TRANSMISSION OF A HUMAN PAPILLOMA TO MONKEYS

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Persistent efforts by numerous investigators seeking to transmit malignant tumors from human beings to laboratory animals have failed to produce adequate proof of their successful transmission or transplantation. Although considerably less work has been devoted to the transfer of benign and precancerous tumors of man, successful results have been obtained much more frequently with these lesions than with malignant tumors. More extensive study along this line would seem to be warranted for, as precancerous lesions, benign tumors may be related to malignant growths. Moreover, many destructive tumors of human beings are made up of cells that are fundamentally benign. Adenomas of the islands of Langerhans and astrocytomas, for example, are benign in the sense that they usually grow rather slowly and do not metastasize; yet both are commonly fatal.

During the past two years we have attempted by various methods to transfer both benign and malignant tumors from human beings to monkeys and rabbits. Most of our experiments have yielded negative results. We have, however, obtained some positive findings that appear significant. In this paper we report the transfer of a cutaneous papilloma of man to M. rhesus, with observations that strongly suggest the presence of a filtrable virus.

Several types of benign proliferative lesions of the human skin have been shown to be caused by filtrable viruses, notably molluscum contagiosum (Wile and Kingery), the common wart (Kingery), the venereal wart (Serra; Lipschütz; Dracoulides), and the laryngeal papilloma. Ullmann (1923–24), using first an emulsion and subsequently a cell-free filtrate from a suspension of finely ground lesions, transmitted laryngeal papillomas serially through two generations in human subjects. He also induced growth of the lesion on the vaginal mucosa of a dog.

Inclusion bodies characteristic of virus diseases have been described as occurring in each of the four types of transmissible, benign, proliferative lesion mentioned above, while positive results of critical experiments in which these tumors were transmitted by cell-free filtrates have firmly established the fact that they are caused by ultraviruses. Many of the benign tumors in lower animals have also been shown to be due to viruses. It would seem, therefore, that some of the benign tumors of man hitherto considered idiopathic may likewise be of virus origin.
SOURCE OF MATERIAL

The tumor used in our experimental study was a small, highly cornified, papilloma on the skin of the left upper eyelid of a man aged seventy-one, who had been treated at the University Hospitals for diabetes mellitus, angina pectoris, arteriosclerosis, and gangrene of the extremities. It was removed Aug. 8, 1938, after a known duration of three years and six months.

Approximately 8 mm. in height and 3 mm. in diameter, the tumor appeared as a slender, non-pigmented, highly cornified cutaneous papilloma. Microscopically, the stratum corneum was seen to be greatly thickened. The stratum granulosum in some parts was made up of several layers of conspicuous cells with numerous granules, but in other places was represented by fewer cells with only a small number of inconspicuous granules. The stratum malpighii was greatly thickened and exhibited numerous mitoses in its basal layers. Slender dermal papillae extended upward into the epithelium. A moderate infiltration of lymphocytes was noted in the connective tissue at the base of the lesion (Fig. 1).

Fig. 1. SECTION OF TUMOR OF UPPER EYELID (UNIVERSITY HOSPITALS PATIENT NO. 662764) SHOWING HAIR FOLLICLES, SEBACEOUS GLANDS, AND THE BASE OF THE TUMOR, WHICH HAS THE APPEARANCE OF A BENIGN CUTANEOUS HORN

PREPARATION OF INOCULUM AND METHOD OF INJECTION

Following excision of the papilloma, a portion of the base was cut off, finely ground with 0.9 per cent sodium chloride in a mortar, and used as the
inoculum. The remainder of the tumor was reserved for paraffin sections. The suspension of ground tumor was injected into the left eye in three adult monkeys, on Aug. 8, 1938. The monkeys had been kept in the laboratory for eighteen months, and had previously been inoculated in parts of the body other than the eye with material from benign and malignant tumors of human origin.

The injection of the tumor suspension was accomplished by passing the needle through the conjunctiva lateral to the limbus of the eye; then medially and anteriorly through the sclera, the outer border of the ciliary body, and the iris, into the anterior chamber. After a small amount of the suspension had been injected into the anterior chamber, the needle was withdrawn, inoculating tissues in its path.

**Experimental Tumors**

Twenty-four hours after inoculation, the eyes of all three monkeys were clear, showing no evidence of bacterial infection. Careful examination thirty-one days later disclosed no sign of infection and no tumorous growths. Sixty days after injection a lesion was present at the site of inoculation on the left eye of each monkey, as follows:

**M. rhesus No. C-521:** On Oct. 12, a pigmented tumor was present at the site of inoculation, lateral to the limbus of the left eye (Fig. 2). It had increased only slightly in size by Dec. 2, when the eye was removed for microscopic study. The tumor was a heavily pigmented elevation approximately 2 mm. in diameter and 1 mm. in height. The histopathology is depicted in Figs. 3 to 7.

