Rabbit Oral Papillomatosis: Ultrastructure of Experimental Infection

E. J. RDZOK, N. L. SHIPKOWITZ, AND W. R. RICHTER

Departments of Pathology and Infectious Disease, Abbott Laboratories, North Chicago, Illinois

Summary

Oral papillomatosis of the rabbit was produced by experimental inoculation with tissue homogenates, its course of development being studied by gross observation and electron microscopy. Prior to the formation of a definite papilloma, the inoculation site was edematous and no virus could be demonstrated. Gross evidence of a well-defined tumor coincided with the finding of the virus at 14 days postinoculation. After 28 days, signs of regression of the tumor were noted. At the termination of the experiment, which was 100 days postinoculation, the tumor was small and less well defined; although regression was pronounced, the virus was still demonstrable. The well-developed tumors of experimentally infected rabbits appeared the same, and the ultrastructure was similar in all respects to the naturally occurring rabbit oral papillomatosis.

Introduction

Oral papillomatosis of the rabbit is a benign tumor usually found on the ventral surface of the tongues of domestic rabbits. These tumors (up to 5 mm in diameter and 4 mm in height) consist of small, gray-white, sessile or pedunculated nodules that are often multiple and sometimes numerous. Filtrates of the papilloma are transmissible to other rabbits but not to other species. There is no cross-immunity relationship with the Shope papilloma.

The condition, originally described by Parsons and Kidd in 1936, has received little attention since that time (8, 9). The tumors are benign, cause little inconvenience to the host, and are not noticed unless the oral mucosa is carefully inspected (4). The virus that causes this tumor has provisionally been placed by Melnick (6) and by Andrewes (2) in the papovavirus group. The agent is filtrable through Berkefeld V and N candles, survives at least 2 years in 50% glycerol at 40°C, and is able to withstand heating at 65°C for 30 min.

In a previous paper (10) we described the appearance of well-developed, naturally occurring lesions by use of light and electron microscopy. The present report deals with experimentally induced lesions and the early stages of papilloma development.

Material and Methods

A naturally occurring rabbit oral papilloma was carefully lifted and cut from the base of the tongue of a white, mature, domestic rabbit. A homogenate of the papilloma was made by grinding 7-10 mg of tissue in a Ten-Broeck grinder with a total of 4 ml of physiologic saline.

The left lateral aspect of the ventral tongue surface of 18 healthy, white, young domestic rabbits was injected with the papilloma cell suspension. Each rabbit’s tongue was inoculated s.c. with 0.05 ml of the triturated suspension. The animals were examined at time intervals ranging from 5 to 100 days postinoculation. At selected periods, the injection site or the resultant papilloma was excised and prepared for ultrathin sectioning and for negative staining (see Table 1).

Fixation of the tissue was in Veronal acetate-buffered 1% osmium tetroxide (7) with the addition of sucrose (3). The tissue was dehydrated and embedded in Epon 812 (5). Ultrathin sections were stained with uranyl acetate for examination by electron microscopy. Sections 0.5-2.0 μ in thickness were stained with toluidine blue and examined by light microscopy. Negative staining was accomplished with 2% phosphotungstic acid adjusted to pH 7.0 with 1 N potassium hydroxide.

A rapid technic for establishing the presence of the virus was also used. The presence of virus in suspected papillomata could be confirmed in less than 15 min with this method. An excised papilloma was triturated in a Ten-Broeck tissue grinder with the addition of 4 ml of distilled water. Equal parts of the homogenate and phosphotungstic acid were mixed and then used to coat collodion membrane, carbon-backed grids. Distilled water rather than saline was used to prepare this homogenate in order to avoid masking the viral particles by salt crystals. (Fig. 8).

