EXPERIMENTAL TRANSMISSION OF CUTANEOUS PAPILLOMA OF THE HORSE *

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Cutaneous papillomas or common warts have been described as benign epithelial tumors.[1] They have been reported most frequently in man, cattle, dogs, and rabbits. Virus-induced papillomas have been reported in various species.[2-6] Kinsley [7] found the skin of the legs and lips to be the common locations in the horse. Cadeac [8] succeeded in transmitting a skin papilloma from one horse to another by applying the ground papillomatous material to a scarified area of the neck. Hagan [9] stated that papillomas are, as a rule, confined to the species of animal in which they originate and there seems to be a considerable measure of species specificity. Goldberg [10] considered papillomas to be the most common of all epithelial tumors, although Feldman [11] has reported them to be less common than malignant forms. According to Willis,[12] infective overgrowths, such as warts, are not true tumors. A study of a number of spontaneous and experimentally induced papillomas in horses constitutes the basis for this report.

Report of Cases

Equine cutaneous papilloma is a disease of horses 1 to 2 years old. The papillomas or warts commonly appear on the nose and about the lips as small, elevated, circumscribed, horny masses from 2 to 10 mm. in diameter. They may number from two or three to as many as 100 or more, covering the entire muzzle. Occasionally, when only a few warts develop, they reach a diameter of 15 to 20 mm. In the horse these warts are apparently of little economic concern. They cause the animal little inconvenience and disappear without leaving scars. Equine cutaneous papilloma has been enzootic in young horses raised at the University of Nebraska for several years. Four cases typical of the disease are described here.

Case 1. The subject was a yearling quarter horse colt. Five small papillomas were found on the nose on July 10, 1948. They slowly increased in size until 60 days later they formed one mass about 15 mm. in diameter. Many smaller growths were present also (Figs. 1 and 2). During the next 3 weeks there was no change in

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the size of the papillomas. However, during the following week they regressed and practically disappeared. There was no recurrence.

Case 2. Papillomas were found on the nose of an 18-months-old quarter horse on February 1, 1949. These grew slowly for 1 month and then rapidly for 3 weeks, reaching a diameter of 10 to 12 mm. Soon after reaching maximum size, regression was evident and all papillomas were gone by May 1, 1949. There was no recurrence.

Case 3. During the last week of September, 1948, several small, scattered papillomas were observed on the nose of a yearling Morgan filly. During the next 2 weeks, warts, 2 to 3 mm. in diameter, had developed over the entire muzzle. They remained for 1 month before there was evidence of regression. During the next month the papillomas disappeared.

Case 4. Seven small, scattered papillomas were noted around the nostrils of a 2-year-old Belgian mare on March 15, 1949. By April 1, two of the papillomas had coalesced to make a mass 8 by 12 mm. in diameter. No other papillomas appeared, but those present grew until May 3, when the smaller ones measured 2 to 3 mm. and the large clump 15 by 20 mm. in diameter. Regression began about the middle of June and 1 month later only a trace of the larger clump remained.

Experimental Transmission

A series of experiments was set up to determine the nature and some of the properties of the causative agent of equine papilloma. Transmissibility, resistance to heat, activity after various storage conditions, and the susceptibility of foreign hosts were investigated.

