6. Trees were sprayed weekly in spring when shoot expansion was greatest. After mid-January, trees were sprayed fortnightly. Concentrations of insecticide and spreader were those suggested by the manufacturer.
7. All the samples could not be dated with the same degree of confidence prior to 1967 (*8*). However, from most of the samples we were able to obtain accurate long-term datings.
8. Most eucalypts are difficult or impossible to date because of poorly defined annual ring boundaries, a high frequency of intra-annual latewood bands (false rings), and a high percentage of parenchyma tissue that obscures or completely masks the discontinuities in tracheid characteristics defining successive growth rings. In slow growing eucalypts and those subject to severe defoliation from drought, fire, or insect attack, some growth rings may be extremely narrow or even locally absent from parts of the tree's circumference (*4*, *17*). However, because of the distinct seasonality of the subalpine environment, *E. stellulata* and *E. pauciflora* are comparatively easy to date by means of standard dendrochronological techniques [M. A. Stokes and T. L. Smiley, *An Introduction to Tree-Ring Dating* (Univ. of Chicago Press, Chicago, 1968)] of counting and cross-dating. In addition to cross-matching of characteristic narrow rings representing the synchronous response of tree growth to shared environmental conditions in certain years, chronological accuracy was ensured by recognition of a distinctive pattern of frost-damage zones that result from freezing of the stem during or shortly after the period of cambial activity [V. C. LaMarche, Jr., *Univ. B.C. Fac. For. Bull.* 7, 99 (1970)]. Because a frost-damage zone records a short spell of unseasonably cold weather during the growing season, it provides a very valuable internal dating check. Within our study area, a distinctive double frost zone is usually contained in the 1967 ring.
9. Ring widths are normally converted to dimensionless growth indices. To obtain these indices we measured ring widths along the radius of maximum length between pith and bark on each slice and divided each value by the mean ring-width value for the period between 1967 and 1972 for that radius.
10. R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, 1969).
11. A period of declining growth began about 1970 in *E. pauciflora* (Fig. 3).
12. Eucalypts are evergreen, and in these species leaf life is 13 to 15 months. An increase in foliage will therefore make a longer term contribution to plant productivity than if the leaves were shed each autumn.
13. The small response in growth increment in the season of treatment suggests that either annual ring growth draws heavily on stored photosynthate or that annual ring formation had been essentially completed by the time the foliage crop resulting from the spray treatment began to contribute exportable quantities of photosynthate. Phenological observations indicate that the growing season begins in November [R. O. Slatyer, *Oecologia* **24**, 357 (1976)] and lasts through March. By late December of the year of treatment (1972), a large increase in leaf area had occurred in response to the spray treatment and increment cores taken at this time showed that annual ring growth was incomplete. Since cambial activity is not restricted to the early part of the growing season it is difficult to explain the virtual lack of ring-width response to increased foliage area immediately following treatment. A possible explanation is that current photosynthate is used for local canopy building and is neither translocated to the canopy of other stem systems [M. G. Cook and L. T. Evans, in *Transport and Transfer Processes in Plants*, I. F. Wardlaw and J. B. Passioura, Eds. (Academic Press, New York, 1976), p. 393] nor used for ring formation during that growing season. Rather, ring growth may utilize photosynthate stored after canopy construction is completed for the season [H. C. Fritts, *Tree Rings and Climate* (Academic Press, London, 1976)]. This explanation assumes that much of the photosynthate is stored in the roots over winter and is redistributed more or less equally among all stems during the following year, thus increasing foliage volumes and increment growth throughout the tree.
14. These eucalypt species are attacked primarily by sawflies (Hymenoptera: Pergidae) and numerous species of Hemiptera and Coleoptera. Of 48 species commonly attacking *E. stellulata*, more than one-half ate only this food plant. On *E. pauciflora*, 39 species were common and 28 percent of these were host-specific. The total number of individuals in the samples from *E. stellulata* was 60 percent greater than from *E. pauciflora*. Details of collecting methods and species identifications are given in (*15*). Measurements of insect damage were made in 1975–1976. *Eucalyptus stellulata* lost 96 percent of its shoots and 50.5 percent of its leaf area to chewing insects, a significantly greater loss than the 76.8 percent of shoots and 36.7 percent of leaf area in *E. pauciflora*. This measure of damage did not include loss of photosynthate to sucking insects, which were much more common on *E. stellulata* (*15*).
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19. P. A. Morrow, in preparation.
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Brain Tumors in Owl Monkeys Inoculated With a Human Polyomavirus (JC Virus)

