9008 C

Tissue Affinity of Shope Papilloma Virus.

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The virus causing papillomatosis in western cottontail rabbits is capable of producing papillomas in other species of rabbits and hares, but not in guinea pigs, rats, mice, dogs, goats or cats.[1] It causes growths which derive from the surface epidermal cells, not the specialized cells of the hair follicles or sebaceous glands, and direct inoculation of the virus into the lining epithelium of the oral cavity, trachea, rectum, genito-urinary tract, or salivary glands of susceptible rabbits has failed to result in lesions, as has intravenous inoculation, save when the virus localizes in the epidermis.[2] Since these observations were made with only a few virus materials it has seemed desirable to expand them, using virus fluids of known high potency and altering the epithelium to be tested in ways that might render it susceptible. In the course of the work an endemic oral papillomatosis of domestic rabbits was encountered which is described in an accompanying paper. The existence of this disease has added a further interest to the delineation of the tissue affinities of the Shope papilloma virus.

Tests of Normal Epithelium Other Than Skin. A 10% suspension of Shope virus of proven activity was tattooed freely into the under surface of the tongue, the cheek, lips, gums, nares, conjunctiva, genital mucous membrane, and skin of the sides of 4 normal, adult, domestic rabbits. In addition, it was tattooed into the buccal mucous membrane at the base of each lower incisor tooth and outwards along a continuous line to the inner surface of the lower lip and thence across the mucocutaneous junction and onto the skin of the lower lip. Virus was also tattooed into the genital mucous membrane and outwards along unbroken lines onto the adjoining skin. Characteristic papillomas appeared in all the animals after 10 to 14 days, but only in the skin where the virus had been inoculated. The growths enlarged progressively and those of the skin about the mouth and genitalia extended to within 1 mm. of the mucocutaneous junction, but never further. The animals were observed over

[1] Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607; Beard, J. W., and Rous, P., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 191.

[2] Rous, P., and Beard, J. W., *J. Exp. Med.*, 1934, **60**, 701.

the next 8 weeks; the skin growths in all animals continued to enlarge but no papillomas developed elsewhere. The experiment was repeated on 3 normal belted Dutch rabbits, using a different active virus material. All developed papillomas where inoculated on the skin, but no growths appeared at the sites of inoculation into the oral or genital mucous membrane, or into the conjunctiva.

Tests of the Susceptibility of Epithelium Keratinizing as a Result of Avitaminosis A. It is well known that widespread epithelial alterations (squamous metaplasia, keratinization) occur in animals deprived of vitamin A for a sufficient period. It was thought that under such circumstances changed epithelial tissues might prove susceptible to the Shope virus, although refractory to its action in their normal state.

A state of vitamin A deficiency, with weight loss and gross xerophthalmia was brought about in 6 of 7 young Dutch rabbits fed 6 to 12 weeks on a diet* consisting of

Regenerated cellulose	20
Casein	15
Sugar	10
Corn starch	37
Wesson oil	4.0
Yeast	10.0
Minerals[3]	4.0

2 drops of viosterol 250 D was added to the above and the diet was supplemented with white turnips carefully selected and pared of green tops. Two to 12 days after gross xerophthalmia had become evident all the rabbits were inoculated with large doses of active Shope virus. The virus was tattooed into the mouth, nares, conjunctivae, and vagina or penis, and in some instances large amounts were given intravenously and *per os.* Four of the animals survived the inoculations 2 weeks or longer and 2 developed characteristic papillomas on the skin, one as a result of localization out of the blood stream into an injured area, the other as a result of direct contamination. The condition of the rabbits was poor and they died 3 to 8 weeks after the virus inoculations. The diet had been maintained, and the elapsed period after inoculation had exceeded the ordinary incubation period of the virus. At autopsy the changes consequent on the vitamin deficiency were pronounced (gross xerophthalmia, weight loss, microscopic keratinization of the cornea,

* We are indebted to Dr. Alwin M. Pappenheimer and his associates, Dr. Madsen and Dr. Goettsch, for guidance as to the diet, as also for generous gifts of ground cellophane (regenerated cellulose).