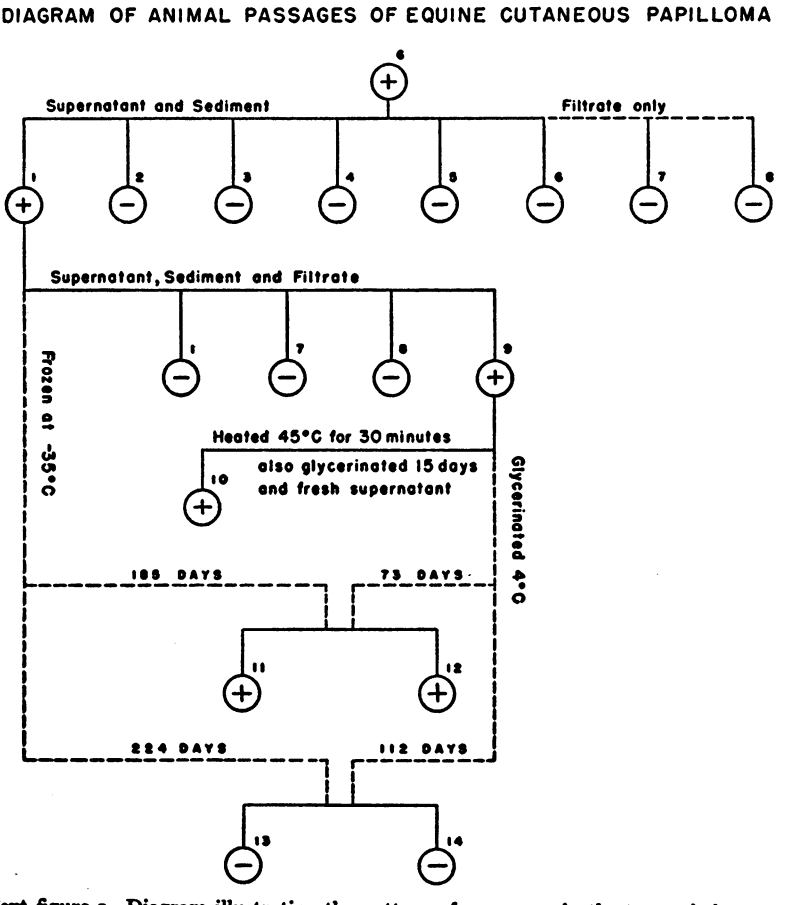
Papillomas used for experimental transmission were cleansed with 70 per cent ethanol and allowed to dry before being removed with scissors. They were ground to a suspension, using approximately 15 cc. of saline solution to each gm. of papilloma. The ground suspension was then centrifuged at a force of about 2400 × gravity to separate the coarse particles. Bacteria-free filtrates were made by filtering the supernatant fluid through Mandler filters (10 lb. test) at a negative pressure of 18 cm. of Hg. These filtrates proved to be bacteriologically sterile when cultured in tryptose broth. The sediment was diluted with saline solution to make the original volume. These three preparations (resuspended sediment, supernatant fluid, and filtrate) were the inocula used in the experiments.

Inoculation sites on the necks of the animals were prepared by shaving an area 2.5 cm. square for each inoculation. The skin was then cleansed with ethanol. Four methods of exposure were used. Intact skin exposure consisted of placing a drop of inoculum on the area which was then massaged with the rounded tip of a glass rod, and covered with adhesive tape to prevent immediate loss of the inoculum. Scarified skin exposures were similar except that small areas 2 to 3 mm. square were scarified with a hypodermic needle until serum exuded before applying the inoculum which was then rubbed in. For intradermal and subcutaneous exposures 0.05 to 0.10 cc. of inoculum was

injected into or under the skin as was desired. All inoculations were made in duplicate.

RESULTS

Sixteen horses were exposed to equine cutaneous papilloma, 14 as shown in Text-figure 1. Horse 6, a 2-year-old mare raised at the University of Nebraska, was naturally infected with equine cutaneous



Text-figure 1. Diagram illustrating the pattern of exposures in the transmission experiments. The circles represent horses which are numbered 1 to 14. A plus sign in the circle indicates that papillomas developed. The inocula used are indicated on the lines leading from the donor to the recipient.