Abstract. *Owl monkeys were inoculated intracerebrally, subcutaneously, and intravenously with JC, BK, or SV40 virus. Two of four adult owl monkeys inoculated with JC virus, a human polyomavirus, developed brain tumors at 16 and 25 months after inoculation, respectively. A grade 3 to grade 4 astrocytoma (resembling a human glioblastoma multiforme) was found in the left cerebral hemisphere and brainstem of one monkey. The second monkey developed a malignant tumor in the left cerebral hemisphere containing both glial and neuronal cell types. Impression smears prepared from unfixed tissue of this tumor showed cells that contained polyomavirus T antigen. Virion antigens were not detected. Tumor cells cultured in vitro also contained T antigen but were negative for virion antigen. Infectious virus was not isolated from extracts of this tumor.*

Man is the natural host for two polyomaviruses: JC virus (JCV) and BK virus (BKV). Cryptic infection with JCV and BKV usually occurs during childhood and is a common event (*1*). The JCV has been isolated from brain tissues of patients with progressive multifocal leukoencephalopathy (PML) and is the virus usually associated with this demyelinating disease (*2*). The BKV has been isolated from urine of immunosuppressed patients (*3*). However, this virus has not been associated with any known disease. Infection in man by the simian polyomavirus SV40 has been reported. A major exposure occurred some years ago when a large number of people were inadvertently inoculated with poliovirus vaccine contaminated with SV40. A variant of this virus, SV40-PML was isolated in two cases of PML (*4*). Tumors have been induced in Syrian hamsters by JCV, BKV, SV40, and SV40-PML. The JCV is particularly notable for its strong predilection for producing tumors in the nervous system. Tumors induced by JCV in the hamster include medulloblastoma, undifferentiated neuroectodermal tumor, glioblastoma, ependymoma, pineocytoma, neuroblastoma, and meningioma (*5*). In contrast, BKV and SV40-PML induce only tumors of ventricular surfaces identified as choroid plexus papillomas or ependymomas (*6*, *7*).

Because of the exposure of large numbers of people to these viruses, and because of the association of these viruses with PML and their ability to induce tumors in hamsters, we decided to test their oncogenicity in subhuman primates. Adult owl monkeys were chosen for the first experiment because they had low or undetectable antibodies against JCV, BKV, and SV40. Serum from other New World monkeys had detectable antibody against SV40 and occasionally JCV. Adult feral Colombian owl monkeys (*Aotus trivirgatus*) were inoculated in the following manner. A small hole was made in the skull over each cerebral hemisphere by means of a dental drill (0.75-mm stainless steel bit). The inoculum, 0.15 ml per hemisphere, was de-

