The Production of a Virus-Induced Tumor in the Central Nervous System of Monkeys*

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In 1958 at Yaba near Lagos, Nigeria, Bearcroft and Jamieson recorded the appearance, spread, growth, and spontaneous regression of a tumor among Indian rhesus monkeys of both sexes in adjacent pens. Transmission was attributed to contact between animals or to arthropod vectors. Andrews et al. believed the causative agent was a virus and demonstrated that filtrates of the neoplastic material and extracts of tissue-culture preparations were active in producing tumors. Niven et al. reported that the inclusion bodies of the neoplastic cells gave positive histochemical tests for desoxyribonucleic acid, and in addition to Owens et al. they found elementary bodies identical with those in other lesions produced by pox virus. Ambrus et al. determined that the virus induced lesions in Asiatic but not in African or in South American monkeys. Intravenous inoculation of cell-free filtrates of tumor produced nodules of tumor in muscle, subcutaneous tissue, heart and lungs; no lesions were found in the brain. Sproul et al. investigated the pathogenesis of the tumor and found that it developed from histiocytes and that regression was an individual cell phenomenon unrelated to circulating neutralizing antibody. The virus has been found by Grace et al. to produce histiocytomas in man. In view of its possible application to neoplastic disease in man, an investigation of the factor influencing development of a brain tumor with the Yaba virus was begun. With different routes of injection and with transplantation of neoplastic tissue to the brain, these experiments have shown that clinically evident tumors of the central nervous system in monkeys could be produced by transplantation of neoplastic tissue to the brain.

Materials and Methods

Two different preparations of Yaba virus were used in these experiments: extracts of tissue-culture media infected with Yaba virus and suspensions of tumor homogenate. No significant difference in the production of tumors was noted between the two preparations. Material for tissue culture was kindly provided by Dr. David Yohn of the Roswell Park Memorial Institute. To prepare the homogenate, the Yaba tumor was excised from an animal 6 to 20 days after inoculation with virus. A suspension of tumor (20 percent weight per volume) in sterile normal saline was homogenized in an omni-mixer at high speed for approximately 5 min. The homogenate then was placed in sterile bottles and stored at -20°C.

Rhesus monkeys caged in pairs were used in all these experiments. Except for subcutaneous injections all procedures and tests were carried out with sterile techniques under pentobarbital sodium anesthesia.

Subarachnoid Injection. In 2 monkeys, a No. 19 needle was introduced into the cisterna magna and spinal fluid was withdrawn into a syringe to confirm location in the subarachnoid space. Two-tenths to 0.3 cc. of extract of tissue culture or tumor homogenate was injected slowly into this space. In 1 animal a sample of cerebrospinal fluid was taken for determination of total protein.

Intracerebral Injection. In 2 monkeys a 2-mm. trephination of the mid-parietal region of the skull was performed, and a No. 19 needle was inserted to a depth of 10–15 mm. through the hole. Aspiration by syringe did not reveal spinal fluid and location in the substance of the brain was therefore assured. Three-tenths to 0.35 cc. of Yaba homogenate or extract of tissue culture was in-
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Injected slowly into the brain to prevent reflux along the tract of the needle through the skull. The trephine hole was closed with bone wax, and the operative site was sutured.

Production of Foreign-Body Reaction. With the knowledge that Yaba virus grows in histiocytes and that talcum powder produced a granulomatous response containing histiocytes in sciatic nerve, talcum powder was introduced into the central nervous system to provide a histiocytic soil in which the Yaba virus might grow. Six days after the introduction of talcum powder into the brain Yaba virus was injected into the same area. To perform this experiment a 2-mm. trephination of the occipital skull was made in 2 monkeys. The polyethylene cannula containing talcum powder was inserted into the hole to a depth of 10 to 15 mm. A second polyethylene cannula was used as a trochar to expel the talc into the brain through holes made in the tip of the larger catheter.

Both cannulae were secured in place so that the virus could be injected into the same area; the overlying skin was sutured. Six days later 0.2 cc. of Yaba homogenate was injected slowly into the large cannula. The smaller cannula was used as a trochar to expel into the brain substance the Yaba contents remaining in the larger cannula. Both cannulae then were removed, bone wax was placed in the trephine holes, and the skin was sutured.

Transplantation. Six monkeys were given epicraniac subcutaneous injections of Yaba homogenate. In 6 to 18 days the tumors were excised for transplantation to the brain. A trephination of the parietal skull was performed with a circular saw 10 mm. in diameter, the dura mater was incised, and the frontoparietal cortex was separated with a periosteal elevator. The surrounding connective tissue was removed from the tumor and a piece of tumor, approximately 2 X 5 X 6 mm., was implanted in the separated cortical area. The dura mater was sewed, the bone fragment was replaced, and the skin was sutured. Various tests such as electroencephalography, lumbar puncture, scan, angiography, and pneumoencephalography were performed on 2 monkeys.

All animals were observed for periods up to 1 year for neurological deficits.

Results

Subarachnoid Injection. Of the 2 animals in this series, 1 is still under observation 1 year after injection, and no clinical signs of a space-occupying lesion have been noted. The second animal, sacrificed 45 days after injection, also had no neurological deficits. A subcutaneous Yaba tumor was found around the area of inoculation in the latter animal. The tumor did not penetrate the skull or invade the central nervous system. No abnormal gross or microscopic changes were noted in the brain.

Intracerebral Injection. One year after injection, 1 animal still has no neurological deficits. A second animal, sacrificed 45 days after injection, had a subcutaneous Yaba tumor mass over the site of injection. The tumor was not invasive, and no gross or microscopic pathologic changes were noted in the brain.

Production of Foreign-Body Reaction. For the 101-day period after injection, both animals had no neurologic deficit or evidence of tumor. At autopsy, brains revealed no tumor, but talcum powder phagocytized by histiocytes or microglia could be seen in the area of penetration.

Transplantation. Of the 6 animals in this series, tumors did not develop in 2. Of the 4 monkeys in which intracranial tumors developed, 2 were given tests before sacrifice to identify the clinical appearance of the lesion. In 2 of 6 animals, intracranial tumors did not grow. The area of implantation in one of these animals was biopsied 21 days after transplantation. Ten months later, this animal showed no clinical signs of a space-occupying lesion of the brain. The second animal was found dead in his cage 23 days after transplantation. On pathological examination, no evidence of growth of the transplant was found. Microscopic examination revealed necrosis of the transplant. Some histiocytes were present, but tumor cells of Yaba type were not seen. The animal had clinical and pathological evidence of colitis.

Intracranial tumors developed in 4 of the 6 remaining monkeys. One of these animals (M-4) had not been observed for 3 days prior to death, but had no neurologic deficit prior to that time. The animal was found dead 47 days after transplantation of tumor. A large Yaba tumor protruded from the right cerebral hemisphere (Fig. 1). No bone fragment was replaced at the site of trephination in this animal.

In the remaining 3 monkeys in this series paresis of the leg or paralysis developed on