papilloma (Fig. 3). One of the papillomas was removed and supernatant fluid, resuspended sediment, and bacteriologically sterile filtrate inocula were prepared as described. Seven horses were exposed with these inocula. Horses 1 to 6 inclusive were exposed with supernatant fluid and resuspended sediment intradermally, subcutaneously, on scarified skin, and on intact skin. Horses 7 and 8 were exposed with the

filtrate on scarified skin. Papillomas were first found on horse 1, a 3-year-old mare, 67 days after exposure (Fig. 4). These had stopped growing and appeared to be regressing 52 days later when the largest one (12 mm. in diameter) was removed to make inocula for another series. No papillomas developed at the site of exposure on intact skin. Small papillomas which developed from one subcutaneous exposure may have been due to a small amount of inoculum being deposited in the skin as the needle was withdrawn. Intradermal and scarified skin exposures gave equally good results. Larger papillomas developed from the supernatant fluid inoculum than from the sediment. Horse 2, a 10-year-old gelding; horse 3, a 10-year-old mare; and horse 4, a 14-year-old gelding, showed no papillomas. Horse 5, a 2-year-old gelding which had been raised at the farm where the disease is enzootic and had undoubtedly been exposed numerous times, also was refractory to the inocula. Horse 6, the donor of the papilloma used for the inocula, had apparently developed an immunity. She still had several papillomas when the experiment started, but they disappeared in about 2 weeks and none developed at the exposure site. Horses 7 and 8 were 12- and 13-year-old geldings, respectively. They showed no evidence of susceptibility; however, they were exposed only to the filtrate.

Supernatant fluid, resuspended sediment, and bacteriologically sterile filtrates were prepared from the papilloma which was removed from horse 1. Four horses were exposed with these inocula by scarification, intradermal injection, and subcutaneous injection. The horses were exposed within 3½ hours after the papilloma had been removed. The remainder of the supernatant fluid was stored at —35° C. to be used at a later date. Horses 1, 7, and 8 which have been previously described were exposed with horse 9, a 1-year-old filly. Horse 1 apparently had developed immunity from the previous induced infection, as she was resistant to the inocula. Horses 7 and 8 again were not susceptible. However, 66 days after exposure, small papillomas were developing from all three inocula in horse 9. Here, again, the supernatant fluid produced larger papillomas than either of the other inocula, although no difference was found in the incubation period. The external horn-like covering began to scale off 48 days after the papillomas first appeared. At this time one of the larger papillomas, which resulted from the supernatant fluid exposure, was removed to prepare inocula for another series. One week later, all of the remaining papillomas had disappeared.

A supernatant fluid inoculum was prepared from the papilloma from horse 9 immediately after its removal. This inoculum was divided into five fractions. Horse 10, a yearling cryptorchid colt, was immediately

exposed intradermally with one fraction. One part was heated to 45° C., another part was heated to 55° C., and still another part was heated to 65° C., each for 30 minutes. Horse 10 was then exposed intradermally with each of these heated fractions. An equal part of glycerol was added to the fifth fraction and it was stored at 4° C. After 15 days and again after 29 days of storage, horse 10 was exposed intradermally to this last fraction. No papillomas were found until about 90 days after the first exposure when small papillomas were found at the sites which had been exposed to the fresh supernatant fluid inoculum, and to the inoculum which had been heated at 45° C. Temperatures of 55° C. and 65° C. for 30 minutes apparently rendered the inoculum inactive as no papillomas were found at these sites. Papillomas were evident at the inoculation sites where 94 days previously the horse had been exposed with inoculum stored in glycerol for 15 days. Soon after this, the horse developed several papillomas on the nose from a natural exposure. No papillomas resulted from exposures to the supernatant fluid stored in glycerol for 29 days. This may have been due to the development of immunity from the previous exposures although the spontaneous infection on the nose occurred at about the time the papillomas should have been developing from this exposure.

Horses 11 and 12, a yearling filly and gelding, respectively, were inoculated with two supernatant fluids. One of these fluids came from horse 1 and had been frozen at —35° C. for 185 days. The other came from horse 9 and had been stored in glycerol at 4° C. for 73 days. The latter was the same inoculum which failed to produce papillomas in horse 10 after 29 days of storage. Small papillomas were evident from both inocula 90 to 95 days after exposure. These papillomas remained small but persistent for a period of 2 months.

Horses 13 and 14 were exposed 39 days later with the same inocula as horses 11 and 12. The frozen inoculum had been stored for 224 days and the glycerinated inoculum had been stored for 112 days at this time. These horses were examined periodically for a period of 5 months but no papillomas developed from the inoculations. However, 4 months after exposure, these horses developed papillomas on the nose from a natural infection which demonstrated that they probably were susceptible at the time of inoculation.