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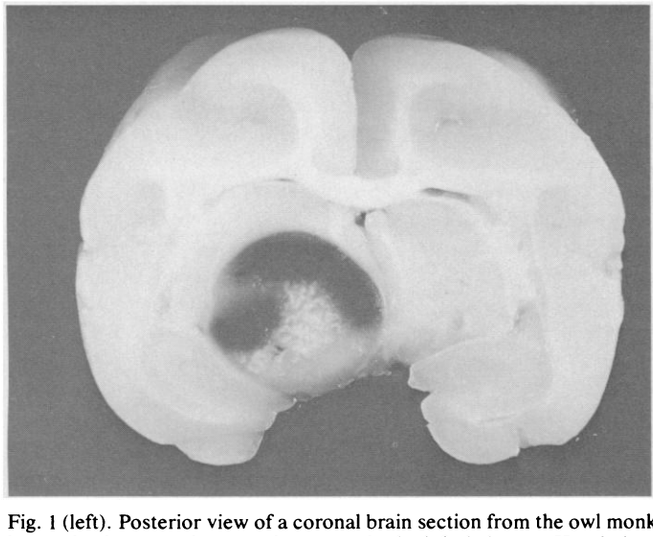
posited deep in the frontal lobes with a 27-guage, 1.5-inch needle. A template enabled us to inoculate approximately the same area in each animal. Additionally, each animal received 1 ml of inoculum intravenously in the saphenous vein and 1 ml subcutaneously in the nape of the neck. Fourteen animals were inoculated with JCV, four with BKV, and four with SV40. The JCV and BKV were grown in primary human fetal glial (PHFG) cell cultures. Viral inocula were prepared as described previously (*8*). Briefly, the procedure included sonication, treatment with receptor-destroying enzyme, and incubation in 1 percent sodium deoxycholate to free virus from cell debris. The hemagglutination titer of the JCV pool was 102,400 per 0.4 ml, and that of the BKV pool was 409,600 per 0.4 ml. The SV40 inoculum was grown in CV-1 cells. The titer of this inoculum was $10^{6.5}$ plaque-forming units per 0.2 ml. To ensure that the cells used to grow the virus inocula did not contain covert agents with oncogenic potential, eight control animals were inoculated in the same manner with an extract of pooled uninoculated PHFG cells and two animals with CV-1 cell extracts. Four additional untreated animals were included in the control group.

Six JCV-inoculated monkeys received immunosuppressive doses of cyclophosphamide and prednisone for 20 weeks: 10 weeks before and 10 weeks after JCV inoculation. Two animals inoculated with PHFG cell extract received immunosuppressants in the same manner as the JCV-inoculated monkeys. Animals inoculated with BKV or SV40 received no immunosuppressant.

Serum specimens were obtained from each animal at monthly intervals. Initially, cerebrospinal fluid samples were also collected monthly from all animals. This procedure was discontinued because it resulted in death of four JCV-inoculated animals. Six other JCV-inoculated monkeys died either of acute subdural hematoma related to a known clotting abnormality or from cyclophosphamide toxicity within 2 months of receiving the virus inoculation (*9*). One animal died 3 months after inoculation with BKV, as a result of nutritional deficiency that was unrelated to the experiment. Six months after inoculation the following monkeys were still alive: four inoculated with JCV, three inoculated with BKV, four inoculated with SV40, eight inoculated with PHFG cell extract, two inoculated with CV-1 cell extract, and two uninoculated controls. Two of the JCV-inoculated owl monkeys and their two controls that received PHFG cell extract had received immunosuppressants.

Samples of serum from each animal were extracted with acetone and titrated for antibodies against JCV and BKV by the hemagglutination-inhibition (HI) technique (*10*). One month after inoculation, all monkeys that received JCV had antibody titers to JCV ranging from 80 to 2560. At the same time, all animals that received BKV had antibody titers to BKV ranging from 160 to 10240. Antibody titers to SV40 virus determined by plaque reduction neutralization test ranged from 80 to $\geq$ 160 in monkeys inoculated with SV40 virus. Animals developed only antibodies that were specific for the virus inoculated. No cross-reacting antibodies were detected by the HI assay. Furthermore, no control or SV40-inoculated animal developed HI antibodies against either JCV or BKV. Two uninoculated monkeys, and animals receiving uninfected CV-1 cell extracts, did not develop SV40 neutralizing antibody. Antibodies against T antigen, detected by an indirect immunofluorescent staining test appeared in the serum of all virus-inoculated animals 3 to 5 months after inoculation. Three years after inoculation (October 1977) all surviving JCV-, BKV-, and SV40-inoculated monkeys had immunofluorescent antibodies against T antigen.