[3] Hawk, P. B., and Oser, B. L., *Science*, 1931, **74**, 369.

conjunctiva, buccal mucous membrane and other epithelial tissues), yet nowhere had the virus produced papillomas save on the skin. Here the growths were characteristic.

Virus Injections into the Mammary Gland. In an attempt to infect the mammary epithelium with Shope virus 3 adult, female, gray-brown domestic rabbits were used. One of the rabbits had suckling young, the others had had litters some time previously but their breasts were inactive. Several areas of mammary tissue on each rabbit were "prepared" for virus infection by injecting into them a saturated solution of Scharlach R in olive oil. Three such injections were made into each area at intervals of 3 to 4 days, with result in firm, fleshy, subcutaneous lumps, which others have shown to consist of proliferating mammary epithelium.[4] Five days after the last Scharlach R injection, active Shope virus was inoculated into the under side of these reactive lumps, and also into several breast regions which had not been treated, by means of a hollow needle inserted through a slit in the skin some distance away. In addition, the ducts of several of the glands were distended with virus fluid inoculated through a blunt needle inserted into the nipple. The animals were observed for 5 to 10 weeks. No mammary growths developed, but in 2 of the rabbits papillomas appeared where the virus had come into contact with the epidermis of the nipple.

Tests with Embryo Skin. Previous findings have indicated that embryo rabbit epithelium is insusceptible to the action of the Shope virus. Two additional experiments, in which the mother was used as the test animal, have corroborated this finding.

The 15-day embryos were removed from a domestic rabbit by hysterectomy. Bits of the skin from several areas were taken from all the embryos. These were pooled and hashed fine with knives, under aseptic conditions. Half the minced fragments were then suspended in ordinary Tyrode under the same conditions. Equal portions of both materials were then injected through the slit skin into comparable situations in the fore and hind leg muscles, the axillae, and groins of the doe from which the embryos had been removed. Small nodules, 3 to 5 mm. in diameter, developed in both groins during the next 3 weeks, but later these gradually dwindled in size. At autopsy 10 weeks after the implantations several tiny nodules up to 1 mm. in diameter were found at the inoculated sites. These consisted mostly of connective tissue cells, phagocytes, and cartilage. No epithelium had survived. Surface papillomas had

[4] Bullock, F. D., and Rohdenburg, G. L., *J. Med. Res.*, 1915, **33**, 53.

appeared on the skin of the mother where virus had come into contact with it on withdrawing the needle. The experiment was repeated in another animal with identical results: no papillomatous or other progressive growths resulted when 25-day embryo skin, secured after hysterectomy and exposed to the virus *in vitro* was implanted into the muscles and subcutaneous tissue of the mother. In previous tests it had been found that implanted embryo epithelium survives and proliferates for several weeks before disappearing.

The results of several collateral experiments emphasize the significance of these tests. Minced portions of normal skin procured from 8 adult, domestic rabbits were exposed *in vitro* to the virus in the same way as was the embryo skin, and auto-implantations of the minced skin were made as before into the muscles and subcutaneous tissues. In every case papillomatous growths developed which were precisely like those resulting from auto-implantation of the Shope papilloma. On the other hand, attempts to infect Brown-Pearce tumor cells with papilloma virus by the same procedure yielded negative results.[5]

Summary. It is plain that the Shope papilloma virus is remarkably specific in its action, affecting only the epidermis of rabbits and hares and failing to influence embryonic epidermis or other kinds of epithelium, even when this is keratinizing as the result of avitaminosis A, or proliferating in consequence of Scharlach R stimulation.

9009 P

A Virus Causing Oral Papillomatosis in Rabbits.

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Papillomas can often be found in the mouths of domestic rabbits. They are usually situated on the under surface of the tongue, rarely elsewhere on the buccal mucous membrane. We have found them in 67 (17.4%) of 385 gray-brown, Dutch-belted, New Zealand, Havana and chinchilla rabbits obtained from various local sources. None was present in 44 Kansas cottontails.

[5] Kidd, J. G., Beard, J. W., and Rous, P., *J. Exp. Med.*, 1936, **64**, 79.