Horses 15 and 16, yearling colts, were placed in a pen with another yearling colt which had a natural infection of equine cutaneous papilloma. After 2 weeks, horses 15 and 16 were isolated and exposed to two inocula. One was supernatant fluid prepared from a papilloma growing on the colt to which they had been exposed. This will be

referred to as strain UN. The other inoculum was made from a papilloma growing on a yearling colt owned by a farmer. This will be referred to later as strain Folsom. Horse 15 was resistant to both inocula and no papillomas developed. Two weeks after inoculation a few papillomas developed on the nose of horse 16. These were probably from the previous exposure to the infected colt. No papillomas resulted from the inoculum from the colt to which horse 16 had been exposed (UN strain), but 58 days after exposure to the Folsom strain, small papillomas were present at the inoculation sites. These papillomas remained for 2 months and then regressed while the spontaneous papillomas were still present on the nose. The UN strain of papilloma may not have contained the causative agent when it was harvested or the horses may have been resistant to it. If the latter is true, there may be an immunologic difference in the strains of equine cutaneous papilloma, as horse 16 was susceptible to the Folsom strain.

Activity of the causative agent was tested in other species using 6 calves, 4 lambs, 3 dogs, 4 rabbits, and 4 guinea-pigs. These were exposed to supernatant fluid inocula that were active in susceptible horses. The calves and lambs were exposed intradermally and on scarified skin of the neck. The rabbits and guinea-pigs were similarly exposed on the skin of the abdomen. The dogs were exposed intradermally on the neck and on scarified oral mucous membrane. These animals were observed frequently for a period of 5 months but no reaction was found at the exposed sites.

Histopathologic Findings

The papillomas consisted principally of hyperplastic epithelium supported on a vascular connective tissue framework. There was elongation of the dermal papillae. Mitotic figures were most frequent in the basal layer of epithelial cells (Fig. 5). There was marked acanthosis or thickening of the prickle cell layer. Sometimes there was proliferation of epithelium about the hair shaft and hair follicles. Generally, the granular layer was thinned and narrower than normal. Nuclei were retained in the stratum corneum, which was thicker than in normal skin. There were areas in nearly every section, usually in the outer portion of the prickle cell layer, where there was degeneration of epithelial cells. These degenerated cells were swollen, the cytoplasm clear (perhaps vacuolated) as in so-called balloon degeneration. Spherical basic staining granules of variable size were present in the cytoplasm of these cells (Fig. 6). These granules may be inclusion bodies of a virus disease.[8] Occasionally the corium underlying the papillomas

was infiltrated with lymphocytes, histiocytes, and polymorphonuclear leukocytes. A mild fibrosis was associated with the cellular infiltration in some instances.

Discussion

Several similarities as well as differences were found in comparing equine cutaneous papilloma with infective papillomas reported by other workers (Table I). Equine cutaneous papilloma usually is confined to the skin of the nose and lips, while cutaneous papillomas of the bovine and rabbit may appear on any part of the body.[2,6] Oral papillomas of the dog and rabbit are confined to the oral mucosa.[3,5] Infectious papillomas occur principally in young animals, although oral and cutaneous papillomas may be found in mature rabbits.[5,6]

Filtration seemed to reduce the activity of the causative agent of equine cutaneous papilloma, as larger papillomas were produced with supernatant fluid than with filtrates. Creech [2] found that filtration reduced the activity of ground bovine papilloma suspensions while Shope [6] reported that filtration seemed to prolong the incubation period of the cutaneous papilloma when wild rabbits were inoculated, although it often shortened the incubation period in domestic rabbits.

An experimental infection with equine cutaneous papilloma apparently produces immunity against another experimental infection (horse 1). However, one horse (no. 10) developed a natural infection while papillomas from two previous experimental infections were still evident. At this time the horse was resistant to a third experimental exposure with known active inoculum. Kidd, Beard, and Rous [13] found the immunity produced in rabbits infected with cutaneous papillomas to be directly proportional to the amount of papillomatous tissue produced. Natural infection with equine cutaneous papilloma was associated with many more papillomas than experimental infections. Perhaps under these conditions more immunity was developed in the horses with the greater number of papillomas.