Sixteen months after inoculation one (No. 49) of the four JCV-infected owl monkeys exhibited opisthotonic posturing. The animal had not received immunosuppressive drugs. Penicillin treatment for an inner ear infection was started. The animal did not improve and was killed. Necropsy revealed no evidence of inner ear infection. The whole brain was fixed in 10 percent formalin for 10 days then sectioned coronally. Two tumors were found on gross examination. A round-shaped, markedly hemorrhagic tumor 10 mm in width was found in the thalamus of the left cerebral hemisphere (Fig. 1). Another, separate, round-shaped gray tumor without hemorrhage and measuring 3 mm in width was located in the left rostral brainstem, involving the midbrain and pons. Sections stained with hematoxylin and eosin (H and E) showed that the cerebral tumor was malignant and was analogous to a grade 3 and grade 4 (glioblastoma multiforme) astrocytoma in the human. There were large numbers of thickened blood ve. sels and numerous areas of ne-



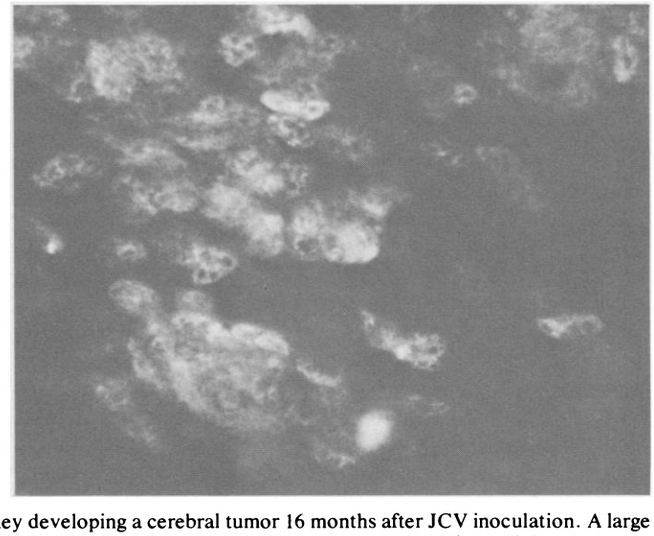


Fig. 1 (left). Posterior view of a coronal brain section from the owl monkey developing a cerebral tumor 16 months after JCV inoculation. A large hemorrhagic, necrotic tumor is present in the left thalamus. Herniation of the tumor across the midline can be seen. Fig. 2 (right). Immunofluorescence showing T antigen in cells of a brain tumor which occurred 25 months after JCV inoculation in an owl monkey. Impression smear stained by indirect anticomplement technique with guinea pig complement and serum from hamsters bearing JCV-induced tumors. Note characteristic sparing of nucleoli.

crosis. Many cells were in mitotic division. Some nuclei were pleomorphic, others of giant size. The features of the brainstem tumor were generally similar to the one found in the cerebral hemisphere. Necrosis, however, was not present in the tumor of the brainstem.

A second owl monkey (No. 26) that received no immunosuppressants developed similar clinical signs 25 months after inoculation with JCV. A mild right hemiparesis and hemisensory deficit were noted. The animal was killed and necropsy performed immediately to obtain fresh specimens for virologic and electron microscopic studies. The cranial vault and meninges appeared normal. The left hemisphere was swollen and a mass was easily palpated. Upon sectioning, a nonhemorrhagic tumor, 15 by 9 mm, was found deep in the left cerebral hemisphere, especially in the thalamus, extending to but not crossing the midline. Portions of this tumor were removed and processed for virus isolation, tissue culture, identification of T and virion antigens, passage in other monkeys, and light and electron microscopy. The remainder of the tissue was fixed in 10 percent formalin. In H and E stained sections the tumor varied in appearance. In some areas, the type and arrangement of neoplastic cells was that of a neuroblastoma. The appearance in other areas was characteristic of an astrocytoma. This tumor was also malignant and there were many cells in mitosis.

Impression smears of the tumor were fixed in acetone for 5 minutes and stained for T antigen by an indirect immunofluorescent method with pooled antiserum from hamsters bearing JCV-induced tumors. Numerous cells containing intranuclear T antigen were observed (Fig. 2). Other smears were fixed in acetone for 15 minutes and treated with hyperimmune rabbit antiserum to JCV with broad specificity. This serum will detect capsid antigens of JCV, BKV, and SV40 by immunofluorescence. Virion antigens were not found in tumor cells. Two attempts to culture virus from a 10 percent extract of the frozen tumor tissue were unsuccessful. Each attempt consisted of three serial passages in PHFG cell cultures. Attempts to isolate JCV by means of cocultivation and fusion are now in progress. The tumor was established in tissue culture and cells of the 17th subculture contained intranuclear T antigen but had no detectable virion antigens.