**M. rhesus No. C-522:** On Oct. 10, a pigmented lesion was present on the surface of the eyeball at the point of inoculation. The eye was removed on that date for paraffin section. The histologic structure of the tumor is shown in Figs. 10 and 11.
M. rhesus No. C-527: On Oct. 10, there was a small area of opacity on the cornea and a pigmented tumor at the site of inoculation. The tumor had not increased in size by Dec. 7, but seemed to have regressed slightly. The eye was removed on that date. In an attempt to retransmit the growth, the cornea and conjunctival lesions were ground in a 0.9 per cent sodium chloride solution and injected into the anterior chamber of the left eye in three monkeys, Nos. C-727, C-728, and C-729. These injections were made through the cornea and not by the lateral approach used in the original inoculations.

The simultaneous appearance of the three tumors at the sites of injection can leave no doubt that the growths were experimentally produced. The lesions appeared after an incubation period of about thirty days and reached their maximum growth in the following thirty days. They must have appeared very soon after the examination on Sept. 9, for they were fully developed on Oct. 10. One tumor was removed 60 days after inoculation, another was excised for section 115 days after injection, and the third was taken for retransmission on the 120th day. There was no significant increase in the size of the latter two tumors between the 60th day and the time they were removed.

The lesions produced in the three monkeys were similar in gross appearance. They varied only slightly in size, all being about 2 mm. in diameter and 1 mm. in height. The uninterrupted continuity of the conjunctiva over the tumors was indicated grossly by their smooth glistening surfaces and was demonstrated by the findings in microscopic sections. The growths were heavily pigmented, brownish-black in color. A deformity of the iris similar to that shown in Fig. 2 was evident in all three monkeys. This fact suggested the possibility that prolapse of the iris might be the cause of the tumor formation, but histologic studies conclusively ruled out this possibility.

Serial sections of the eye removed from monkey No. C-521 provide a complete picture of the histopathology of the tumor produced in this animal (Figs. 3-7). The tumor is covered with conjunctiva, beneath which there is a dense vascular stroma composed of connective tissue and chromatophores derived from the underlying uvea. Penetrating downward through the center of the tumor is a cystic epithelial growth continuous with and derived from the conjunctiva. The epithelial processes were found to be more extensive than the superficial tumor seen grossly, as they penetrated through the sclera and followed the line of inoculation between the choroid and the sclera to the ciliary body. Serial sections showed that the longest process was a slender epithelial tube that extended, underneath the sclera, 1.2 mm. beyond the fold of conjunctiva from which it originated and 0.65 mm. beyond the border of the superficial tumor.

In the same eye, at the base of the iris, close to the limbus and some distance from the lesion at the site of inoculation was a small circumscribed mass of cells that appeared to be a separate tumor. These cells were obviously derived from chromatophores, as they had abundant cytoplasm in which numerous granules of brown pigment were present (Figs. 8 and 9).

Sections of the eye removed from monkey No. C-522 revealed a tumor with a histologic structure markedly different from that of monkey No. C-521. As shown in Figs. 10 and 11, focal proliferation of conjunctival epithelium had caused the tumorous projection seen grossly, as well as the formation
Figs. 3 and 4. Serial Sections from Tumor in Monkey No. C-521. X 45

Fig. 3 (serial section 620) is a cross-section from the center of the tumor, showing the growth to be composed of several cellular elements. From an epithelial surface fold 0.6 mm. in length (serial sections 620-695) epithelium penetrates into the deeper structures.

Fig. 4 (serial section 600) is a section taken at a distance of 160 microns from that in Fig. 3. The cystic epithelium is seen to extend directly through the sclera.
Fig. 5 (serial section 575) is a section extending through the superficial lesion near its border, 360 microns distant from the section shown in Fig. 3. It shows an epithelial process growing into the deeper tissues below the sclera.

Fig. 6 (serial section 520) shows an extension of the epithelial tumor into tissues below the sclerotic coat and beyond the border of the superficial lesion.

of irregular masses of epithelial cells penetrating into the subconjunctival connective tissue. There was a moderate proliferative reaction of the connective tissue concurrent with the epithelial changes.

As already mentioned, no sections were available from the third monkey,
FIG. 7. (SERIAL SECTION 520) FROM MONKEY NO. C-521: HIGHER MAGNIFICATION OF THE DEEP TUMOR PROCESS ILLUSTRATED IN THE PRECEDING SECTIONS, SHOWING THE TYPICAL EPITHELIAL STRUCTURE OF THE LESION. × 540

No. C-527, as all of the pathologic tissue from this animal was used in attempted retransmission.