Summary

Equine cutaneous papilloma is an infectious disease normally confined to the skin of the nose and lips. Susceptible horses were infected by inoculating the skin of the neck with suspensions of ground papillomatous tissue and with bacteriologically sterile filtrates made from these suspensions.

The active agent was not affected when held at a temperature of 45° C. for 30 minutes but was rendered non-infectious when held at 55° C. and 65° C. This agent remained active for 73 days when stored

TABLE I

A Comparison of Some Virus Papillomas of Animals

	Cutaneous papilloma of horses	Cutaneous papilloma of cattle	Shope (cutaneous) papilloma of rabbit	Oral papilloma of rabbit	Oral papilloma of dogs
Usual location	Nose and lips	Shoulders[2], head, neck	Neck, shoulders, inner thighs, abdomen[6]	Mouth[5]	Mouth[3]
Usual age of susceptibility	1 to 3 years	Young[2]	All ages[6]	No age difference[5]	Young dogs[3]
Incubation period	2 to 3 months	2 months[2]	6 days[6]	6 to 38 days[5]	30 to 33 days[3]
Duration of papillomas	2 months	5 months[2]	6+ months[6]	2 to 13 months[5]	1½ to 3 months[3]
Immunity produced	After infection	Not cited	High degree after infection[14]	Solid after infection[5]	Solid after infection[3]
Storage period of virus	(a) 185 days at −35°C. (b) 73 days at 4°C. in 50% glycerol	Not known	106 days in glycerol–saline at 4°C.[6]	(a) 40 days at −22°C. (b) 703 days in 50% glycerol in Locke's solution at 4°C.[5]	(a) 50% glycerol 64 days at 10°C. (b) Dried frozen *in vacuo* 63 days at 10°C.[3]
Virus survived temperature of	45°C. for 30 min.	Not known	65°C. for 30 min.[6]	65°C. for 30 min.[5]	45°C. for 1 hour[3]
Virus inactivated at temperature of	55°C. for 30 min.	Not known	70°C. for 30 min.[6]	75°C. for 30 min.[5]	50°C. for 1 hour[3]
Virus filterable through	Mandler filter	Berkefeld N[2]	Berkefeld V, N, and W, positive; Seitz, negative[6]	Berkefeld V or N[5]	Berkefeld N[3]

in 50 per cent glycerol at 4° C., but was inactive after 112 days. In a suspension frozen at —35° C., the causative agent remained active for 185 days, but not for 224 days.

Some degree of immunity seems to be produced by experimental infections. Natural infections induce solid immunity.

Six calves, 4 lambs, 3 dogs, 4 rabbits, and 4 guinea-pigs were resistant to inoculations with known active suspensions of equine cutaneous papilloma.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 170

FIG. 1. Cutaneous equine papilloma, case 1. Photograph taken 60 days after lesions were first noted. The variation in size and also coalescence of small papillomas into larger masses are evident.