Only central nervous system tumors were found in the two nonimmunosuppressed owl monkeys described above. One immunosuppressed JCV-inoculated owl monkey (No. 30) developed a mild right hemiparesis 27 months after inoculation. The animal was killed 29 months after receiving JC virus. Needle tracts were found bilaterally in the deep cerebral hemispheres. In the left hemisphere the needle tract was located lateral to the thalamus and in part within the internal capsule. A zone of tissue destruction and cellular reaction surrounded this tract. A cerebral tumor was not present. We thought that the mild right hemiparesis might be the result of tissue destruction in the left internal capsule. The remaining JCV-inoculated monkey, which had been immunosuppressed, is still living. This animal has failed to develop clinical signs of neurologic disease although it continues to have antibody to T antigen 3 years after inoculation. All monkeys inoculated with BKV and SV40 remain seropositive for their respective viruses at this time. All BKV and SV40 inoculated and control animals remain clinically normal. One control animal inoculated with PHFG cell extract was killed. Gross and histological examinations failed to reveal any abnormalities of the brain in the monkey.

The occurrence of brain tumors in two of four owl monkeys inoculated with JCV without the appearance of tumor in control animals or in monkeys inoculated with BKV or SV40 suggests that the oncogenicity of JCV extends to nonhuman primates. This conclusion is supported by several points: (i) to our knowledge neither spontaneous nor experimentally induced brain tumors have been reported previously in owl monkeys; (ii) the occurrence of multiple tumors, as seen in one monkey, and the histologic diversity found in three tumors fits the characteristics of JCV oncogenicity in hamsters; and (iii) the presence of T antigen in tumor cells and the lack of capsid antigen or infectious virus suggests that JCV is not merely a passenger in the tumor but is present in the tumor cells in a state analogous to that in JCV-induced hamster tumors and in cells transformed by polyomaviruses.

Additional evidence suggests a possible relation between the primate polyomaviruses and human brain tumors. Giant bizarre astrocytes resembling malignant astrocytes of human glioblastomas have been seen frequently in brain lesions of PML patients (*11*). Multiple astrocytic gliomas have also been found in a PML patient (*12*). Early passage cell cultures of two human meningiomas have been shown to contain SV40-related antigen (*13*). Meinke and co-workers using DNA reassociation techniques found evidence of SV40-related DNA in a human glioblastoma (*14*). Furthermore, DNA from 5 of 12 human tumors and three of four human tumor cell lines was reported to contain information related to DNA of BKV (*15*). The significance of these hybridization results and other observations, although suggestive in their implication, must be critically evaluated to assess whether these viruses have any etiologic relation to human cancers.

The question of an etiologic association between the primate polyomaviruses (JCV, BKV, and SV40) and human cancer has also been studied serologically. The T antigens of JCV, BKV, and SV40 show a high degree of antigenic similarity (*16*). Animals bearing tumors induced by these viruses usually contain antibodies to T antigen in their serums. Serums from human cancer patients, including patients with tumors of the nervous system, were tested for antibodies to T antigens of BKV and SV40. A low percentage of serums from cancer patients was found to contain antibodies to BKV T antigens (*17*). Cells from human tumors have been examined for the presence of SV40 and SV40-PML T antigens (*7*, *13*). Of the variety of human brain tumors studied, only meningiomas (two of seven) were found to contain T antigen when tested with SV40 antiserums. These studies suggested that polyomaviruses were not widely associated with human cancer. This generalization may not be justified in regard to brain tumors since they are believed to be relatively inaccessable to immunologic responses.

It is hoped that studies of JCV in owl monkeys will confirm its oncogenicity for primates and lead to a more thorough understanding of the role of this virus in human neoplasia and chronic central nervous system disease.

