal flora of brackish water; therefore, man is a chance victim of the pathogenic O1 serovars. The difficulty in establishing the O1 serovars as normal flora is that no reliable method is available for isolating specifically the O1 serovars from water or other specimens. For analyzing water, either the Moore swab (1) or filtration (2) when used in conjunction with alkaline peptone broth is selective for *Vibrio* spp. but not for the O1 serovars. Of the vibrios in water the non-O1 serovars predominate (3, 4), and O1 serovars of *V. cholerae* are isolated rarely and only by chance.

We have adapted the principles of serologically specific electron microscopy (5) and the enzyme-linked immunosorbent assay (ELISA) (6) to develop a

method for isolating O1 serovars of *V. cholerae* from environmental water. We used rabbit antiserum to boiled cells of *V. cholerae* Inaba, so that the antiserum contained antibodies against the O1 somatic antigens but not against the *V. cholerae* common flagellar antigens. Initial experiments were done with antibody-coated plastic cover slips which were exposed to dilute suspensions of culture. Bacterial cells that adsorbed to the cover slips were viable as determined by overlaying the cover slips with agar medium and observing colony formation. In later experiments we used as absorbent surfaces microtiter plates used for ELISA (Cook Laboratories, Alexandria, Va.) which had been cut into single well pieces. These were difficult both to prepare and to wash free of excess antibody and nonadsorbed bacterial cells.

We found that the best matrix for immobilizing antibody in the isolation of O1 serovars was polystyrene beads (2.5 by 4.0 mm) (7). Antibody was adsorbed to the beads by incubating 25 g of beads in 100 ml of 0.06M bicarbonate-carbonate buffer, pH 9.6 (6), in a 500-ml Erlenmeyer flask to which 1 ml of antiserum was added. Adsorption was effected over a 6-hour period at 34°C with gentle agitation on a rotary shaker. The beads were then washed three times with 300-ml quantities of 0.01M tris(hydroxymethyl)aminomethane buffer, pH 7.2, containing 0.15M NaCl and 0.1 percent Tween 20 (TST) to adjust the pH of bead surfaces and to remove unadsorbed material. The beads were dried and stored at -60°C until used.

To isolate O1 serovars from water we developed the following method. To a glass column (1.5 by 20 cm) fitted with a Teflon supporting screen, antibody-coated polystyrene beads were added to a height of 10 cm and were covered with TST. One liter of water sample with Tween 20 added to a final concentration of 0.1 percent was passed through the column at a flow rate of about 1 liter per

Table 1. Serological and hemolytic characteristics of isolates from water. The O1 antiserum agglutinates the O1 serovar; Inaba antiserum is specific for the Inaba serovar; Ogawa antiserum is specific for the Ogawa serovar. Agglutinations were performed by slide agglutination (8). Hemolytic activity was determined as described in (8). The isolates W-11, W-15, and W-16 have been identified as toxigenic, classical Inaba of the same phage type as our stock strain Inaba CA 401 (9).

Charac- terization	Isolates					
	W-1	W-11	W-12	W-15	W-16	W-19
Antiserum						
O1	+	+	+	+	+	+
Inaba	+	+	+	+	+	-
Ogawa	-	-	+	-	-	-
Hemolysin	+	-	+	-	-	+