Results

Definite papillomata were 1st observed at 14 days postinoculation and were seen in all animals examined after this (Table 1). Four rabbits sacrificed prior to the 14th day showed no evidence of papilloma formation even though an extensive search of the tissue was made. The inoculation site on the tongue was edematous, but the overlying epithelium showed no histologic alterations or presence of the virus. Tissue homogenates made from the injection sites also failed to show virus particles.

The papillomata 1st observed at 14 days postinoculation were well defined; they were about 0.5-1.0 mm in size and could be raised from the surface of the tongue with ease. The tumors generally reached a maximum diameter of 1-2 mm at the end of 1 month, after which gradual regression was noted. At 100 days postinoculation the lesions were smaller and less well defined, but observations were not made beyond 100 days. The experimentally produced papillomata had the same gross appearance as those occurring in a natural infection. Extensive experimentally produced papillomatosis is illustrated in Fig. 1.

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FIG. 1. An experimentally produced rabbit oral papillomatosis. X 5.
FIG. 2. Oral papillomatosis of the rabbit. Light microscopy. X 450.
FIG. 3. The viral inclusions as shown with oil immersion. X 2400.
FIG. 4. Twenty-eight days postinoculation. Tightly packed crystalline inclusions. The horizontal line represents 1 μ. X 22,500.
TABLE I

**Correlation of Gross Evidence of Papilloma and the Presence of Virus in Experimentally Infected Rabbits**

<table>
<thead>
<tr>
<th>Postinoculation interval (day sacrificed)</th>
<th>Gross evidence of papilloma</th>
<th>Virus particles observed by electron microscopy</th>
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* Two animals were used for each group.

Thick sections (1–2 μ) of embedded papillomata gave the typical appearance of a benign epithelial neoplasm with numerous foci of proliferating cells arranged concentrically around a core of connective tissue and capillaries (Fig. 2). Intranuclear inclusions were most advantageously seen by using somewhat thinner sections (0.5 μ) and oil immersion light microscopy (Fig. 3).

By comparing adjacent sections with the light and electron microscopes, it was established that these inclusions consisted of virus particles usually in a crystalline arrangement (Fig. 4). Inclusions were relatively common in all lesions, and it was possible to screen papillomata for the presence of virus by checking for inclusions with the light microscope. Whenever inclusions were detected by light microscopy it was possible to find virus by means of electron microscopy, but it was never possible to find virus in tissues where inclusions were not observed. Nuclei containing inclusions were found in a narrow 1- or 2-layer zone of the epithelium. The depth of the infected layer in the epithelium varied from animal to animal, but inclusions never occurred in the basal cell layer.

Papillomata examined 14 and 17 days postinoculation were small but histologically indistinguishable from older, experimentally produced or naturally occurring tumors. At this time (14–17 days) the intranuclear inclusions were not as numerous or as large as those found in the older lesions. The inclusions consisted of a random arrangement of viral particles (Fig. 5), or occasionally a loose crystal grouping or packing (Fig. 6). The cell layer containing virus was always found deep in the epithelium just above the basal cell layer and never near the surface at 14–17 days postinoculation. This infected layer was 2–4 cells thick rather than the 1 or 2 cells thick found in older, experimentally produced and naturally occurring lesions.

At 21 and 28 days the papillomata became progressively larger, reaching a diameter of 1–2 mm. Intranuclear inclusions were more numerous and larger at this time. In addition, they were more densely packed with viral particles and the crystalline arrangement was more pronounced. These inclusions occurred in cell layers that varied in position from animal to animal. In some lesions they occurred just above the basal layer, while in others they occurred near the epithelial surface. However, the depth of the infected cell layer was constant in a single lesion; it varied only from animal to animal or from papilloma to papilloma.

Except in those cases where the infected layer was near the basal layer, the nuclei were heavily packed with virus particles. Infected nuclei near the epithelial surface were often completely filled with virus. The nucleolus was inversely related in size to the amount of virus present, being virtually nonexistent in the nuclei of virus-filled cells. Cells in which the nuclei were filled with virus had numerous invaginations and irregularities, as well as small vacuoles or vesicles and clumping of chromatin at the nuclear wall (Fig. 7).