RETRANSMISSION

Three *M. rhesus* monkeys were inoculated with a suspension of the pathologic tissues of the eye of monkey No. C-527 on Dec. 7, 1938. The injection was made by passing the needle through the cornea into the anterior chamber, so that inoculation of the anterior chamber and the cornea only was accomplished.

Following are brief protocols of the three animals inoculated:

*M. rhesus No. C-727*: One eye of this monkey was inoculated Dec. 7, 1938, as described above. The animal died of pneumonia Dec. 28, 1938. At necropsy the eye was clear, and sections failed to show any evidence of incipient tumorous growth.

*M. rhesus No. C-728*: On Dec. 7, 1938, an injection was made into the anterior chamber of one eye of this animal, as described above. On Jan. 6 and 12, 1939, the eye appeared normal and showed no sign of a tumor. The animal died on April 3 with no evidence of papillomatous growth in the eye.

*M. rhesus No. C-729*: The anterior chamber of one eye was inoculated through the cornea on Dec. 7, 1938. Examination on Jan. 6, 1939, revealed what seemed to be a small growth on the iris and a strand of tissue extending between the iris and the cornea. The eye was removed, and the pathologic tissues were ground in saline solution for injection. Inoculation of this suspension into the eyes of two monkeys yielded negative results.

Inasmuch as the attempt to retransmit the tumor was clearly a failure in
two of the three monkeys, and since the significance of the lesion in the eye of the third was questionable, the tumor was not considered to have been transmitted beyond the first generation. The only tumor used in the attempted retransmission was excised two months after its period of growth had ended. This delay might well have been sufficient to cause the death or attenuation of a virus present in the lesions of the first generation. Moreover, the route of injection in the second generation was much less conducive to a successful
"take," because it failed to include that tissue which, in the first generation, had formed the tumors; *i.e.*, the conjunctiva.

**Discussion**

From the evidence here presented it is apparent that a human papilloma has been reproduced in monkeys. The origin of the tumors experimentally produced may be explained logically in either of two ways. They may have resulted from heterotransplantation of human papillomatous cells or they may...
have been caused by the stimulation of cellular growth by a virus derived from the human papilloma. Each of these processes should produce characteristics sufficiently well defined to permit identification of the type of transfer.

Although it is possible that a heterotransplantation from man to monkey took place in our experiments, this is doubtful, for it is highly improbable that this would have occurred so uniformly in all three animals inoculated. It is well known that the direct heterotransplantation of tissues is rarely, if ever, successful among higher mammals (except, perhaps, as an independent tissue growth in the ocular chambers). Intensive study of serial sections of two of the three tumors produced in monkeys reveals that the lesions are composed of hyperplastic epithelium continuous with the conjunctival epithelium. It seems most unlikely that human epithelium would merge with the epithelium of the monkey to produce such continuity.

It is noteworthy that three tumors were produced by three inoculations of the same material. This fact, in view of general failure of the transfer of human tumors to animals, precludes an accidental result. All three lesions appeared simultaneously after an incubation period of about thirty days and all reached maximum or nearly maximum growth within another thirty days. These observations point to a uniform process as a basis of the growth of the tumors and suggest the presence of a virus.

The two tumors sectioned were alike in that both were derived from conjunctival epithelium, but the details of structure were quite dissimilar. Moreover, accompanying one of the papillomas was a small tumor of pigmented cells, possibly an anomaly but more probably a result of the process that caused the main tumor. It is known from work with the Shope papilloma that a virus may produce different types of tumor in a single animal. Smooth, fleshy tumors and hard, rough horns may occur on a rabbit infected with this virus. The variations in the experimentally produced tumors in monkeys may be analogous to the diversity of tumors produced by the virus of Shope.

It seems significant, also, that the three similar experimental tumors developed simultaneously in the three monkeys after an identical period of incubation. In reports of the transmission of other benign proliferative lesions of man (common warts, laryngeal papillomas, and venereal warts), incubation periods of one to six months have been observed. Accordingly, the incubation period of about thirty days noted in our animals is well within the usual range for lesions of this type. It seems reasonable to conclude from these considerations that the tumors were produced by an infective agent, perhaps a virus derived from the original tumor and capable of stimulating proliferation of the conjunctival epithelium of monkeys, rather than by a heterotransplantation of cells.

**Summary**

1. Inoculation of the eyes of three monkeys with a saline suspension of a finely ground, cutaneous papilloma from a human patient resulted, in all three cases, in the proliferation of conjunctival epithelium to form small tumors at the inoculation site.

2. The tumors developed simultaneously after an incubation period of
about thirty days, and attained maximum growth within the following thirty
days.

3. The formation of the tumors by proliferation of the conjunctival epi-
theelium of the experimental animals is highly suggestive of the presence of an
infectious agent, perhaps a virus, in the human papilloma.

Bibliography