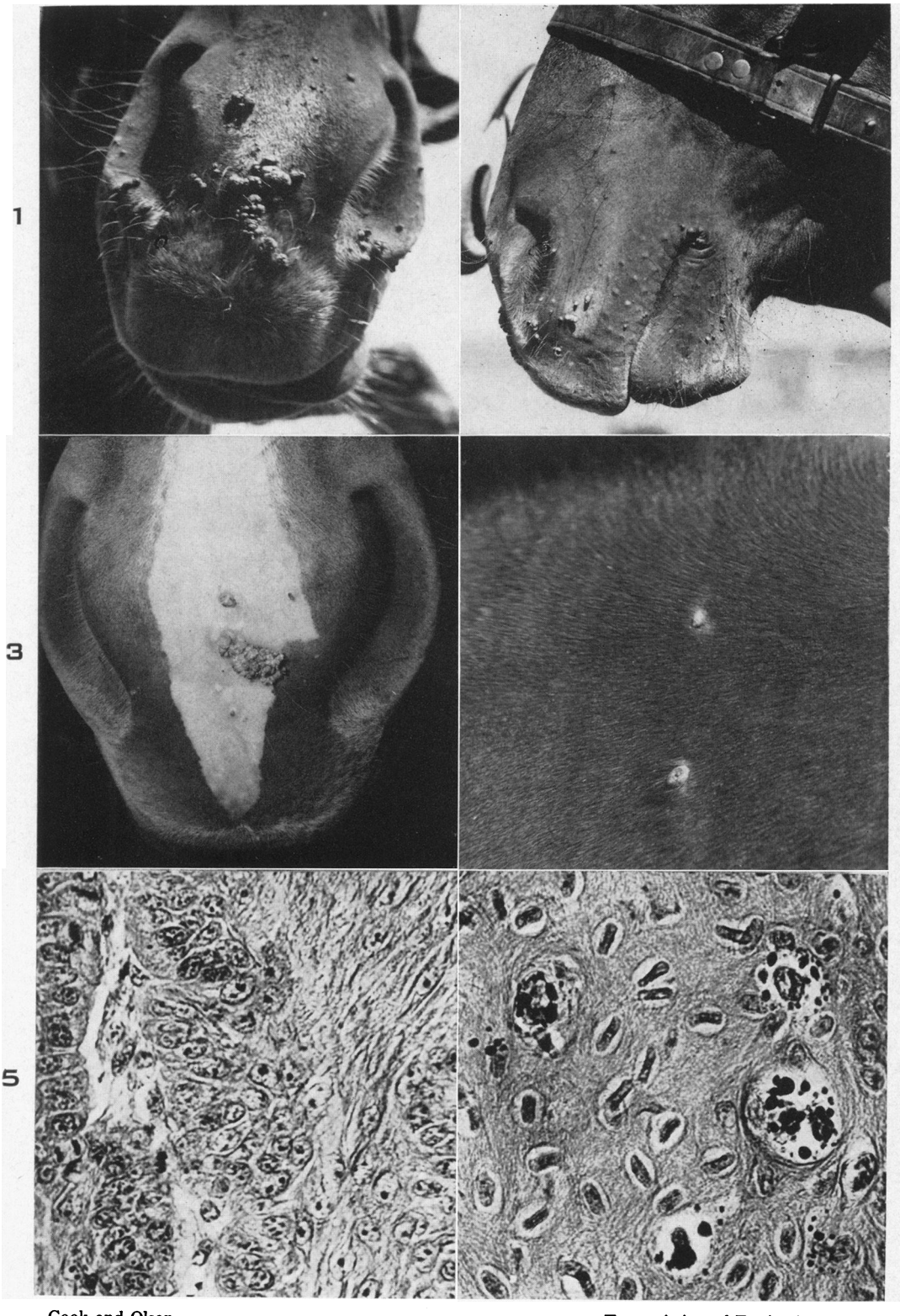
FIG. 2. Side view of case 1 showing small papillomas at corner of mouth and on chin.

FIG. 3. A natural case of equine cutaneous papilloma. The large papilloma to the left of the median line was used to make inocula for the original series of transmissions. The papillomas had been present about 3 months at the time the photograph was made.

FIG. 4. These papillomas, on the neck of horse 1, resulted from intradermal injection of a supernatant fluid inoculum prepared from the large papilloma shown in Figure 3. The photograph was made 87 days after the animal was exposed and 20 days after the papillomas were first apparent.

FIG. 5. Section of papilloma shown in Figure 4. The field includes epithelial cells adjacent to a dermal papilla. Mitotic figures are evident in the basal cells. × 450.

FIG. 6. A cross section of the prickle cell area of a papilloma in Figure 4. Granules of varying size may be noted in the clear cytoplasm of some swollen epithelial cells. These may be "inclusion bodies" of a virus. × 450.



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Transmission of Equine Papilloma