In animals examined more than 28 days postinoculation there was gross evidence of regression in the size of the papillomata. This was especially true with lesions examined at 100 days postinoculation. Histologically and ultrastructurally, there was little difference between the lesions at 100 days and those at 28 days postinoculation. The major difference seemed to be the size of the over-all lesion. The older lesions still contained a narrow zone of epithelial cells, 1–2 cell layers thick, that contained virus-filled nuclei.

In addition to embedded and sectioned material, a small amount of each papilloma was ground as previously described, and negatively stained preparations were studied for the presence of virus. Virus was seen by this rapid screening technic in all cores in which tissue sections also contained virus. Table 1 indicates the correlation of gross evidence of papilloma and the presence of virus. Thus it was possible to detect or recognize the presence of virus by examination of ground tissues, by detection of nuclear inclusions, or by electron microscopy of the tissue sections.

The diameter of the virus was found to be 50–52 nm on negative-stained preparations and 40 nm when measured center to center in well-formed crystals in sectioned material. The diameter of the area of density as seen in these sections, or in loose aggregates in the sectioned material, was 38 nm. These particles appeared to be somewhat hexagonal in section, and the density at the center of the viral particle was lower than that at the periphery. Negative-stained preparations of the virus indicated that it had an icosahedral symmetry and that numerous capsomers were evident on the surface. It was not possible to determine the exact number of capsomers because the entire capsid was never seen in detail. Specifically, there were no particles in which 2 capsomers of 5-fold symmetry could be identified; thus an accurate count was not possible. The size of this virus is in the same general range as that reported for Shope papilloma (6) and human wart virus (1).

**Discussion**

The results concerning the ultrastructure of experimentally produced lesions of oral papillomatoses in the rabbit indicate that the experimental condition is similar to the naturally occurring condition. Furthermore, little difference between the early and late stages of the lesion was observed. In the earliest developing papillomata, which were detectable approximately 14 days postinoculation, the viral particles were not numerous or densely packed in well-formed crystals within the nuclei. By 28 days postinoculation, all infected cells had well-defined crystals of virus particles in their nuclei. From 28 to 100 days there was no difference in the ultrastructure of the lesion; the main differences were in gross size. Because virus-infected cells were found in narrow zones that varied with their depth in the epi-
Fig. 5. The 1st demonstration of the virus 14 days postinoculation, showing a random arrangement of viral particles. The horizontal line represents 1 \( \mu \)m. \( \times 30,000 \).

Fig. 6. Fourteen days postinoculation. The virus is beginning to aggregate into loose crystals. The horizontal line represents 1 \( \mu \)m. \( \times 16,000 \).
FIG. 7. Twenty-eight days postinoculation. Extensive packing of nucleus with viral crystalline inclusions. The horizontal line represents 1 μ. × 25,000.

FIG. 8. Negative stain of the virus as prepared by the rapid method described in the text. The horizontal line represents 1 μ. × 100,000.
the epithelium, it would seem that the development of the virus is cyclic in nature, as suggested in our previous report. Thus, the virus would begin its development in a layer of cells just above the basal layer, then develop to the point of filling the nuclei as this layer, or several layers, of cells migrated toward the surface of the epithelium to be sloughed away eventually. A new layer, or cycle of viral activity, would then begin when a previous cycle was sloughed from the surface cells.

The screening of tissue for electron microscopy was greatly enhanced by the use of 0.5-μ, stained sections studied with oil immersion light microscopy. Viral inclusions were readily observed. As a result, in future studies only selected blocks with inclusions need be used for ultrathin sectioning. This method enabled us to conclude that virus-filled cells are limited to a narrow zone of the epithelium no more than 2 cell layers thick in well-developed papillomata or 2-4 cell layers thick in the earliest stages.

References
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