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Extreme Myopia Produced by Modest Change in Early Visual Experience

Abstract. *Chicks whose vision was restricted to the frontal visual field became extremely myopic (mean, −10 diopters; maximum, −24 diopters) and had eyes of increased axial length. Animals restricted to lateral field vision did not differ from normal animals. Monocular deprivation of form vision also produced myopia and eye enlargement and, in addition, produced increased anterior chamber depth.*

Myopia is an extremely prevalent human condition which, when severe, is associated with progressive retinal pathology leading to blindness (*1*). For many years environmental factors have been theorized to play a role in the development of myopia. One such hypothesis is that close visual attention leads to myopia. This relationship is weakly suggested by (i) the tendency of children to become more myopic from the age of six, when schoolwork starts (*2*); (ii) the high incidence of myopia in Eskimo schoolchildren whose parents were illiterate and tended to be hyperopes (*3*); and (iii) the tendency of men working in the close quarters of missile launch facilities to develop myopia related to their length of service (*4*). Young tested this close-work theory by keeping pig-tailed macaques seated in a monkey chair in an enclosed visual space for about a year; he reported that these animals developed a small amount of myopia (adults, 0.75-diopter change; young, 1.75-diopter change), which was retained for many months after their removal from the chair (*5*). In addition, cats raised in cages are about 2 diopters myopic compared with feral cats (*6*).

The amount of myopia found in all of these studies on environmental influences is very small. It is possible, therefore, that some of these myopias arise from an increased tonus of the muscles of accommodation, whereas the higher myopias found clinically have an anatomical origin. In part for this reason the hypothesis that there can be experiential influences on myopia has not fared well in comparison with the so-called biological theory that refractive errors are a genetic-embryological phenomenon (*7*).

We now report that restricting the vision of chicks to their frontal visual field produces extreme changes in ocular refraction (to a maximum of 24 diopters of myopia), which are not produced by restriction to the lateral visual field.

To restrict chicks to their frontal field of view, we developed lightweight, translucent, hemispherical occluders that fit over each eye and were glued to the skin with collodion (*8*). A trapezoidal notch cut in the front of the occluder permits the birds to have frontal vision (Fig. 1A). To restrict birds to lateral vision in one eye, an opaque vinyl cylinder 7 mm high was glued around the eye with collodion (Fig. 1B). Both types of occluders were put on at hatching and were exchanged for successively larger ones as the animals grew.

When the animals were 4 to 7 weeks old, their eyes were refracted by one of us (Trachtman), who is a retinoscopist familiar with refraction of the eyes of small animals. Measurements were made by streak retinoscopy in the horizontal meridian, 90° to the sagittal plane of the head, which is approximately 30° temporal to the optic axis. Neither the retinoscopist nor the person holding the animals knew to which group the animal belonged. We assessed the reliability of the measurements by twice determining the refractions on 19 animals ($r = .95$).

Postmortem measurements by means of an ocular micrometer in a dissecting microscope were made on formalin-perfused enucleated eyes from a sample including the animals refracted. The anterior chamber depth was approximated in this series of measurements and was also roughly estimated in living animals by a photographic technique (*9*).

The animals whose visual experience was confined to the frontal visual field were extremely myopic (mean, −10 diopters), unlike the lateral-field animals (mean, +1.9 diopters) (Mann-Whitney U test, $P < .001$), which did not differ from the normal animals (Fig. 2) (*10*). Similarly, the axial length of the eyes of the frontal-field animals was significantly greater than that of either the normals ($P < .01$) or the lateral-field animals ($P < .05$) (analysis of variance with Newman-Keuls test), which did not differ from each other (Fig. 3A).

It seems reasonable to suppose that the eyes of the frontal-field birds experienced more close vision than did the restricted eye of the lateral-field birds. In the frontal-field birds, vision was limited to the area around the beak, and the beak is a chick's only means of feeding and tactile exploring; in contrast, when the lateral-field birds approached an object, the eye with the occluder looked off to one side. There is evidence that in pigeons the frontal field is normally used for close vision and the lateral field for distant vision (*11*). The myopia is not analogous to a monocular visual deprivation effect, involving the animal's suppressing one eye in favor of the other,

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